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Original Research

Circulating 1,3-Beta-D-Glucan is Associated with Lung Function, Respiratory Symptoms, and Mediators of Matrix Degradation in Chronic Obstructive Pulmonary Disease

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Abstract

Introduction: Factors beyond cigarette smoke likely contribute to chronic obstructive pulmonary disease (COPD) pathogenesis. Prior studies demonstrate fungal colonization of the respiratory tract and increased epithelial barrier permeability in COPD. We sought to determine whether 1,3-beta-d-glucan (BDG), a polysaccharide component of the fungal cell wall, is detectable in the plasma of individuals with COPD and associates with clinical outcomes and matrix degradation proteins.

Methods: BDG was measured in the plasma of current and former smokers with COPD. High BDG was defined as a value greater than the 95th percentile of BDG in smokers without airflow obstruction. Pulmonary function, emphysema, and symptoms were compared between COPD participants with high versus low BDG. The relationship between plasma BDG, matrix metalloproteinases (MMP) 1, 7, and 9, and tissue inhibitor of matrix metalloproteinases (TIMP) 1, 2, and 4 was assessed adjusting for age, sex, and smoking status.

Results: COPD participants with high BDG plasma levels (19.8%) had lower forced expiratory volume in 1 second to forced vital capacity ratios (median 31.9 versus 39.3, p=0.025), higher St George's Respiratory Questionnaire symptom scores (median 63.6 versus 57.4, p=0.016), and greater prevalence of sputum production (69.4% versus 52.0%) and exacerbations (69.4% versus 48%) compared to COPD participants with low BDG. BDG levels directly correlated with MMP1 (r=0.27, p<0.001) and TIMP1 (r=0.16, p=0.022) in unadjusted and adjusted analyses.

Conclusions: Elevated plasma BDG levels correlate with worse lung function, greater respiratory morbidity, and circulating markers of matrix degradation in COPD. These findings suggest that targeting dysbiosis or enhancing epithelial barrier integrity may have disease-modifying effects in COPD.

Abbreviations: chronic obstructive pulmonary disease, **COPD**; 1,3-beta-d-glucan, **BDG**; matrix metalloproteinases, **MMP**; tissue inhibitor of matrix metalloproteinases, **TIMP**; pathogen-associated molecular pattern, **PAMP**; computed tomography, **CT**; Specialized Center of Clinically-Oriented Research, **SCCOR**; body mass index, **BMI**; forced expiratory volume in 1 second, **FEV_1**; forced vital capacity, **FVC**; St George's Respiratory Questionnaire, **SGRQ**; diffusing capacity of the lungs for carbon monoxide, **DLCO**; percentage predicted, **%pred**; interquartile range, **IQR**; inhaled corticosteroids, **ICSs**; dendritic cell-associated C-type lectin-1, **dectin-1**; Global initiative for chronic Obstructive Lung Disease, **GOLD**

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Introduction

Chronic obstructive pulmonary disease (COPD) affects more than 5% of the population and is one of the leading causes of death in the United States.[1-4] Although cigarette smoke exposure is a major risk factor, other factors likely play an important role in COPD pathogenesis as only a subset of individuals who smoke develop airflow obstruction. Microbial translocation, i.e., the movement of microbes or their products across an impaired epithelial cell barrier into the systemic circulation, has been linked to numerous chronic diseases.[5-10] The role of microbial translocation in COPD remains a relatively unexplored area, although studies demonstrating increased gut permeability in COPD patients and alterations of the lung and gut microbiome linked to COPD outcomes suggest that microbial translocation may contribute to COPD pathogenesis.[11-15]

