ORIGINAL RESEARCH LETTER

Circulating desmosine as a biomarker of azithromycin treatment response: a *post hoc* analysis of the COLUMBUS randomised controlled trial

To the Editor:

Elastin degradation in the lung, leading to emphysema, and elastin degradation in blood vessels, leading to atherosclerosis, are key mechanisms in the development and progression of chronic obstructive pulmonary disease (COPD) [1]. Elastin degradation can be measured in blood through quantification of desmosine and its isomer iso-demosine, which are released specifically through cleavage of mature elastin [2]. Severe COPD is frequently associated with chronic neutrophil-mediated inflammation and airway microbial dysbiosis [3]. Neutrophils release harmful proteases through the processes of degranulation and neutrophil extracellular trap formation that degrade extracellular matrix, including elastin, leading to disease progression [3, 4].

The management of COPD seeks to reduce symptoms with long-acting bronchodilators and pulmonary rehabilitation and to reduce exacerbations [5]. Recent data suggest that the most widely used anti-inflammatory drugs, inhaled corticosteroids, predominantly reduce eosinophilic airway inflammation, reduce exacerbations and slow lung function decline in patients with elevated blood eosinophils [5–7]. To date there are no treatments that are indicated specifically for the treatment of neutrophilic COPD and none have been shown to slow neutrophil-driven elastin degradation. Macrolide antibiotics such as azithromycin may, however, be a potential therapy to target neutrophilic inflammation [7–9]. They enhance the clearance of apoptotic neutrophils, reduce neutrophil oxidative burst and enhance neutrophil chemotaxis among their other effects on innate immunity [6–8]. The impact of these anti-inflammatory and antimicrobial effects is the demonstrated efficacy of macrolides to reduce exacerbations in patients with neutrophil-dominated lung diseases such as bronchiectasis and cystic fibrosis [9, 10].

The COLUMBUS randomised controlled trial was a double-blind placebo-controlled single-centre study in the Netherlands performed between May 2010 and June 2013 [11]. Patients had a diagnosis of COPD by Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria and a history of at least three exacerbations in the previous year. All patients were receiving standard care for frequently exacerbating COPD including inhaled corticosteroids at baseline. Patients were randomised to receive either 500 mg azithromycin three times per week or matched placebo three times per week. The total follow-up duration was 12 months. The primary end-point of the original trial was the rate of moderate and severe COPD exacerbations by intention to treat. The trial was registered at ClinicalTrials.gov with identifier NCT00985244 [11].

The study randomised 92 patients to azithromycin (n=47) or placebo (n=45) for 12 months, and found a significant reduction in exacerbations favouring treatment with azithromycin (rate ratio 0.58, 95% CI 0.42–0.79; p=0.001) [11].

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Seeking evidence that macrolides may be effective in subgroups of patients with neutrophil-mediated lung disease, and that macrolides may slow the progression of COPD by reducing elastin degradation, we conducted a *post hoc* analysis of the COLUMBUS trial participants. Serum samples had been obtained at baseline (prior to macrolide treatment) and at 12 months (end of study).

Total serum desmosine (sDES) concentration was measured using a liquid chromatography with mass spectrometry method [2]. Our exploratory objectives were: 1) to determine if baseline sDES predicted response to azithromycin in terms of exacerbation reduction; and 2) to determine if azithromycin treatment resulted in a reduction in sDES from baseline compared to placebo. Finally, in a subgroup of patients, we measured whether sDES changed at onset of acute exacerbation.

A priori, we determined that we would split patients into two groups based on sDES levels above and below the median level in the population (0.39 $ng{\cdot}mL^{-1}$). This included n=46 for >0.39 $ng{\cdot}mL^{-1}$ (n=26 azithromycin-treated, n=20 placebo) and n=45 for <0.39 $ng{\cdot}mL^{-1}$ (n=21 azithromycin-treated, n=24 placebo). As has been previously reported, sDES was strongly correlated with age (r=0.53, p<0.0001). Inhaled corticosteroid dose was not different between the two groups (p=0.7).

We chose to split exacerbations into moderate and severe exacerbations, based on recent data from bronchiectasis suggesting that elevated circulating desmosine was a predictor of severe exacerbations [4]. We analysed frequency of exacerbations using Poisson regression.

The patient characteristics have been previously reported. The mean age was 65 years, patients had a mean of four exacerbations per year at baseline and the mean lung function suggested severe airflow obstruction. Patients were commenced on azithromycin or placebo at baseline, while clinically stable and free from exacerbation, as previously described [11].

The median level of sDES was 0.39 $ng{\cdot}mL^{-1}$ (interquartile range (IQR) 0.25–0.51 $ng{\cdot}mL^{-1}$). sDES was similar at baseline between azithromycin and placebo groups (median (IQR) 40 (28–51) *versus* 37 (24–54) $ng{\cdot}mL^{-1}$, respectively; p=0.5).

