A double-blind, randomised, placebo-controlled trial of long-term doxycycline therapy on exacerbation rate in patients with stable COPD

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Author contributions

JPA, BHV, SEB, PPW, PMAC, GCD and JAW contributed to the study design, protocol and study materials. JPA, BHV, SEB, LJF, LA-M, EB, PPW, contributed to patient recruitment and collection of study data at participating centres. ML and GB performed the statistical analysis. JPA wrote the first draft of the manuscript. All authors contributed to interpretation of the data and revision of the manuscript.

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Running title

Use of Long-term doxycycline treatment in COPD.

Abbreviation List

CAT	COPD assessment test
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein, serum
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GOLD	Global initiative for chronic obstructive lung disease
IQR	Interquartile range
NIHR	National Institute of Health Research
SGRQ	St George's Respiratory Questionnaire
95% CI	95% Confidence interval

Research in context

Evidence before this study

Current clinical guidelines recommend considering prophylactic azithromycin therapy to reduce the frequency of chronic obstructive pulmonary disease (COPD) exacerbations. Azithromycin is sometimes contraindicated and while sparse evidence supports the use of alternative antibiotic choices, in practice some patients receive prophylactic doxycycline. A Cochrane review, including studies up to January 2020, identified 17 randomised controlled trials examining the effect of prophylactic antibiotics upon COPD exacerbation frequency, and concluded that macrolides prolonged time to next exacerbation but that the effect of doxycycline was unclear. A Pubmed search, including the terms ("COPD" or "chronic obstructive pulmonary disease") AND "exacerbation" AND "doxycycline", completed in 03/10/2022 identified no further comparable trials examining the effect of doxycycline prophylaxis on exacerbation frequency. Further, we found no study considered interactions between eosinophil count at baseline (non-exacerbating state) and the effect of antibiotic prophylaxis on exacerbation frequency.

Added value of this study

We performed a multicentre, double-blind, randomised, placebo-controlled 12 month trial of doxycycline 100mg daily. This included 222 individuals with at least moderate grade COPD, experiencing one or more exacerbations during the preceding year. Overall, doxycycline did not reduce exacerbation frequency or improve quality of life. However, in pre-planned analyses we found interactions between prophylactic doxycycline therapy and both baseline disease severity and baseline eosinophil count. These interactions suggested doxycycline may better reduce exacerbation frequency among those with severe COPD and among those with a baseline eosinophil count <300 cells/μL.

Implications of all the available evidence

Prophylactic doxycycline did not significantly reduce exacerbation rate, over 12-months, in participants with COPD, who exacerbated regularly. However, it may prove more effective for those with a lower baseline eosinophil rate or severe disease. Further research is required to understand the potential benefits of this therapy in specific patient phenotypes.

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Abstract

Rationale: COPD exacerbations are a major cause of morbidity and mortality and preventing them is a key treatment target. Long-term macrolide treatment is effective at reducing exacerbations but there is a paucity of evidence for other antibiotic classes. To assess whether 12-month use of doxycycline reduces exacerbation rate in people with COPD.

Methods: People with moderate to very severe COPD and an exacerbation history were recruited from 3 UK centres and randomised to 12-months doxycycline 100mg once daily or placebo. The primary study outcome was exacerbation rate per person year.

Results: 222 people were randomised. Baseline mean FEV_1 was 1.35 (SD 0.35) L; 52.5 (SD 15.9) % predicted. Median number of treated exacerbations in the year before the study was 2 (1-4). 71% of patients reported ≥2 exacerbations. 81% were already prescribed inhaled corticosteroids at baseline. COPD exacerbation rate did not differ between the groups – doxycycline/placebo rate ratio 0.86 (0.67, 1.10); p=0.23. No difference was seen if only treated exacerbations or hospitalisations were considered. In pre-planned sub-group analysis, doxycycline appeared to better reduce the exacerbation rate among people with severe COPD (RR:0.36 (0.15, 0.85); p=0.019), and in those with an eosinophil count <300 cells/μL (RR:0.50 (0.29, 0.84); p=0.01). Health status measured by SGRQ was 5.2 points worse in the doxycycline group at 12-months (p<0.007).

Conclusions: Doxycycline did not significantly reduce exacerbation rate, over 12-months, in participants with COPD, who exacerbated regularly, but may have benefitted those with more severe COPD or blood eosinophil counts <300 cells/μL.