Most COPD studies to date have focused on the bacterial microbiome, yet fungal colonization of the respiratory tract occurs in COPD and is linked to lung function.[16,17] 1,3-beta-d-glucan (BDG) is a key polysaccharide component of the fungal cell wall of many clinically relevant fungi. BDG can act as a pathogen-associated molecular pattern (PAMP), defined as a molecule associated with pathogen infections that serves as a ligand for host response, by binding pattern recognition receptors on immune cells, particularly macrophages, leading to cell activation and release of inflammatory mediators as part of the host's immune response to fungal infection.[18] While BDG levels are used clinically for the diagnosis of invasive fungal infections, a growing body of literature links elevated BDG levels to disease outcomes in the absence of active fungal disease.[17,19,20] Building on studies showing fungal colonization and impaired epithelial barrier integrity in COPD, we aimed to assess the association of circulating BDG levels with measures of lung disease severity on spirometry and chest computed tomography (CT) imaging, symptoms, and respiratory exacerbations in individuals with COPD caused by tobacco exposure. Our secondary objective was to examine the correlation of plasma BDG levels with levels of circulating matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), proteins largely secreted by macrophages and important in the pathogenesis of COPD.[21,22] We hypothesized that high BDG levels would associate with worse lung function and respiratory symptoms and that BDG levels would correlate with circulating MMPs and TIMPs.

Methods

Participants and Data Collection

The study sample consisted of participants in the COPD Specialized Center of Clinically Oriented Research (SCCOR) cohort at the University of Pittsburgh. SCCOR is a single center cohort recruited between July 2008 and June 2010. All SCCOR participants were 40 years of age or older with a minimum 10 pack-year tobacco history at enrollment. Exclusion criteria included clinical or radiographic evidence of another pulmonary diagnosis (e.g., interstitial lung disease), history of lung cancer or a new, suspicious nodule on CT scan, and body mass index (BMI) greater than 34. All eligible participants were ambulatory. We selected a convenience sample, based on availability of stored blood samples and complete clinical data, of 247 SCCOR participants with COPD, defined by a post-bronchodilator forced expiratory volume in 1 second to forced vital capacity ratio (FEV_1/FVC) of <0.70. Fifty SCCOR participants without evidence of airflow obstruction on spirometry (controls) were selected to establish the range of BDG levels in individuals with tobacco exposure but no evidence of COPD. These 50 control participants were not included in the main analyses. The study protocol was approved

by the University of Pittsburgh Institutional Review Board. Written informed consent was obtained for each participant.

Measures

Each participant completed a chest CT scan, pre- and post-bronchodilator spirometry, measurement of lung diffusion capacity, and demographic and medical history questionnaires. Symptoms were assessed with the St George's Respiratory Questionnaire (SGRQ). The SGRQ total score has a range of 0 to 100 with higher scores reflecting greater symptoms and a difference of 4 being the minimal clinically important difference.[23] Participants were asked if they had 1 or more acute exacerbations of COPD, defined as 3 or more days of increased dyspnea, cough, and/or sputum production requiring treatment with either antibiotics or steroids, and the presence or absence of sputum production within the preceding 12 months. Blood specimens were collected on the morning of the study visit with the participant in a semi-fasting state.

Computed Tomography Imaging

Non-contrast CT examinations were performed with a GE Healthcare LightSpeed VCT (64-detector) scanner. The presence of emphysema was assessed from the CT images using semi-quantitative visual scoring. A single chest radiologist, blinded to participant identities and characteristics, visually assessed the presence and severity of emphysema using a previously validated, 6-point semi-quantitative scoring system to define emphysema severity (0, none; 1, trace; 2, mild; 3, moderate; 4, severe; 5, very severe) corresponding to 0%, less than 10%, 10%–25%, 26%–50%, 51%–75%, and greater than 75% visually assessed emphysema.[24] Participants were grouped into 3 categories based on visual emphysema score: none (visual score, 0), trace/mild (visual score, 1 or 2), or moderate/severe (visual score, 3, 4, or 5).

1,3-Beta-D-Glucan and Biomarker Measurements

A BDG-specific limulus amebocyte zysate assay for plasma levels of BDG was performed on blood samples obtained at the study visit using the Fungitell assay (Associates of Cape Cod Incorporated, East Falmouth, Massachusetts).[25,26] Serum samples, in volumes of 5 microliters, were tested in duplicate on each plate according to the manufacturer's instructions. Standard curve concentrations were adjusted to 7.8pg/mL to 250pg/mL. The average of duplicate samples was reported. If there was a greater than 20% difference between duplicate samples, the assay was repeated. The lower limit of detection of the assay was 7.8pg/ml. Where sample titers exceeded the upper limit of the standard curve, the samples were diluted in glucan-free water and re-tested and the results multiplied by the dilution value.

