



Haemoglobin as a biomarker for clinical outcomes in chronic obstructive pulmonary disease

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Haemoglobin is nonlinearly associated with clinical outcomes in COPD, with increased morbidity at either extreme of the observed range, suggesting that therapeutic correction of anaemia might be most beneficial if targeting normal haemoglobin values <https://bit.ly/3ovl7Lt>

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Abstract

In COPD, anaemia is associated with increased morbidity, but the relationship between haemoglobin over its entire observed range and morbidity is poorly understood. Such an understanding could guide future therapeutic targeting of haemoglobin in COPD management. Leveraging the COPDGenE study, we conducted a cross-sectional analysis of haemoglobin from COPD participants, examining symptoms, quality of life, functional performance, and acute exacerbations of COPD (AECOPD). Haemoglobin was analysed both as a continuous variable and categorised into anaemia, normal haemoglobin, and polycythaemia groups. Fractional polynomial modelling was used for continuous analyses; categorical models were multivariable linear or negative binomial regressions. Covariates included demographics, comorbidities, emphysema, diffusing capacity, and airflow obstruction. From 2539 participants, 366 (14%) were identified as anaemic and 125 (5%) as polycythaemic. Compared with normal haemoglobin, anaemia was significantly associated with increased symptoms (COPD Assessment Test score: $p=0.006$, modified Medical Research Council (mMRC) Dyspnoea Score: $p=0.001$); worse quality of life (St. George's Respiratory Questionnaire (SGRQ) score: $p<0.001$; Medical Outcomes Study Short Form 36-item Questionnaire (SF-36) General Health: $p=0.002$; SF-36 Physical Health: $p<0.001$), decreased functional performance (6-min walk distance (6MWD): $p<0.001$), and severe AECOPD ($p=0.01$), while polycythaemia was not. Continuous models, however, demonstrated increased morbidity at both ends of the haemoglobin distribution ($p<0.01$ for mMRC, SGRQ, SF-36 Physical Health, 6MWD, and severe AECOPD). Evaluating interactions, both diffusing capacity and haemoglobin were independently associated with morbidity. We present novel findings that haemoglobin derangements towards either extreme of the observed range are associated with increased morbidity in COPD. Further investigation is necessary to determine whether haemoglobin derangement drives morbidity or merely reflects systemic inflammation, and whether correcting haemoglobin towards the normal range improves morbidity.

Introduction

Derangements in haemoglobin are common in COPD, with prevalence of anaemia and polycythaemia reported as high as 33% and 6%, respectively [1, 2]. Numerous studies have previously described an association between anaemia and COPD morbidity, including symptoms, quality of life, exercise



performance, and mortality [3–8]. Further, COPD patients with anaemia have increased healthcare use [9]. As a result, interventions targeting correction of anaemia have been considered to improve outcomes in COPD patients. To that end, however, a deeper understanding of morbidity across the spectrum of haemoglobin values in a COPD population would be a key step towards identifying potential thresholds that optimally demarcate morbidity.

Few studies have examined associations with COPD morbidity across a range of haemoglobin values that spans both anaemia and polycythaemia [2, 6], and to our knowledge prior studies have not evaluated haemoglobin as a continuous variable across its observed range. This is probably related to the varied pathophysiology represented by haemoglobin derangements; anaemia is believed to be related to chronic inflammation [10–12], whereas polycythaemia is often secondary to chronic hypoxaemia. In the era of long-term oxygen therapy, polycythaemia is noted to be less frequent [13, 14], and the clinical relevance of polycythaemia is poorly understood. Furthermore, physiological gas transfer interactions with anaemia and polycythaemia as they pertain to morbidity are unknown. Despite these complexities, understanding the association between haemoglobin as a continuous variable across its range and COPD morbidity is an important step in establishing the relevance of a given haemoglobin value in a clinical setting.

This study aims to evaluate the association between haemoglobin and COPD morbidity across the observed range, hypothesising that both anaemia and polycythaemia are associated with increased COPD morbidity. The COPDGene study offers an ideal opportunity to evaluate these associations in a large, well-phenotyped stable COPD cohort.

Methods

Study population and design

COPDGene is a prospective observational study of participants aged 45–80 years with or without prior smoking history conducted across 21 clinical centres. The COPDGene study methodology has been previously reported [15]. Initial enrolment occurred from 2007–2012, with follow-up visits scheduled every 5 years. The present investigation is a cross-sectional study of participants with COPD (defined as forced expiratory volume in 1 s (FEV₁)/forced vital capacity <70% with ≥10 pack-years smoking history) for whom complete blood count (CBC) data at the visit 5 years after original enrolment was available. From the 6284 participants who completed the 5-year visit, 2539 met inclusion criteria (figure 1). COPDGene was approved by institutional review boards at all participating centres. Each participant provided written informed consent.