In the first analysis of whether sDES could predict macrolide response, there was a significant reduction in the frequency of exacerbations (incident rate ratio (IRR) 0.52, 95% CI 0.35–0.79), a reduction in moderate exacerbations (IRR 0.52, 95% CI 0.33–0.83) and a reduced time to first exacerbation (hazard ratio (HR) 0.36, 95% CI 0.18–0.70) comparing azithromycin *versus* placebo in patients with sDES >0.39 $ng{\cdot}L^{-1}$. There was a trend to reduction in severe exacerbations (IRR 0.53, 95% CI 0.22–1.31) (figure 1).

In those with the lower sDES levels, there was no significant reduction in the frequency of exacerbations (IRR 0.74, 95% CI 0.51–1.07), moderate exacerbations (IRR 0.63, 95% CI 0.40–1.00), time to first exacerbation (HR 0.58, 95% CI 0.31–1.10) or severe exacerbations (IRR 1.02, 95% CI 0.53–1.96).

Exploratory tests of interaction between these groups were statistically nonsignificant, but this was to be expected with this sample size (interaction for total exacerbations −1.26, p=0.1; severe exacerbations −1.16, p=0.1; time to first exacerbation −1.00, p=0.1).

The apparent difference in response was not driven by desmosine enriching for frequently exacerbating patients in the high-desmosine group, as baseline desmosine did not correlate with risk of exacerbations

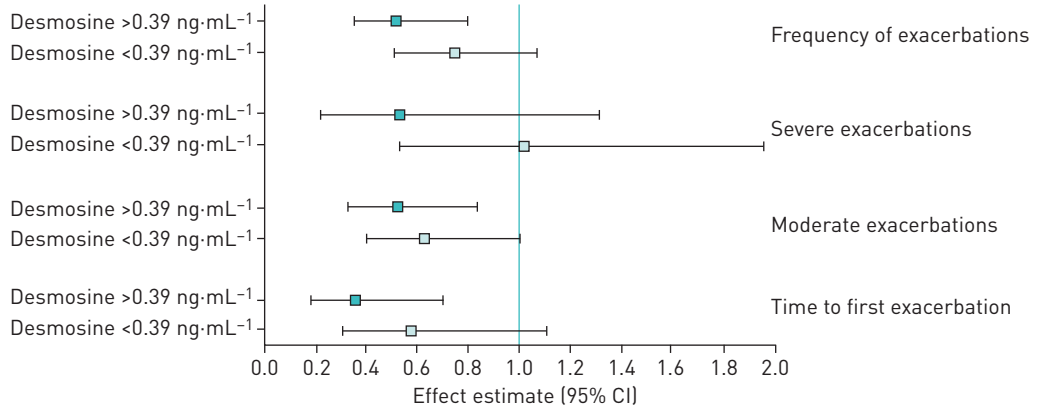

FIGURE 1 Forest plot displaying the effect estimates comparing azithromycin and placebo treatment in subgroups defined by above (>0.39 $ng{\cdot}mL^{-1}$) or below (<0.39 $ng{\cdot}mL^{-1}$) the median level of serum desmosine for the COLUMBUS trial population. The effect estimate shown is incident rate ratio for frequency of exacerbations, severe exacerbations and moderate exacerbations, and hazard ratio for time to first exacerbation.

(IRR 0.92, 95% CI 0.70–1.20; IRR 0.90, 95% CI 0.68–1.17 after adjustment for treatment allocation) or time to first exacerbation (HR 0.88, 95% CI 0.56–1.38; HR 0.93, 95% CI 0.59–1.47 after adjustment for treatment allocation).

For our secondary objective of determining whether azithromycin reduced sDES after 12 months of treatment, we observed that desmosine reduced over the study by -0.03 ng·mL^{-1} in the azithromycin group (standard deviation of change 0.23) but did not change in the placebo group (mean difference 0, standard deviation of change 0.17). When the statistical difference between groups was evaluated, azithromycin did not reduce sDES (p=0.4).

We compared desmosine in 24 patients at baseline and during an acute exacerbation. No difference in sDES was observed (median (IQR) 0.36 (23–47) *versus* 0.36 (23–48) ng·mL^{-1}; p=1.0).

We conclude that azithromycin treatment does not have an impact upon elastin degradation in patients with COPD, but that patients with higher levels of sDES may have a greater response to azithromycin treatment when compared to patients with lower sDES, in terms of reducing exacerbations. This study was exploratory and should be regarded as hypothesis-generating, as the COLUMBUS study was not designed to test for biomarkers of azithromycin response [11]. We observed that sDES did not change during acute exacerbations of COPD and that sDES was not a predictor of future risk of exacerbations or time to first exacerbation. This study is among the first to seek biologically relevant determinants of macrolide response in COPD. Several studies have now shown the value of macrolides to reduce exacerbations in patients with COPD [11–14]. Clinical predictors of macrolide response were sought by Han *et al.* [14], who examined data from a trial in the USA of 1142 patients with COPD treated with azithromycin 250 mg daily or placebo. They found few clinical predictors of macrolide response, with no difference in response detected by lung function, sex, presence of chronic bronchitis or prior therapy. Interestingly, only current smoking and age >65 years were associated with improved response. sDES and other biological markers were not measured in the study [14].