Circulating MMPs, including MMP1, MMP7, and MMP9, and TIMP1, TIMP2, and TIMP4 were measured in stored plasma samples using commercial multiplex assays according to manufacturer's instructions (Human MMP Luminex Performance Assay kit, R & D Systems; Human TIMP Luminex Performance Assay, R & D Systems).

Statistical Analysis

The 95th percentile of the BDG distribution in the 50 control participants without airflow obstruction was used as the threshold to categorize COPD participants as having high versus low BDG levels. The primary exposure of interest was BDG level (high versus low and continuous value). The primary outcomes of interest were lung function (FEV_1 percentage predicted [% pred], FEV_1/FVC ratio, and diffusing capacity of the lungs for carbon monoxide [DLCO] % pred), respiratory symptoms (SGRQ total score, sputum production in the preceding 12 months), and a history of 1 or more exacerbations within the last year. The secondary outcome of interest was circulating MMP and TIMP levels. Fisher's exact test was used to compare categorical variables, and Student's *t*-test was used to compare continuous variables for the primary outcomes of interest. The association between circulating BDG and protein levels were assessed with linear regression analysis. The models were adjusted for age, sex, and current smoking status. BDG levels were log-transformed and circulating protein levels were log-transformed when the data were skewed. Sensitivity analyses were performed with the removal of participants who were active smokers, given the potential of tobacco exposure to impact the outcome measures. All statistical analyses were performed with Stata 16.1 (StataCorp LP).

Results

Participant Characteristics

Participants with COPD (n=247) had a median age of 64 (interquartile range [IQR] 59–68) and equal sex distribution (Table 1). Greater than three-fourths of the cohort had severe airflow obstruction, defined as an FEV_1<50% predicted. Seventeen percent of participants were actively smoking at the time of study entry and approximately one-half of cohort participants reported a history of acute exacerbations and daily sputum production within the preceding year. Characteristics of COPD participants and control participants without airflow obstruction were similar, with the exception of a higher percentage of participants reporting active smoking within the control group (Table S1 in the online supplement).

Association of Circulating 1,3-Beta-D-Glucan with Lung Function and Respiratory Symptoms

BDG levels were similar between individuals with COPD (median [IQR] 110pg/mL [58–260]) and control participants without airflow obstruction (median [IQR] 100pg/mL [67–175] Figure 1). A BDG value of 304pg/mL corresponded to the 95th percentile of BDG in the control participants. Forty-nine (19.8%) participants with COPD had a BDG level ≥304pg/mL and were categorized as high BDG. Participants with high BDG levels were younger (median [IQR] 61 [56–67] versus 64.5 [59–69], p=0.0029), but had similar sex distribution (n [%] female 20 [41] versus 99 [50]), body mass index (BMI) (median [IQR] 26.4 [23.6–32.9] versus 26.1 [22.4–29.4]), current smoking status (n[%] 5[10] versus 37 [19]), and total pack years (median [IQR] 55 [33–68] versus 50 [34–71]) compared to those participants with BDG levels below the 304pg/mL threshold (all p>0.05). There were no differences in reported use of inhaled corticosteroids (ICSs), systemic steroid use within the past 30 days, or current oral steroid use between high and low BDG groups (all p>0.05, Table S2 in the online supplement). BDG levels in participants reporting current use of ICSs or oral steroids or use of systemic steroids in the last 30 days were similar to participants without steroid use (data not shown). Those participants with high BDG levels had a lower median FEV_1/FVC ratio, more exacerbations, greater sputum production, and higher median symptom scores (Table 2, Figure 2). FEV_1 %pred trended lower in the high BDG group, although the difference was not statistically significant (median [IQR] 27% [23%–45%] versus 35% [24%–62%], p=0.055). A sensitivity analysis removing participants who were current smokers showed similar associations between BDG and lung function, exacerbations, sputum production, and symptom scores (Table S3 in the online supplement).