Anaemia and polycythaemia characterisation

Haemoglobin concentrations, haematocrit, mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration were obtained from a CBC drawn at the 5-year visit. Anaemia was defined as a haemoglobin concentration <12 g·dL⁻¹ in females, and <13 g·dL⁻¹ in males [16], while polycythaemia

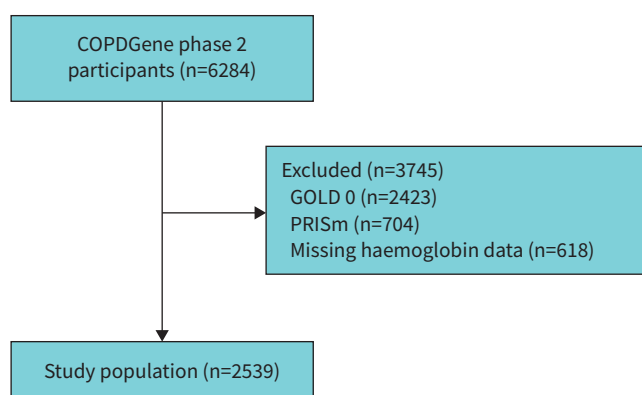


FIGURE 1 Participant selection. Excluded participants include individuals with a forced expiratory volume in 1 s (FEV₁)/forced vital capacity ratio ≥0.7 who had an FEV₁ >80% predicted (GOLD 0) or had impairment in FEV₁ <80% predicted (Preserved Ratio Impaired Spirometry, PRISm), and those without available haemoglobin data. Study population included only those with COPD (GOLD 1–4) and haemoglobin data. GOLD: Global Initiative for Chronic Obstructive Lung Disease.

was defined as haemoglobin $>16 \text{ g}\cdot\text{dL}^{-1}$ in females, and $>16.5 \text{ g}\cdot\text{dL}^{-1}$ in males [17]. Haematocrit values were not included in definitions for the purposes of consistency across analyses, as haemoglobin is the primary measure of interest.

Physiological testing

Spirometry and diffusing capacity of the lung for carbon monoxide (D_{LCO}) measurements were conducted using the ndd EasyOne Pro in accordance with European Respiratory Society (ERS)/American Thoracic Society (ATS) guidelines [18], with standardisation of protocols and quality control procedures across clinical sites. Only subjects with tests judged acceptable and reproducible were included. FEV_1 and D_{LCO} % predicted values were calculated using Global Lung Initiative reference equations [19, 20], with D_{LCO} values adjusted for haemoglobin and altitude [20]. Six-minute walk tests (6MWTs) were conducted per ATS guidelines [21].

Computed tomography

Computed tomography (CT) scans were acquired using individual site CT scanners, with previously published protocols for each scanner type [22]. Total % emphysema (%LAA₋₉₅₀) was defined as the per cent of voxels with an attenuation at or below -950 Hounsfield units (HU), quantified using Thirona software (<http://www.thirona.eu>).

Patient-reported outcomes

COPD Assessment Test (CAT) [23], St. George's Respiratory Questionnaire (SGRQ) [24], modified Medical Research Council (mMRC) Dyspnoea Score [25], and Medical Outcomes Study Short Form 36-item Questionnaire (SF-36) [26] were administered and scored to assess quality of life and impact of symptoms.

Exacerbations

Exacerbation rate was determined from self-reported episodes of increased COPD symptoms requiring antibiotics or steroids in the 12 months preceding this evaluation. Severe exacerbations were defined as the subset of exacerbations requiring an emergency room (ER) visit or hospitalisation, while moderate exacerbations were those that did not.

Statistical analysis

Participant characteristics were described using means with standard deviations, unless otherwise specified. Prevalence of anaemia and polycythaemia were described by COPD disease severity using the Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification [27]. We analysed morbidity using haemoglobin as both a categorical and continuous variable. For categorical analyses, multivariable linear and negative binomial regression models were constructed for outcomes as appropriate with the normal haemoglobin group set as reference. All models were adjusted for age, sex, ethnicity, education, body mass index (BMI), pack-years smoking history, smoking status, FEV_1 % predicted, D_{LCO} % predicted, and %LAA₋₉₅₀. Further, models were adjusted for self-report of the following comorbidities: congestive heart failure (CHF), hypertension (HTN), diabetes mellitus (DM), and chronic kidney disease (CKD). A sensitivity analysis was performed excluding non-normocytic individuals to address possible differences due to microcytic or macrocytic anaemia. Given the small sample sizes for these groups, formal statistical interaction testing was not performed. A second sensitivity analysis included oxygen use and resting oxygen saturation as covariates to address confounding with regard specifically to the relationship between polycythaemia and morbidity.