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Introduction

Acute exacerbations of COPD are common[1] and drive healthcare utilisation, hospitalisation, disease progression[2,3] and mortality.[4] Preventing exacerbations is a key approach towards improving quality of life and reducing healthcare utilisation.[5] Preventative, long-term antibiotic treatment has been shown to prevent exacerbations among those with COPD.[6] Most trials have studied macrolides (predominantly azithromycin),[7-13] although one studied moxifloxacin[14] and one four-armed trial studied azithromycin, doxycycline and moxifloxacin.[15] Guidelines support the use of long-term macrolides to prevent exacerbations[5] but azithromycin is contraindicated in some patients and the effectiveness of other antibiotics remains uncertain. Furthermore, except for current smokers, where azithromycin appears to be ineffective, it is unclear which sub-groups of patients benefit most from long-term antibiotic therapy.[7]

Although the effectiveness of long-term non-macrolide antibiotics remains uncertain, we found that doxycycline was the second commonest long term-antibiotic therapy used by patients with COPD in the UK.[16] Doxycycline possesses both bacteriostatic antibacterial properties and complex anti-inflammatory actions.[17] Long-term doxycycline is already used for several non-respiratory conditions, can reduce airway bacteria in COPD patients, and compares favourably with Azithromycin and Moxifloxacin in regards to adverse events, bacterial resistance, and adherence profiles.[15] However, the beneficial effect of macrolides may relate in part to their immunomodulatory effects, a feature not shared by many other antibiotic classes.[18]

Studies assessing the efficacy of non-macrolide antibiotics, in particular doxycycline, are needed to help clinicians decide which long-term antibiotics to prescribe and identify those patients who might benefit from such therapy.[6,19] In this study we assessed whether 12-month use of doxycycline reduced

143 COPD exacerbation rate and whether there were different responses among particular patient sub-
144 groups.

Methods

Study Design

We enrolled outpatients attending three UK centres: The Royal Brompton Hospital (London), University Hospital Aintree (Liverpool) and St George's Hospital (London) from both primary and secondary care.

This was a double-blind, randomised, placebo-controlled trial of 12 months of doxycycline 100mg daily conducted between 15/07/2014 and 12/07/2017. The primary study outcome was COPD exacerbation rate per person-year. Secondary outcomes included time to first COPD exacerbation; annual rate of corticosteroid and/or antibiotic treated exacerbations; number and duration of COPD-related hospital admissions; change in spirometry, respiratory health status (SGRQ) and C-Reactive protein (CRP) during the study period. The relationship between the primary outcome and important co-variates was explored.

Research ethics approval was granted by the Hampstead Research Ethics Committee and all patients provided their written informed consent.

Inclusion and Exclusion Criteria

Patient enrolled were aged 45 years or older, had Global Initiative for Chronic Obstructive Lung Disease (GOLD) grade 2-4 (moderate to very severe) COPD (post-bronchodilator ratio of forced expiratory volume in 1 second (FEV_1) to forced vital capacity (FVC) of <0.7 and a postbronchodilator FEV_1 <80% predicted), reported at least a 10-pack year history of smoking and had received treatment with antibiotics and/or corticosteroids for at least one COPD exacerbation in the preceding 12 months. All patients were enrolled when clinically stable, being well for at least 4 weeks after their last COPD exacerbation. Exclusion criteria are listed in the supplementary appendix.

Recruitment

Patient medical history, concomitant medications and oxygen saturations were recorded, post-bronchodilator spirometry measurements were made according to ATS/ERS criteria[20] and an ECG was

performed. Blood samples were collected to record full blood count (FBC) and CRP, and to assess safety (liver function, renal function and coagulation). Patients were trained to complete daily symptom diary cards, to record any change in treatment (antibiotics and/or steroids) and to record adverse events, as previously reported.[21] Patients also completed the St Georges Respiratory Questionnaire (SGRQ) and the COPD Assessment Tool (CAT). Chronic bronchitis was defined as a productive cough lasting at least three months per year for at least two consecutive years.

Randomisation

Patients were randomised 1:1 to treatment or control (placebo) groups using a computer-generated permuted block system of variable sizes. Randomisation was stratified by site and smoking status (current or ex-smoker) to ensure group balance within each site based on smoking status. Over encapsulated placebo and doxycycline (100mg) tablets were visually identical. Unblinding for analysis was undertaken after data entry was completed and locked.