Table 1. COPD Participant Characteristics[a]

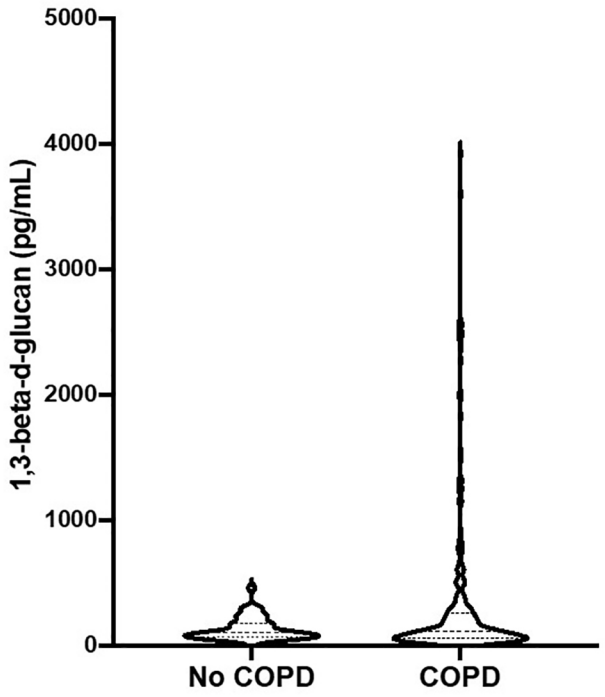
Age, median (IQR)	64.0 (59.0–68.0)
Female, n (%)	119 (48.2)
BMI, median (IQR)	26.2 (22.6–29.7)
Diabetes, n (%)	40 (16.3)
Current Smoker, n (%)	42 (17.0)
Pack Years, median (IQR)	50.0 (33.0–70.0)
ICS, n (%)	165 (66.8)
Oral Steroid Use in Past 30 Days, n (%)	76 (30.8)
Current Oral Steroid Use, n (%)	59 (23.9)
FEV_1% Pred, median (IQR)	32.2 (23.5–59.5)
FEV_1/FVC, median (IQR)	37.8 (30.2–52.2)
DLCO % Pred, median (IQR)	37.2 (26.3–51.5)
GOLD Category, n (%)	
1	21 (8.4)
2	53 (21.5)
3	59 (23.9)
4	114 (46.2)
SGRQ, median (IQR)	60.3 (37.4–71.6)
Visual Emphysema, n (%)[b]	
0=None	10 (4.1)
1-2=Trace/Mild	58 (24.1)
3-5=Moderate/Severe	173 (71.8)
Any Prior Exacerbations, n (%)	129 (52.2)
Any Sputum Production, n (%)	137 (55.5)

[a]N=247
[b]n=241

COPD defined by post-bronchodilator FEV_1/FVC ratio <0.7.
Any prior exacerbations=categorical yes/no for exacerbation in last 12 months.

COPD=chronic obstructive pulmonary disease; IQR=interquartile range; BMI=body mass index; ICS=current use of inhaled corticosteroids; FEV_1=forced expiratory volume in 1 second; % pred=percentage predicted; FVC=forced vital capacity; DLCO= iffusing capacity of the lung for carbon monoxide; GOLD=Global initiative for chronic Obstructive Lung Disease; SGRQ=St George's Respiratory Questionnaire

Figure 1. Violin Plot of 1,3 Beta-D-Glucan Distribution in Participants Without Airflow Obstruction Versus Participants With COPD



COPD=chronic obstructive pulmonary disease

Association of Circulating 1,3-Beta-D-Glucan with Matrix Metalloproteinases and Tissue Inhibitor of Matrix Metalloproteinases