For models with haemoglobin treated as a continuous variable, fractional polynomial regression modelling was used to characterise nonlinear relationships. Using the “fp” package (StataCorp), powers for the haemoglobin term were selected from the set $\{-2, -1, -0.5, 0, 0.5, 1, 2, 3\}$ to identify the best-fitting polynomial model. Point estimates and 95% confidence intervals displayed in the figures are predicted values for the adjusted associations between haemoglobin and outcomes using the “marginscontplot” package (StataCorp) [28, 29]. Fractional polynomial models were similarly adjusted for the covariates used in the categorical analyses.

We conducted interaction analyses using the “mfpigen” package (StataCorp) [30], which uses fractional polynomial modelling to test and visualise interaction effects between continuous predictors. We explored the unadjusted interaction between haemoglobin and D_{LCO} % predicted to evaluate the combined and independent effects of impaired gas transfer and alterations in haemoglobin on morbidity. Our D_{LCO} % predicted values were corrected for haemoglobin, to better capture the effects of membrane diffusivity alone and minimise correlations between haemoglobin and D_{LCO} in analyses.

All analyses were conducted using Stata 15.1 (StataCorp LLC, College Station, TX, USA) [31], with significance for main effects set at a p-value of <0.05 and for interactions set at a p-value <0.1.

Results

Participant characteristics

The study population included 2539 participants with COPD GOLD spirometry stage 1–4 with haemoglobin measurements (figure 1). Haemoglobin values in the study cohort ranged from 6.2–19.6 g·dL⁻¹, with a median (interquartile range (IQR)) of 14 (13 – 15). Approximately 14% of participants were anaemic and 5% were polycythaemic. Baseline characteristics categorised by clinical definitions of anaemia, normal haemoglobin, and polycythaemia are presented in table 1. A higher percentage of anaemic participants were African American and had cardiovascular and renal comorbidities than those with normal haemoglobin. Polycythaemic participants were over 80% male, and a majority were current smokers. Approximately 20% of participants used oxygen in the normal haemoglobin and polycythaemia groups, compared with 34% of anaemic participants. Average MCV tended to be higher in the normal haemoglobin and polycythaemia groups. When categorised into microcytic (MCV <80 fL), normocytic (80–100 fL), and macrocytic (MCV >100 fL), a greater percentage of anaemic participants were microcytic than those with polycythaemia or normal haemoglobin (supplemental table 1). Conversely, polycythaemic participants had a higher percentage with macrocytosis (supplemental table 1). Proteomic analyses of C-reactive protein (CRP), a measure of systemic inflammation, and transferrin, a biomarker reflecting iron

TABLE 1 Participant characteristics

Characteristic	Anaemia	Normal haemoglobin	Polycythaemia
Subjects n	366	2048	125
Age years	70±9	68±8	66±8
Female	151 (41)	956 (47)	24 (19)
African American	131 (36)	451 (22)	20 (16)
Education			
Eighth grade or less	12 (3)	40(2)	2 (2)
High school, no diploma	45 (12)	177 (9)	6 (5)
High school graduate or GED	93 (25)	505 (25)	29 (23)
Some college, no degree	113 (31)	561 (27)	43 (34)
College or technical degree	75 (20)	548 (27)	32 (26)
Master's or Doctoral degree	28 (8)	217 (11)	13 (10)
BMI kg·m ⁻²	29±7	28±6	28±5
Smoking status			
Former smoker	267 (73)	1321 (65)	55 (44)
Current smoker	99 (27)	727 (36)	70 (56)
Smoking history pack-years	53±26	50±25	50±21
Oxygen use	123 (37)	453 (22)	24 (19)
Resting oxygen saturation %	95±4	95±3	94±4
Congestive heart failure	49 (13)	87 (4)	5 (4)
Coronary artery disease	55(15)	221 (11)	17 (14)
Hypertension	252 (69)	1069 (52)	68 (54)
Diabetes	81 (22)	326 (16)	16 (13)
Chronic kidney disease	34 (9)	58 (3)	1 (1)
Sleep apnoea	38 (10)	242 (12)	15 (12)
FEV ₁ % predicted	57±22	61±23	59±20
FVC % predicted	79±20	83±20	80±18
FEV ₁ /FVC ratio	0.53±0.13	0.54±0.12	0.55±0.12
D _{LCO} % predicted	62±22	67±22	67±23
%LAA ₋₉₅₀ , median (IQR)	5 (2–16)	6 (2–16)	5 (2–12)
LAA ₋₉₅₀ >5% [#]	159 (51)	994 (53)	55 (48)
MCV fL	89±8	92±5	94±4

Data are presented as mean±SD or n (%), unless otherwise stated. Study population of COPD participants, Global Initiative for Chronic Obstructive Lung Disease 1–4 with anaemia and polycythaemia groups defined by haemoglobin cut-off values. BMI: body mass index; FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide; GED: General Education Diploma; IQR: interquartile range; LAA₋₉₅₀: low attenuation area <-950 HU, representing emphysema; MCV: mean corpuscular volume. #: emphysema was available for n=2299.

stores, demonstrated higher CRP among anaemic participants than participants with normal haemoglobin (supplemental figure 1). No differences were noted across groups in transferrin.