Follow-up

Patients were reviewed face-to-face at 3, 6, 9 and 12 months. At all visits, diary cards were reviewed and any exacerbations, treatment for exacerbations and/or adverse events were assessed and recorded. Study medication compliance was assessed from pill counts of remaining medication at each visit. Participants were contacted by telephone two weeks after enrolment and within one month following study completion to further record adverse events.

At the final visit (12 months), concomitant medications were recorded; spirometry repeated and the SGRQ questionnaires completed. Blood sampling was repeated at each visit. Patients were asked to attend follow-up visits, even if they prematurely discontinued the study treatment.

Definition of exacerbations and treatment

The onset of an exacerbation was defined from diary card data as the development or worsening of at least two symptoms (including at least one major symptom) which lasted for at least two consecutive days.[11] Major symptoms included dyspnoea, increased sputum volume and increased sputum purulence.

Minor symptoms included sore throat, cold (nasal discharge and/or nasal congestion), fever (>37.5° Celsius), increased cough or increased wheeze/chest tightness. An exacerbation was defined as lasting from the day of onset until the last day before two consecutive symptom-free days.

In the absence of a completed diary card, where available patient reported healthcare utilisation was also used to define exacerbation occurrence. In such cases the exacerbation onset was defined as the first day of treatment with antibiotics and/or steroids or the first day of hospitalisation. As in previous studies, exacerbation identification from diary cards was adjudicated in a blinded manner by an author not involved in data collection during the study.[11]

Statistical analysis

We estimated that enrolment of 156 patients would provide 90% power to detect a significance difference in exacerbation rate between the Doxycycline and placebo groups using Poisson regression. This assumed an exacerbation rate ratio (erythromycin to placebo) of 0.648 from Seemungal,[11] a mean exacerbation rate of 2.26 (unpublished) and overdispersion of 1.29 from Hurst.[22] Assuming a 30% drop-out or non-adherence rate we planned to recruit a sample of 222 individuals.

Differences in exacerbation rate per person-year between the doxycycline and placebo groups were analysed using negative binomial regression taking account of time periods when patients are exacerbating, and hence cannot develop an exacerbation, and adjusting for both the number of exacerbations in the previous year and by site.[23] The mean exacerbation rate in each group was estimated by dividing the total number of exacerbations in each group by the follow-up time of each group. Supporting analyses included adjustments for baseline covariates (FEV_1, SGRQ score, and sex) and then adjusting further for additional covariates which proved unbalanced at baseline.[24]

With regards to the primary outcome, differences in therapy impact were assessed according to belonging to the following subgroups: age; sex; baseline smoking status (current or ex-smoker); COPD severity (FEV_1 percent of predicted <30% vs. 30-50%, <30% vs. >50%); baseline blood eosinophil count (<300 vs ≥300 cells/μL); exacerbation rate within the previous year (0-1 vs 2+).

Secondary endpoints were analysed as sensitivity analyses. Three analyses were undertaken for each endpoint. Firstly, adjusting for the baseline value of the studied variable alongside study centre. Secondly, models were further adjusted for baseline values imbalanced at baseline (exacerbation rate in preceding year, FEV_1 percent predicted, SGRQ score and sex). Thirdly, models were further adjusted for enrolment FEV_1/FVC; FEV_1 and oxygen saturation.

Data are presented as mean and standard deviation (SD) or 95% confidence interval (CI) as appropriate. All tests were two sided and significance set at $p<0.05$. In the event of the primary outcome being non-significant, all other p values reported were considered to be of only nominal significance.

Results

Study population

Study flow and completion is shown in figure 1 with 355 people screened, 222 randomised and 183 completing the study (18% dropout). There were no differences in the withdrawal rates between the two study arms (online data supplement). From the 222 randomised patients, 107 were enrolled at the Royal Brompton Hospital (London), 111 at Aintree Hospital (Liverpool) and 4 at St George's Hospital (London).

The baseline characteristics of the randomised patients are shown in table 1. Mean age was 67 years, 57% were male and 34% were current smokers. The doxycycline groups had greater tobacco exposure (50 vs. 41 pack years). The FEV_1/FVC ratio was slightly lower in the doxycycline group (FEV_1/FVC 0.43 vs. 0.47). Mean FEV_1 and FEV_1 percent of predicted had lower values in the doxycycline relative to the placebo group (FEV_1: 1.25L vs. 1.44L; and FEV_1 percent predicted: 50.6% vs. 54.4%; respectively). The number (percentage) of subjects with moderate, severe and very severe COPD was 121 (55%), 83 (37%) and 18 (8%) respectively.