Participants with high BDG had higher levels of MMP1 (median [IQR] 2667pg/mL [1513–7029] versus 1879 pg/mL [1318–3277[, p=0.0001), lower levels of MMP7 (median [IQR] 10017pg/mL [7518–14224] versus 13098 pg/mL [9662–18958], p=0.019) and lower levels of TIMP2 (median [IQR] 60363pg/mL [51429–67245] versus 65828pg/ml [58747–73834], p=0.04) (Table S4 in the online supplement). BDG levels were directly associated with logMMP1 (r 0.27, p<0.001) and TIMP1 (r=0.16, p=0.022), associations that remained significant after adjustment for age, sex, and smoking status. LogMMP7 was associated with BDG in unadjusted (r -0.15, p=0.027), but not adjusted, analysis (Table 3, Figure 3). There was no association between BDG and MMP9 or TIMP4.

Table 2. Clinical Outcomes in COPD Participants with High Versus Low 1,3-Beta-D-Glucan Levels

	Low BDG N=198	High BDG N=49	*p*-value
FEV_1 %pred, median (IQR)	35.0 (23.7–62.1)	27.3 (23.3–45.3)	0.055
FEV_1/FVC, median (IQR)	39.3 (31.4–54.2)	31.9 (27.3–46.6)	**0.025**
DLCO %pred, median (IQR)	39.3 (27.5–52.7)	32.8 (23.1–46.7)	0.13
GOLD Category, n (%)			**0.029**
1	19 (9.6)	2 (4.1)	
2	47 (23.7)	6 (12.2)	
3	50 (25.3)	9 (18.4)	
4	82 (41.4)	32 (65.3)	
SGRQ, median (IQR)	57.4 (32.6–70.7)	63.6 (57.8–73.0)	**0.016**
Visual Emphysema, n (%)[a]			**0.040**
0=None	10 (5.1)	0 (0)	
1-2=Trace/Mild	48 (24.6)	10 (21.7)	
3-5=Moderate/Severe	137 (70.3)	36 (78.3)	
Prior Exacerbations, n (%)	95 (48.0)	34 (69.4)	**0.010**
Sputum Production, n (%)	103 (52.0)	34 (69.4)	**0.037**

[a]n=195 Low BDG; n=46 High BDG

P-values in bold are significant.

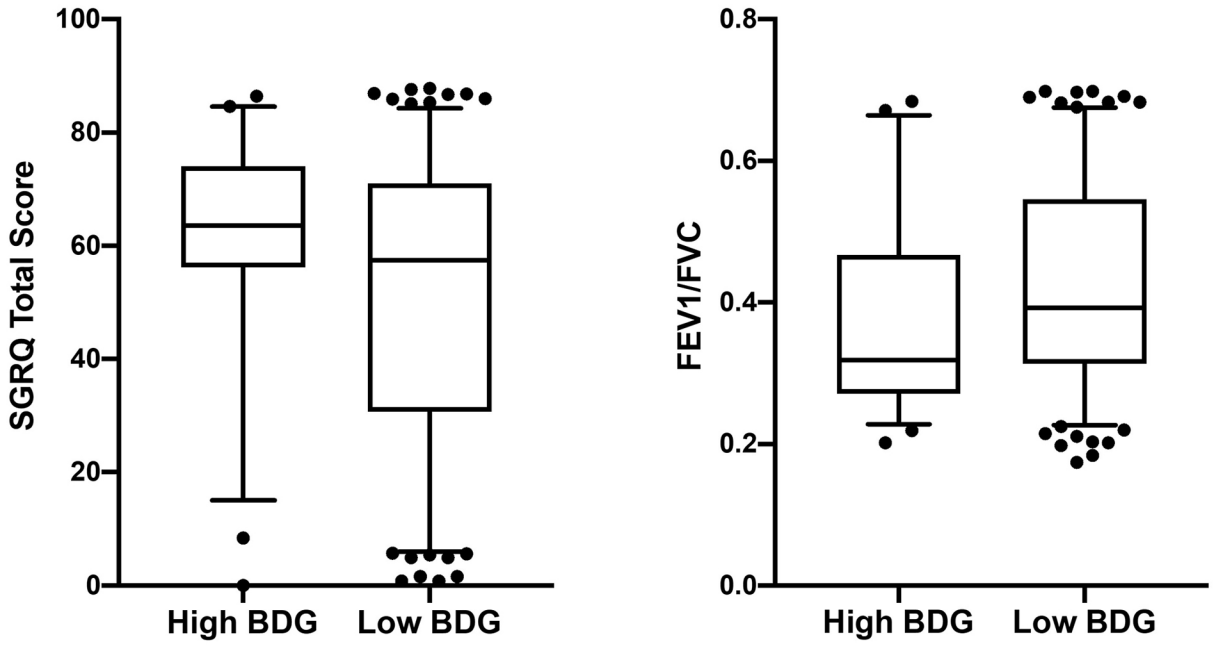
Prior exacerbations=categorical yes/no report of any exacerbation in the last 12 months. BDG=1,3-beta-d-glucan; FEV_1=forced expiratory volume in the 1 second; % pred=percentage predicted; IQR=interquartile range; FVC=forced vital capacity; DLCO=diffusing capacity of the lung for carbon monoxide; GOLD=Global initiative for chronic Obstructive Lung Disease; SGRQ=St George's Respiratory Questionnaire