The study population had on average moderate airflow obstruction, with all three groups demonstrating similar mean FEV₁ % predicted values (57%, 61%, and 59% for anaemic, normal, and polycythaemic participants, respectively). Gas transfer, after correction for haemoglobin and altitude, tended to be more impaired among the anaemic participants (D_{LCO} 62% predicted) compared to normal and polycythaemic participants (D_{LCO} 67% predicted). The three groups had similar degrees of emphysema, with roughly half of all participants demonstrating greater than 5% low attenuation areas on CT.

Using the GOLD classification of disease severity [27], we observed increasing anaemia prevalence with greater COPD disease severity. In contrast, no clear pattern of polycythaemia prevalence emerges with disease severity (supplemental figure 2).

Anaemia is independently associated with COPD morbidity

Multivariable models comparing anaemic participants to those with normal haemoglobin demonstrated independent associations with symptoms, quality of life, and functional performance by 6-min walk distance (6MWD) (table 2). Anaemic participants demonstrated on average an SGRQ score 4.19 points (95% CI 1.86–6.51, $p < 0.001$) higher than those with normal haemoglobin; compared to a minimum clinically important difference (MCID) of 4 [32], this association is both statistically and clinically significant. Similarly, on the 6MWT, anaemic participants walked on average 51 m less than normal haemoglobin participants (95% CI 36.6–65.4, $p < 0.001$), which is again a clinically relevant difference compared to an MCID of 25 m [33, 34]. In addition, anaemia was associated with a 63% higher rate of severe exacerbations (rate ratio (RR) 1.63, 95% CI 1.1–2.4, $p = 0.01$) as compared with normal haemoglobin (table 2). In sensitivity analyses excluding individuals with microcytosis or macrocytosis, similar results were observed although anaemia no longer demonstrated statistically significant associations with CAT score, SF-36 mental health component score, or severe exacerbations (supplemental table 2).

There were no significant associations between polycythaemia and morbidity (table 2) compared with normal haemoglobin. Polycythaemia tended towards higher rates of severe exacerbations (RR 1.24, 95% CI 0.64–2.38, $p = 0.52$) but did not achieve statistical significance for either moderate or severe exacerbations (table 2). Additional sensitivity analyses adjusting for resting oxygen saturation and long-term oxygen use, demonstrated similar results (supplemental table 3).

Haemoglobin demonstrates a nonlinear association with clinical morbidity

Examining haemoglobin as a continuous variable using flexible modelling methods provided evidence of nonlinear relationships between haemoglobin and COPD morbidity across the observed range (figure 2). Specifically, morbidity was increased at both extremes of the range of haemoglobin values for most

TABLE 2 Anaemia is associated with COPD morbidity

	Anaemia		Polycythaemia	
	β (95% CI) [#]	p-value	β (95% CI) [#]	p-value
CAT score	1.40 (0.40–2.41)	0.006	−0.31 (−1.80–1.19)	0.69
mMRC dyspnoea score	0.28 (0.12–0.44)	0.001	0.07 (−0.16–0.31)	0.55
SGRQ score	4.19 (1.86–6.51)	<0.001	0.49 (−2.95–3.94)	0.78
SF-36 general	−2.14 (−3.47–−0.81)	0.002	1.24 (−0.73–3.21)	0.22
SF-36 physical function	−2.80 (−4.13 –−1.48)	<0.001	0.40 (−1.57–2.37)	0.69
SF-36 mental health	−1.47 (−2.92–−0.01)	0.048	−0.37 (−2.52–1.79)	0.74
6MWD m	−51.0 (−65.4–−36.6)	<0.001	−0.2 (−21.4–20.9)	0.98
Moderate exacerbations [#]	1.06 (0.76–1.49)	0.72	0.71 (0.41–1.24)	0.23
Severe exacerbations [#]	1.63 (1.10–2.40)	0.01	1.24 (0.64–2.38)	0.52

Models adjusted for age, sex, ethnicity, education, body mass index, pack-years smoking, smoking status, congestive heart failure, hypertension, diabetes mellitus, chronic kidney disease, % emphysema, forced expiratory volume in 1 s % predicted, and diffusing capacity of the lung for carbon monoxide % predicted. Coefficients and p-values are in comparison to normal haemoglobin. β coefficient units are points for CAT, mMRC, SGRQ, and SF-36 scores, and metres for 6MWD. CAT: COPD Assessment Test; mMRC: modified Medical Research Council Dyspnoea Score; SGRQ: St. George's Respiratory Questionnaire; SF-36: Medical Outcomes Study Short Form 36-item Questionnaire; 6MWD: 6-min walk distance. [#]: RR (95% CI) for moderate and severe exacerbations.