Our study also adds to the available data regarding sDES as a biomarker of COPD. Previous studies in the ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points) cohort and other large cohorts found associations between desmosine and age, coronary artery calcification, Medical Research Council dyspnoea score and airflow obstruction, but did not demonstrate a link with emphysema or emphysema progression. Desmosine nevertheless was an independent predictor of mortality in 1177 COPD patients [1, 15].

In summary, we show that azithromycin use does not reduce sDES over 12 months, but patients with higher levels of sDES may be more responsive to azithromycin treatment. This would justify larger studies of sDES to phenotype patients in COPD.

James D. Chalmers[1], Remco S. Djamin[2], Marjolijn Schouten[2], Holly R. Keir[1], Brandon Tan[1], Joachim G.J.V. Aerts[3], Jeffrey T-J. Huang[1] and Menno van der Eerden[3]

[1]Scottish Centre for Respiratory Research, University of Dundee, Ninewells Hospital and Medical School, Dundee, UK. [2]Dept of Respiratory Medicine, Amphia Hospital, Breda, The Netherlands. [3]Dept of Respiratory Medicine, Erasmus Medical Centre, Rotterdam, The Netherlands.

Correspondence: James D. Chalmers, Division of Molecular and Clinical Medicine, University of Dundee, Dundee, DD1 9SY, UK. E-mail: jchalmers@dundee.ac.uk

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References

1 Rabinovich RA, Miller BE, Wrobel K, *et al.* Circulating desmosine levels do not predict emphysema progression but are associated with cardiovascular risk and mortality in COPD. *Eur Respir J* 2016; 47: 1365–1373.
2 Huang JT, Chaudhuri R, Albarbarawi O, *et al.* Clinical validity of plasma and urinary desmosine as biomarkers for chronic obstructive pulmonary disease. *Thorax* 2012; 67: 502–508.
3 Dicker AJ, Crichton ML, Pumphrey EG, *et al.* Neutrophil extracellular traps are associated with disease severity and microbiota diversity in patients with chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 2018; 141: 117–127.
4 Chalmers JD, Moffitt KL, Suarez-Cuartin G, *et al.* Neutrophil elastase activity is associated with exacerbations and lung function decline in bronchiectasis. *Am J Respir Crit Care Med* 2017; 195: 1384–1393.
5 Vogelmeier CF, Criner GJ, Martinez FJ, *et al.* Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report: GOLD Executive Summary. *Eur Respir J* 2017; 49: 1700214.
6 Barnes NC, Sharma R, Lettis S, *et al.* Blood eosinophils as a marker of response to inhaled corticosteroids in COPD. *Eur Respir J* 2016; 47: 1374–1382.
7 Agusti A, Bel E, Thomas M, *et al.* Treatable traits: toward precision medicine of chronic airway diseases. *Eur Respir J* 2016; 47: 410–419.
8 Wedzicha JA, Calverley PMA, Albert RK, *et al.* Prevention of COPD exacerbations: a European Respiratory Society/American Thoracic Society guideline. *Eur Respir J* 2017; 50: 1602265.
9 Chalmers JD, Aliberti S, Blasi F. Management of bronchiectasis in adults. *Eur Respir J* 2015; 45: 1446–1462.
10 Mustafa MH, Khandekar S, Tunney MM, *et al.* Acquired resistance to macrolides in *Pseudomonas aeruginosa* from cystic fibrosis patients. *Eur Respir J* 2017; 49: 1601847.
11 Uzun S, Djamin RS, Kluytmans JA, *et al.* Azithromycin maintenance treatment in patients with frequent exacerbations of chronic obstructive pulmonary disease (COLUMBUS): a randomised, double-blind, placebo-controlled trial. *Lancet Respir Med* 2014; 2: 361–368.
12 Seemungal TA, Wilkinson TM, Hurst JR, *et al.* Long-term erythromycin therapy is associated with decreased chronic obstructive pulmonary disease exacerbations. *Am J Respir Crit Care Med* 2008; 178: 1139–1147.
13 Albert RK, Connett J, Bailey WC, *et al.* Azithromycin for prevention of exacerbations of COPD. *N Engl J Med* 2011; 365: 689–698.
14 Han MK, Tayob N, Murray S, *et al.* Predictors of chronic obstructive pulmonary disease exacerbation reduction in response to daily azithromycin therapy. *Am J Respir Crit Care Med* 2014; 189: 1503–1508.
15 Huang JT, Bolton CE, Miller BE, *et al.* Age-dependent elastin degradation is enhanced in chronic obstructive pulmonary disease. *Eur Respir J* 2016; 48: 1215–1218.