Discussion

Our study is the first to show that elevated plasma levels of BDG are associated with poor respiratory outcomes and circulating matrix degradation proteins in stable COPD. We demonstrate that BDG levels are elevated in the blood of individuals with COPD in the ambulatory setting in the absence of clinical fungal infection, and that elevated BDG levels correlate with lower lung function, worse respiratory symptoms, and more frequent acute exacerbations of COPD. We also show that plasma BDG levels correlate with circulating matrix degradation proteins independent of age, sex, and smoking status.

BDG is the most abundant polysaccharide contained in the cell wall of many fungal organisms, including *Candida, Aspergillus,* and *Pneumocystis.* Clinically, detection of BDG in the blood is used to aid in diagnosis of invasive fungal infections, as components of the cell wall enter the circulation and can be detected using

Figure 2. Higher Symptom Scores and Lower Forced Expiratory Volume in 1 Second to Forced Vital Capacity Ratio in Participants with High Circulating 1,3-Beta-D-Glucan Levels



(a) Box and whisker plots of SGRQ total scores (Student's *t* test, p=0.016) and (b) FEV_1/FVC ratio (Student's *t* test, p=0.025) in participants with COPD with high versus low BDG levels.

High BDG defined as a BDG level >304pg/mL.

SGRQ=St George's Respiratory Questionnaire; BDG=1,3-beta-d-glucan; FEV_1=forced expiratory volume in 1 second; FVC=forced vital capacity

the Fungitell assay.[27] Emerging literature suggests that elevations of circulating BDG occur in the absence of fungal infection and are associated with poor disease outcomes. In persons living with HIV without invasive fungal disease, elevations in BDG levels correlate with worse lung function, increased pulmonary artery systolic pressure, and higher plasma cytokines.[17] In chronic kidney disease, elevations in BDG have been correlated with worsening renal function, with the highest levels detected in those individuals on dialysis.[28] BDG has also been studied as a prognostic marker after major abdominal surgery and in critical illness, with higher levels associated with worse outcomes.[29,30] We found significant BDG elevations in current and former smokers both with and without airflow obstruction and, as in other chronic diseases, BDG elevations appear to correlate with respiratory morbidity and worse outcomes in COPD.