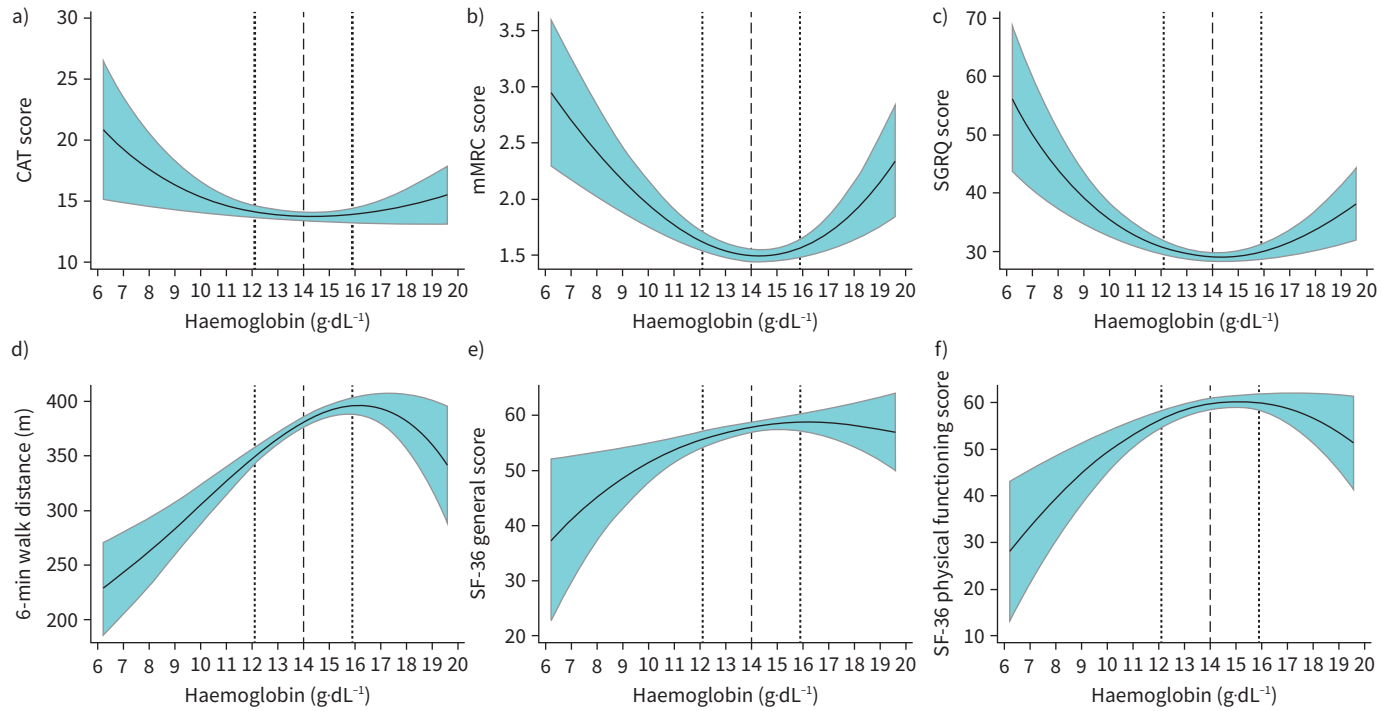


FIGURE 2 Morbidity across the range of haemoglobin values: **a)** COPD Assessment Test (CAT), **b)** modified Medical Research Council Dyspnoea Score (mMRC), **c)** St. George's Respiratory Questionnaire (SGRQ), **d)** 6-min walk distance, and **e, f)** Medical Outcomes Study Short Form 36-item Questionnaire (SF-36). Models adjusted for age, sex, ethnicity, education, pack-years smoked, smoking status, body mass index, congestive heart failure, hypertension, diabetes mellitus, chronic kidney disease, forced expiratory volume in 1 s % predicted, diffusing capacity of the lung for carbon monoxide % predicted, and % emphysema. Dashed line represents median (14 g·dL⁻¹), and dotted lines represent 10th (12.1 g·dL⁻¹) and 90th (15.9 g·dL⁻¹) percentiles of haemoglobin.

outcomes. This pattern was most evident with mMRC dyspnoea score, SGRQ score, 6MWD, and SF-36 physical functioning score (figure 2b–d, f). In contrast with the categorical analyses, participants in both the anaemia and polycythaemia ranges had increased symptoms, decreased quality of life, and reduced functional performance.

Moderate exacerbations tended to be stable across the observed range of haemoglobin values, while severe exacerbations were increased among severely anaemic and polycythaemic participants (supplemental figure 3). Statistical testing of the combined haemoglobin terms in the fractional polynomial models for all outcomes are presented in supplemental table 4.