Across the year preceding enrolment, subjects recalled suffering a median of 2 exacerbations (IQR 1-4), with 158 (71%) individuals recalling at least 2 exacerbations. Subjects reported a high symptom burden and poor health status (mean CAT score 21.1; mean SGRQ score 51.7). At enrolment, 180 (81%) subjects were already prescribed inhaled corticosteroids and 144 (65%) were using triple inhaled therapy. Baseline prescriptions appeared similar across the doxycycline and placebo groups but four subjects, all randomised to receive doxycycline, were already prescribed long-term oral corticosteroids at enrolment. At enrolment, blood eosinophil count was ≥300 cells/μL among 59 (27%) subjects and <300 cells/μL among 163 (73%) subjects and was similar across treatment arms (blood eosinophil count was ≥300 cells/μL in 26% of subjects in the doxycycline group and 27% in the placebo group).

Overall, FEV_1 and FEV_1 percent predicted values were lower among subjects enrolled in Liverpool relative to London (mean FEV_1 1.21 (0.50)L vs. 1.48 (0.52)L; mean FEV_1 percent predicted: 50.5 (15.9)% vs. 54.5 (15.7)%).

Exacerbation rate

During the study, subjects experienced a calculated median 3.2 (IQR: 1.0-5.4) exacerbations per person-year with median 1.4 (IQR: 0-3.4) treated exacerbations per person-year.

There was no statistically significant difference in the arithmetically calculated overall COPD exacerbation rate between the doxycycline and placebo groups: median 2.28 (IQR: 1.03, 4.68) vs 3.35 (IQR: 1.03, 5.68) events per person year respectively resulting in an unadjusted negative binomial rate ratio of 0.86 (95% CI: 0.67, 1.10); p=0.23. Adjustment for baseline smoking status, exacerbation rate during the previous year (number of AE or frequent (2+) vs. not frequent (<2) exacerbation status), sex and SGRQ did not change this conclusion, the adjusted rate ratio was 0.85 (95% CI: 0.67, 1.07); p=0.16. Additional analysis adjusting for study site, lung function and oxygen saturations at baseline also did not significantly alter these results.

The rate of treated exacerbations did not differ significantly between the doxycycline and placebo groups: median (IQR) 1.27 (0, 3.21) vs 2.01 (0, 3.91) events per person year respectively, giving a rate ratio of 0.87 (95% CI: 0.65, 1.16); p=0.34 by negative binomial analysis, a finding unchanged following adjustment for the co-variates listed above. Doxycycline did not alter the number of hospitalisations (26 in the doxycycline vs 21 in the placebo group) or time spent in hospital. Figure 2 shows the Kaplan Meier plot of time to first exacerbation with groups stratified according to treatment and enrolment site. There was no significant difference in the time to first exacerbation between the groups.

In pre-planned sub-group analyses we examined the effect of doxycycline according to COPD severity (moderate or severe vs. very severe), eosinophil count (<300 vs ≥300 cells/μL), smoking status (current-smokers vs. ex-smokers), age (>67 vs. ≤67years) and sex (male vs. female).

Significant interactions were detected between baseline COPD severity and treatment arm suggesting doxycycline had a greater impact on exacerbation rate among those with severe COPD. Among those enrolled with severe COPD (FEV_1 30-50% predicted), doxycycline reduced exacerbation rate by a factor of 0.36 (95%CI 0.15 to 0.85; p=0.019) relative to those with very severe COPD (FEV_1<30% predicted). No significantly greater doxycycline effect was detected among those with moderate COPD

(FEV_1 50-80% predicted), the exacerbation rate factor being 0.51 (95%CI 0.22 to 1.17; p=0.11). These data are shown in figure 3.

A significant interaction was also detected between enrolment blood eosinophil count and treatment. Doxycycline had a bigger impact on those with eosinophil counts lower than 300 cells/μL relative to those with ≥300 eosinophils/μL. Among those with a blood eosinophil count at enrolment of <300 cells/μL, doxycycline reduced exacerbation rate by a factor of 0.50 (95%CI: 0.29 to 0.84; p=0.01) relative to those with eosinophil counts ≥ 300 cells/μL. These data are shown in figure 3.