Whether BDG is merely an indicator of disease severity or has functional effects once gaining access to the systemic circulation is unknown, although in-vitro co-exposure models evaluating toll-like receptor-induced cytokine production have demonstrated an increased inflammatory response in the presence of BDG.[31-33] Beyond its role as a marker of invasive fungal infection, BDG is also a PAMP that binds to receptors on immune cells, including macrophages, neutrophils, and T cells, and can trigger a host immune response. While the binding of BDG to its recognition receptor dendritic cell-associated C-type lectin-1 (dectin-1) on immune cells is important in the host's defense against invasive fungal disease, binding in the absence of infection may lead to immune cell activation, contributing to disease pathogenesis.[34] Recent studies suggest that dectin-1 plays an important role in allergic airway inflammation,[35-37] inflammation in inflammatory bowel disease,[38,39] and inflammation following ischemic stroke and myocardial infarction.[40-42] The macrophage is a key target of BDG, with BDG binding to the dectin-1 receptor leading to macrophage activation and mediator release.[20,43] MMPs and TIMPs secreted by activated macrophages regulate matrix degradation and apoptotic pathways and have

Table 3. Association of Log-transformed 1,3-Beta-D-Glucan with Circulating Matrix Metalloproteinases

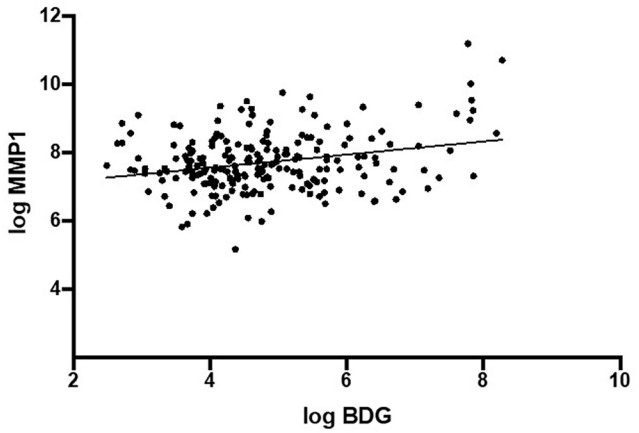
	Unadjusted Analysis		Adjusted for Age, Sex, and Current Smoking	
	r[a]	*p*-value	r[a]	*p*-value
logMMP1	0.27	**<0.001**	0.28	**<0.001**
logMMP7	-0.15	**0.027**	-0.13	0.058
logMMP9	-0.07	0.29	-0.08	0.24
TIMP1	0.16	**0.022**	0.15	**0.027**
TIMP2	-0.10	0.14	-0.07	0.32
TIMP4	-0.09	0.19	-0.07	0.32

[a]Pearson's correlation coefficient
[b]Partial correlation coefficient

P-values in bold are significant.

MMP=matrix metalloproteinase; TIMP=tissue inhibitors of metalloproteinases

Figure 3. Circulating 1,3-Beta-D-Glucan is Directly Associated with Circulating Matrix Metalloproteinase 1 Levels



Association of log 1,3 beta-d-glucan and MMP 1.

Linear regression, $p<0.001$.

MMP=metalloproteinase 1; BDG=1,3 Beta-D-Glucan

been implicated in the development of emphysema in humans and murine models.[21,44-46] Our study found that BDG directly correlated with circulating MMP1 and TIMP1, and inversely correlated with MMP7 in unadjusted and adjusted models. Elevations of circulating MMP1 and TIMP1 in COPD that inversely correlate with lung function and directly associate with emphysema severity have been described by others.[46,47] While plasma levels of MMP7 have likewise been shown to inversely correlate with FEV_1 in COPD due to biomass smoke exposure, studies have demonstrated a downregulation of MMP7 expression in the airway epithelium and alveolar macrophages of healthy smokers and smokers with COPD.[45,48,49] Our group is conducting further research to understand the mechanisms underlying these complex associations.