D_{LCO} does not modify the association between haemoglobin and morbidity

To evaluate the interrelated physiological processes of anaemia and gas transfer, we examined the interaction between D_{LCO} and haemoglobin on morbidity. Of note, D_{LCO} % predicted was corrected for haemoglobin, to better capture diffusivity specifically. The association between haemoglobin and morbidity was found to be consistent across a wide range of D_{LCO} values, and with greater D_{LCO} impairment, there was a consistent increase in morbidity across all outcomes (figure). Significant interactions were identified between D_{LCO} and haemoglobin for mMRC (figure 3b) and SGRQ score (figure 3c), but not for CAT score, 6MWD, SF-36 (figure 3a, d–f), moderate exacerbations ($p=0.94$), or severe exacerbations ($p=0.21$) (exacerbation plots not shown). For mMRC and SGRQ, the effect of haemoglobin on morbidity is attenuated below a D_{LCO} of ~30% predicted, with minimal variation in morbidity scores across the haemoglobin range. In other words, with very severe reduction in D_{LCO} , breathlessness is high, and quality of life is poor, irrespective of haemoglobin value.

Discussion

In this well-characterised cohort of COPD participants, haemoglobin derangements (anaemia or polycythaemia) were found to be prevalent (19%) and associated with increased morbidity including symptoms, quality of life, functional exercise performance, and hospitalisations for COPD exacerbations. We demonstrate that clinical categorisation of anaemia and polycythaemia incompletely describes the

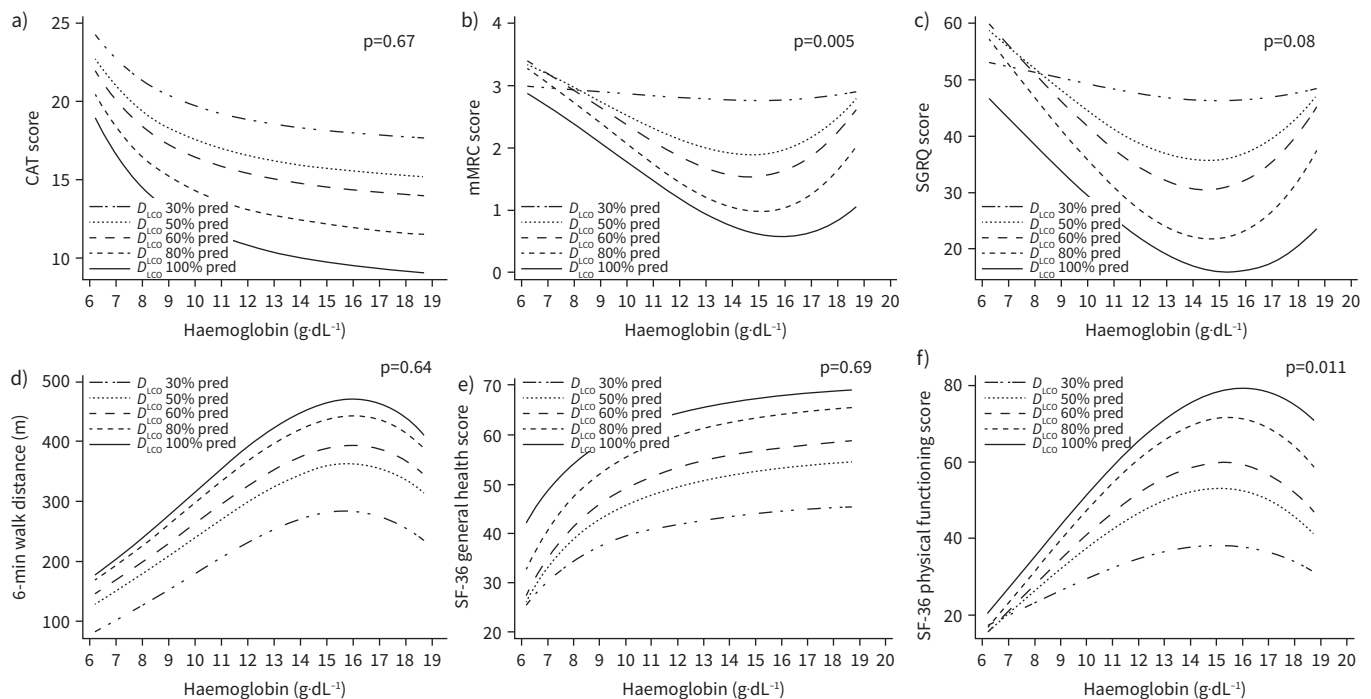


FIGURE 3 Relationship between haemoglobin and COPD outcomes by level of gas transfer impairment. **a)** COPD Assessment Test (CAT), **b)** modified Medical Research Council Dyspnoea Score (mMRC), **c)** St. George's Respiratory Questionnaire (SGRQ), **d)** 6-min walk distance, **e)** Medical Outcomes Study Short Form 36-item Questionnaire (SF-36) general health score and **f)** SF-36 physical functioning score. Models were unadjusted regressions of clinical outcomes on the interaction between haemoglobin and diffusing capacity of the lung for carbon monoxide (D_{LCO}) % predicted. Plots are for marginal predictions at D_{LCO} % predicted values of 30, 50, 60, 80, and 100% predicted. D_{LCO} % predicted is adjusted for altitude and haemoglobin. p-values correspond to the interaction term.