With respect to the primary endpoint, no interaction was detected between treatment arm and smoking status, age or sex (figure 3).

Other secondary outcomes

There was no effect of doxycycline on FEV_1 at the end of the study. Health status, measured by SGRQ, was worse at the end of the study among those taking doxycycline relative to placebo by 5.2 (1.44 to 9) points; p=0.007, when adjusted for enrolment site, baseline SGRQ and time on study. Health status worsened slightly in those treated with doxycycline and improved in those treated with placebo and was not affected by blood eosinophil count.

Adverse events

There was no substantial difference in overall number of adverse events with 270 in the placebo subjects and 250 in the doxycycline subjects as (Table 2). Gastrointestinal disorders were more common with doxycycline (39 vs. 25) due to the more frequent occurrence of dyspepsia, nausea and vomiting but this was not significant statistically. The occurrence of diarrhoea was the same in both groups. There was no difference in hepatic, dermatological or cardiac adverse events but nervous system adverse events were more common in placebo subjects (21 vs. 9) as were infections (55 vs. 28).

Discussion

In this randomised, double-blind placebo-controlled trial enrolling patients with at least moderate COPD who experienced one or more exacerbations during the previous year, prophylactic doxycycline did not significantly change the exacerbation rate or lung function and was associated with a worse health status at one year compared to placebo therapy. However, the therapeutic impact of doxycycline varied according to patient characteristics at study entry. Compared with those with very severe COPD, patients with severe COPD appeared to benefit from antibiotic treatment while those with moderate COPD did not benefit. Additionally, doxycycline reduced exacerbation rates among patients with baseline eosinophil counts <300 cells/μL when compared to patients with eosinophil counts of 300 cells/μL or more. Although not helpful for every patient, these findings suggest doxycycline may reduce COPD exacerbation rate among specific patient sub-groups.

This is the first randomised controlled study to investigate the effect of prophylaxis with doxycycline on future COPD exacerbations. A recent Cochrane meta-analysis of fourteen completed trial aiming to reduce exacerbations of COPD using antibiotic prophylaxis found long-term antibiotics reduced exacerbations by a factor of 0.57 (95%CI: 0.42 to 0.78; P<0.001).[6] These trials mostly studied how macrolides reduce exacerbation rates and international guidelines advocate using azithromycin prophylaxis for this purpose.[5,25] Our study design and sample compare favourably with these preceding studies. Although smaller than the largest macrolide study,[7] our study is larger, similar in design and contains a similar study population to the positive macrolide study performed by Seemungal et al.[11] With a study sample of just 109 patients, Seemungal et al were able to show that erythromycin significantly reduced the exacerbation rate with a rate ratio relative to placebo of 0.65. While Seemungal et al focussed upon moderate/severe exacerbations, our negative finding was unaltered after restricting our analysis to treated exacerbations only. Significant macrolide effects have been found in even smaller study samples enriched for more frequent exacerbators,[26] however, the proportion of our subjects who experienced at least 3 exacerbations per year already appears comparable to Seemungal et al (47% vs 35%).[11] Therefore our findings suggest that long-term doxycycline is broadly less effective than macrolides at reducing COPD exacerbation frequency.

Our negative primary outcome is important because, despite limited evidence, long-term doxycycline has been more commonly prescribed than azithromycin to patients with COPD within the UK.[16] While our headline findings may deter this generic approach, this may also obscure a useful role for doxycycline within certain subsets of patients. Age, smoking status and gender did not influence response to doxycycline, but we found that doxycycline reduced exacerbations among those with severe COPD better than among those with moderate or very severe COPD. Weaker exacerbation reduction among those with worse spirometry measurements has been observed previously in bronchodilator studies,[27,28] perhaps reflecting differences in exacerbation pathophysiology accompanying increasing disease severity.