The source of BDG in the blood of COPD patients is unknown, although we speculate that BDG enters the circulation by translocation across an impaired gut or lung epithelial cell barrier in individuals with dysbiosis of the aerodigestive tract. Although most studies have focused on changes in the gut or lung microbiome in COPD, evidence of alterations of the COPD mycobiome exist.[13-15,50,51] Morris et al showed that *Pneumocystis* colonization of the lower airways, detected by polymerase chain reaction, was associated with greater severity of airflow obstruction in stable COPD.[16] Overrepresentation of *Pneumocystis jirovecii*, in addition to other fungi, has also been described in microbial communities isolated from bronchoalveolar lavage fluid from individuals with HIV-related airflow obstruction.[52] Others have described a distinct fungal population in the respiratory mycobiome in patients with COPD at baseline and noted changes in fungal communities with acute exacerbations.[53] Individuals with frequent exacerbations of COPD likely have greater steroid and antibiotic exposure, which may predispose to fungal colonization of the aerodigestive tract. We did not see increased circulating BDG in participants reporting ongoing or recent steroid use, but BDG levels were higher in participants with a history of acute exacerbations (median [IQR] 126pg/mL [63–330]) compared to those without exacerbations (median [IQR] 93pg/mL [57–195], $p=0.023$), suggesting that distant steroid or antibiotic exposure may lead to prolonged fungal colonization. Diabetes and chronic kidney disease have likewise been associated with fungal colonization, but the prevalence of diabetes and creatinine levels were similar between high and low BDG groups.[8,9,54]

Increased epithelial barrier permeability during activities of daily living, in the setting of an acute exacerbation of COPD, or with localized tissue hypoxia resulting from cigarette smoke exposure, as previously described in the literature,[55-57] may then predispose to the translocation of fungal organisms and/or cell wall components across the aerodigestive tract and

into the systemic circulation. Although data regarding microbial translocation in COPD is sparse, translocation of microbes or microbial products is linked to numerous other chronic diseases, including heart failure, kidney disease, and comorbidities in persons living with HIV.[6,9,10] Elevated BDG levels have also been associated with histologic findings in inflammatory bowel disease, with decreased circulating BDG levels corresponding to a therapeutic response.[58] A greater understanding of the role of microbial translocation in COPD pathogenesis is warranted given its potential as a novel therapeutic target in COPD.

While the findings of our study are novel, we recognize several important limitations. We are unable to draw conclusions about causality due to the cross-sectional design of our study. Further investigations are needed to assess whether elevated BDG levels are associated with COPD progression, and whether these findings can be replicated across cohorts. Notably, most of our cohort participants had severe obstructive lung disease and were classified as either Global initiative for chronic Obstructive Lung Disease (GOLD)[59] stage 3 or GOLD 4. Future studies will need to examine the prevalence and significance of BDG elevations in earlier disease. BDG levels in COPD participants were higher than anticipated, with most levels well above the clinical threshold for invasive fungal infection. False elevations of circulating BDG can be caused by gauze contamination due to gauze's high BDG content.[60] However, the likelihood of gauze contamination in this population of stable COPD patients is low, and we would expect contamination to be random and not associated with clinical outcomes. Although the age of stored blood samples may potentially affect protein measurements, our internal data on the storage of serum samples demonstrates excellent stability of BDG under years-long storage at -80°C (data not shown) and, although intracellular degradation has been shown to occur through presumed hydrolytic mechanisms, this should not occur in cell-free preparations.[61] Finally, we lacked a true control group of individuals with similar demographics but no history of tobacco exposure.

Conclusions

Our study demonstrates that elevated BDG levels in COPD patients correlate with worse lung function, greater respiratory morbidity, and circulating markers of matrix degradation. These novel findings suggest that translocation of fungal organisms and/or cell wall components may contribute to COPD pathogenesis in some, and that therapies that target dysbiosis or enhance epithelial cell barrier integrity may have disease-modifying effects in COPD.

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Author contributions: MG and DS are co-first authors who contributed equally. MG, DS, SMN, and JB were responsible for study concept and design. MF and Yonglong Zhang (YoZ) performed the 1,3-beta-d-glucan assays and assisted with interpretation of the data. Yingze Zhang (YiZ) performed the MMP and TIMP assays and assisted with interpretation of the data. JP performed the chest CT analyses and assisted with interpretation of the data. MG, DS, SMN, and JB performed the statistical analyses and data interpretation. MG, DS, DC, GDK, AM, FCS, and JB provided intellectual input and made significant contributions to the writing and editing of the manuscript. All authors contributed significantly to the intellectual content of the article and gave final approval of the submitted version.

Declaration of Interest

None of the authors have any conflicts of interest to declare.

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