relationship between haemoglobin and clinical morbidity outcomes in COPD. Importantly, we note that haemoglobin is associated with morbidity in a nonlinear fashion, and we observe an optimal range of haemoglobin values. Finally, we identify haemoglobin derangement and gas transfer as distinct factors associated with morbidity. With very severe impairment in gas transfer, dyspnoea is high, and quality of life is poor, irrespective of haemoglobin. These novel results better characterise haemoglobin derangements in an epidemiologic study of stable COPD patients and further generate hypotheses regarding the relationship between haemoglobin and morbidity outcomes.

In the COPDGene cohort, we demonstrate a 14% prevalence of anaemia, and a 5% prevalence of polycythaemia, similar to prior studies in COPD [1, 2, 8–10, 35, 36]. In SPIROMICS, another large well-characterised cohort of COPD, PUTCHA *et al.* [10] described a prevalence of 7.5% for normocytic anaemia while our cohort had ~13% with normocytic anaemia. This difference may be attributable to the enrichment for African-Americans in the COPDGene cohort; anaemia in COPD has been observed to be more common among African-Americans [10, 37, 38]. Prevalence of polycythaemia in our cohort is lower than prior estimates ranging from 6% to 8%, but previous studies may have inadvertently been enriched for polycythaemia, with predominantly male and long-term oxygen therapy COPD patients [2, 8]. The presented results offer prevalence estimates in a general COPD population.

We observed microcytosis was present in a higher percentage of anaemic participants, while macrocytosis was more frequent in polycythaemic individuals, which is consistent with purported mechanisms of development of anaemia and polycythaemia in COPD. Systemic inflammation and impaired iron utilisation are implicated in development of anaemia in COPD [10, 11, 35, 39], which would present as either normocytic or microcytic anaemia. In concordance with this, higher CRP levels were noted among anaemic participants as compared to individuals with normal haemoglobin. Meanwhile, chronic hypoxia with subsequent erythropoietin stimulation leads to release of larger, immature red blood cells [40]. Consistent with this, polycythaemic patients had lower resting oxygen saturations and had more macrocytosis. Additionally, 56% of polycythaemic participants were current smokers, and previous studies have described smoking-related elevations in carboxyhaemoglobin leading to development of polycythaemia [41, 42].

In analyses comparing anaemia and polycythaemia to a normal group, we identified significant independent associations between anaemia and morbidity across multiple domains. There have been numerous studies demonstrating the associations between anaemia and increased symptoms [2, 3, 8, 10], worse quality of life [2, 3, 10], decreased exercise performance [2, 3, 35], increased hospitalisations [5, 8, 36], and increased mortality [4, 8, 36, 43]. The presented findings confirm the association between anaemia and morbidity outcomes. Categorical analysis also identified no significant associations between polycythaemia and morbidity. COTE *et al.* [2] conducted a study of COPD participants recruited from a Veterans Affairs clinic and described no significant association between polycythaemia and functional status by mMRC and 6MWD. Our categorical results confirm these existing findings in the setting of a relatively low prevalence of polycythaemia compared with anaemia, and further explore the range of haemoglobin values in our continuous analyses.

We demonstrate novel findings of a nonlinear relationship between haemoglobin and morbidity across all domains. A nonlinear relationship between haemoglobin and morbidity has been described [10], but that study notably excluded those with polycythaemia or non-normocytic anaemia. Our results extend those findings by examining haemoglobin continuously across its entire observed range and demonstrating increased morbidity at either end of the haemoglobin spectrum, departing from the categorical analyses previously published [2, 8] and described in this cohort. The findings of increased morbidity with haemoglobin values in the polycythaemia range, to our knowledge, have not been previously described, although studies preceding the routine use of long-term oxygen therapy describe improvement in exercise tolerance among COPD patients after phlebotomy to a haematocrit below 55% [44, 45]. The discrepancy between this finding and the presented categorical analyses may be related to individuals with haemoglobin values close to the definition cut-off, minimising differences between those categorised as polycythaemic *versus* normal haemoglobin. The mechanism behind the increased morbidity associated with polycythaemia is not explored in this analysis, but may be related to untreated hypoxaemia, or active smoking, which generates carboxyhaemoglobin and can yield a functional anaemia that limits oxygen carrying capacity and thereby functional performance [41, 42].