Our data also suggest that the effectiveness of doxycycline in reducing exacerbations was influenced by the patient's baseline eosinophil count. While not considered in previous comparable COPD prophylactic antibiotic studies,[7,8,11,14] a similar interaction has been observed in one three-month study of Azithromycin and trials of long-term antibiotics targeting other inflammatory respiratory diseases. In a post-hoc analysis, Vermeersch et al. found that long-term azithromycin commenced at the onset of a severe exacerbation reduced treatment failure, particularly among those with blood eosinophil counts <300 cells/μL at the exacerbation onset.[29] An elevated blood eosinophil count in chronic rhinosinusitis was found to predict a poor response to long-term macrolide therapy[30] and long-term doxycycline may worsen outcomes among those with co-existing asthma or elevated baseline serum IgE levels.[31] Furthermore, although tetracyclines offer immunomodulatory benefits to some asthmatics, these benefits may be absent among those with co-existing COPD.[32]

The mechanism underlying the interaction between doxycycline and baseline eosinophil count deserves further study and may reflect the dual bacteriostatic and anti-inflammatory action of tetracyclines.[33,34] Doxycycline's bacteriostatic properties may prove most beneficial to patients with lower eosinophil counts, perhaps treating an associated heightened susceptibility to infection contributing to exacerbation occurrence.[35] However, in other patients, baseline eosinophilic inflammation may be a more prominent driver of exacerbations.[35] Current COPD treatment strategies use a blood eosinophil count threshold of 300 cells/μL[36] to help identify steroid responsiveness.[37,38] Plausibly, eosinophil levels might also predict

response to other anti-inflammatory agents, such as tetracyclines. In vivo, doxycycline exhibits anti-inflammatory effects,[39] including downregulation of eosinophil degranulation[40] and decreased nitric oxide production,[41] which could theoretically impair defence against some infections. Variation in airway microbiome is also linked to blood eosinophil counts and Proteobacteria may dominate in those with low eosinophil counts. Suppression of these organisms with doxycycline therapy may be beneficial, but a reduction in the Firmicutes and Streptococcus dominating at higher eosinophil counts might be disadvantageous.[42] Future trials will be needed to test such hypotheses.

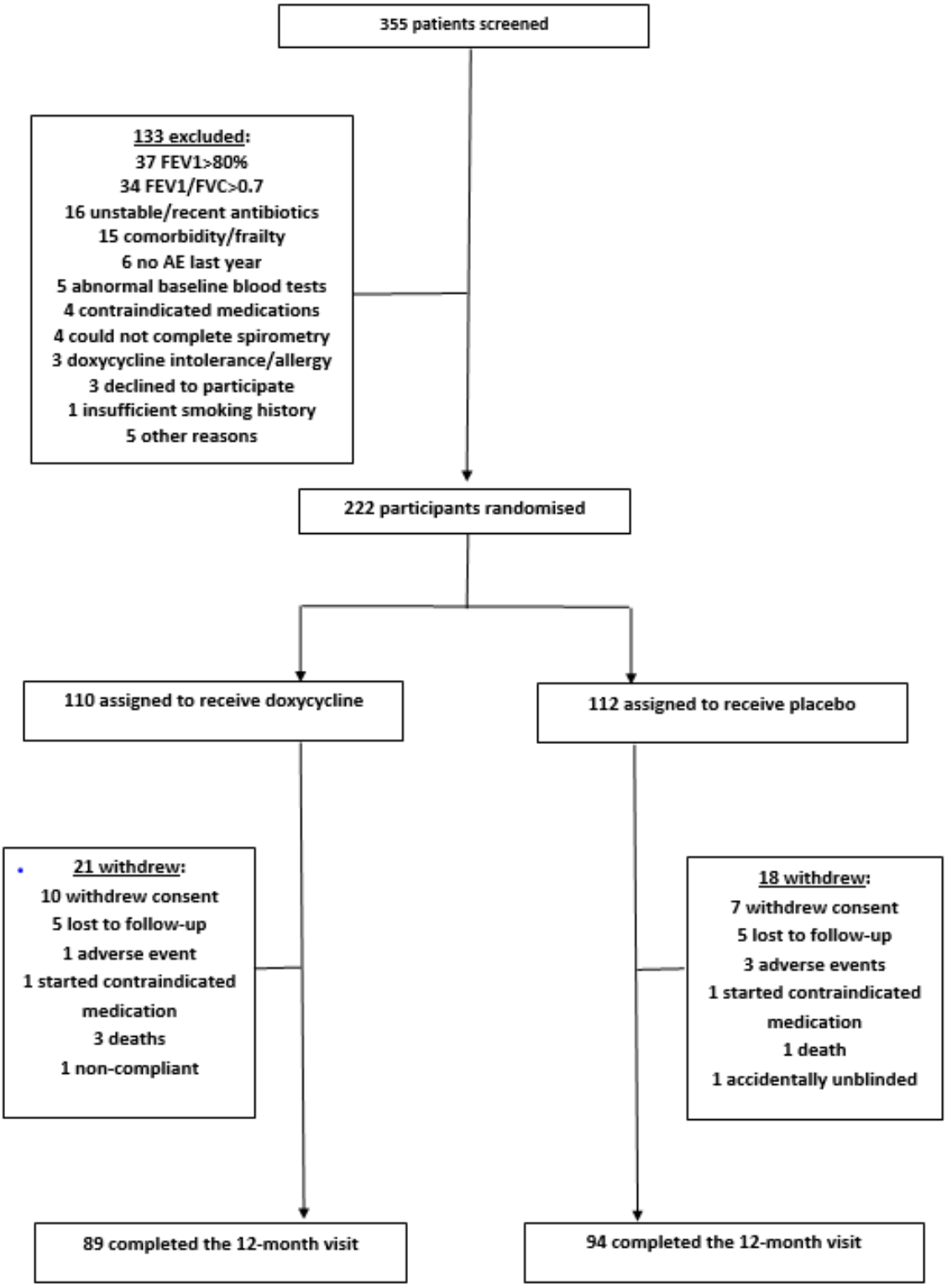
At enrolment our patients reported significant impairment of health status as identified by both the SGRQ and CAT questionnaires. SGRQ did not change in those receiving doxycycline but improved in those randomised to placebo. These differences may be explained by selective withdrawal of the sicker patients taking placebo, as observed in the trials of inhaled corticosteroids.[43] The difference is not explained by blood eosinophil count where health status worsened to a similar degree in those receiving doxycycline and improved to a similar degree in those receiving placebo, regardless of whether baseline blood eosinophil count was higher or lower. The pattern of adverse events reported with doxycycline was similar to that of the placebo treatment. The reasons behind the apparent failure of doxycycline to improve health status remain unclear.