Acknowledging the nonlinear relationship between haemoglobin and morbidity allows for identification and development of appropriate haemoglobin targets for interventions seeking to address anaemia in management of COPD. Such interventions include blood transfusions, iron administration, erythropoiesis-stimulating agent (ESA) administration, or novel small-molecule therapies. To date, few

studies have been published evaluating the utility of treatment of anaemia in COPD. One small study noted that blood transfusions in stable COPD patients improved minute ventilation and reduced work of breathing [46]. Similarly, in a case series of five COPD patients in an intensive care unit setting, blood transfusions to an average haemoglobin concentration of $12 \text{ g}\cdot\text{dL}^{-1}$ were associated with more rapid weaning off a ventilator in COPD patients [47]. A small trial of intravenous iron therapy in patients with COPD noted improvements in dyspnoea and walk distance as compared with placebo [48]. Interestingly, the study was conducted in COPD patients irrespective of anaemia or iron deficiency, with an average haemoglobin level of $14.5 \text{ g}\cdot\text{dL}^{-1}$ and only 10% iron-deficient at baseline. Given our presented findings, the somewhat modest effects noted in this trial may be due to inappropriate selection of participants. Another small observational study of a combination of iron and ESA therapies in anaemic COPD patients noted an improvement in dyspnoea that correlated with the improvement in haemoglobin [49]. Finally, to date there are no studies in a COPD population of ESA therapy alone or small-molecule therapies that inhibit hypoxia-inducible factor, both of which are used or are being evaluated in CKD. The presented results offer information on appropriate selection of individuals who may benefit the most from management of haemoglobin derangements with such therapies, establishing a foundation for future studies in this arena.

Another key finding of this study was that both haemoglobin derangement and gas transfer impairment independently associated with morbidity outcomes, as demonstrated by a consistent relationship between haemoglobin and morbidity across various D_{LCO} levels for all outcome domains except dyspnoea and quality of life. For these outcomes, beyond very severe gas transfer impairment, morbidity was high irrespective of haemoglobin level. The lack of effect modification between haemoglobin and D_{LCO} suggests that each measure is distinctly associated with morbidity. While potential mechanisms for the associations with morbidity were not assessed in this study, this generates hypotheses regarding D_{LCO} as a reflection of parenchymal lung damage and haemoglobin possibly reflecting an inflammatory state in COPD. The relationships between D_{LCO} and haemoglobin are complex, with likely variable associations across the spectrum of anaemia to polycythaemia, and the presented findings suggest that both D_{LCO} and haemoglobin are relevant clinical factors to consider with respect to morbidity in COPD.

The present study is limited by the cross-sectional study design, precluding discussion of directionality of the associations identified, and self-report for many of the outcomes including COPD exacerbations. However, the consistency of the associations across multiple morbidity outcomes and with prior publications across multiple different cohorts suggests that these results are robust. Another key limitation of this study is incomplete characterisation of haemoglobin derangements. Microcytosis and macrocytosis prevalence were low among anaemic and polycythaemic individuals, limiting our ability to conduct analyses assessing effect modification of MCV across the spectrum of haemoglobin values. The lack of data on iron indices and erythropoietin levels limits discussion regarding mechanisms of haemoglobin derangements and the impact on morbidity. Similarly, measures of renal insufficiency would provide more accurate estimation of CKD in this population, a key comorbidity known to alter haemoglobin levels. However, as a surrogate, self-reported CKD and other comorbid conditions were included to limit confounding from multiple comorbid conditions including renal insufficiency. Finally, the present study, while suggestive of values that may define an optimal haemoglobin range for COPD patients, is a cross-sectional study without formal threshold analysis, in part due to the low prevalence of polycythaemia. Future studies across multiple large cohorts may better establish cut-off values of clinical relevance in a COPD population.

The presented findings demonstrate in a large cohort of COPD participants that haemoglobin derangements are prevalent and are independently associated with varying degrees of increased morbidity across the spectrum of observed haemoglobin values. Examination of haemoglobin as a continuous variable suggests that values in both the anaemia and polycythaemia ranges are associated with increased morbidity, and that there may be an optimal range of haemoglobin values that is relevant for a COPD population. These results offer support for future investigation of whether haemoglobin derangements drive morbidity or are markers of underlying disease severity and further exploration of factors that contribute to derangements in haemoglobin among this population. Additionally, these findings lay the foundation for research regarding potential therapeutic targeting of haemoglobin in management of COPD. In summary, this study establishes the association between haemoglobin and morbidity in COPD and supports consideration of haemoglobin measurement in evaluation of symptomatic individuals with COPD.

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