A major strength of study is that patients reported a significant number of exacerbations during their observation period, identified using a well-tried methodology which allowed us to compute an event rate in an unbiased way.[23] Although not powered to investigate the subset of exacerbations that were treated, this study showed similar trends, albeit statistically non-significant, towards doxycycline reducing exacerbation rate using either definitions. As expected due to their prior history of exacerbations, most of our patients were already using inhaled corticosteroid and bronchodilator therapy and this precluded an exploration of interactions between these treatments and maintenance antibiotic therapy. Further, we were not able to conduct detailed surveillance of antibiotic resistance in our patients, a concern which may limit the wider use of antibiotic prophylaxis.[15]

In conclusion, doxycycline 100mg daily did not significantly reduce overall exacerbation frequency or improve quality among those with at least moderate COPD. However, the impact of long-term

386 doxycycline varied according to baseline blood eosinophil count. Future studies are needed to confirm
387 whether the potentially substantial effect of doxycycline on exacerbation numbers is present in patients
388 prospectively identified as having a blood eosinophil count below 300 cells/μL.

389 **Figure 1: Consort diagram showing patient flow.**



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391 **Table 1: Demographic features of the study population reported for all participants (and by**
392 **treatment arm) at enrolment**. Data are expressed as means, with 95% confidence intervals (95%CI)
393 or standard deviations (SD), or medians, with interquartile ranges (IQR).

	Overall	**Placebo**	**Doxycycline**
Number of subjects	222	112	110
Mean age (SD), years	66.7 (7.6)	66.1 (7.6)	67.3 (7.7)
Number of males (%)	126 (57%)	62 (55%)	64 (58%)
Median weight [IQR], kg	74.4 [62.3, 88.0]	74.3 [62.8, 92.9]	74.5 [61.3, 84.1]
Median BMI [IQR], kg/m^2	26.3 [23.2, 30.9]	26.5 [23.3, 32.0]	26.2 [23.2, 30.4]
Number of current smokers (%)	75 (34%)	38 (34%)	36 (34%)
Median pack years (IQR)	45 [30, 56]	41 [30, 50]	50 [35, 58]
Number of previous regular drug smoker (%)	24 (11%)	11 (10%)	13 (12%)
Mean FEV_1 (SD), L	1.35 (0.53)	1.44 (0.56)	1.25 (0.48)
Number with FEV_1 <30% (%)	18 (8%)	7 (6%)	11 (10%)
Number with FEV_1 30–49.9% (%)	83 (37%)	38 (34%)	45 (41%)
Number with FEV_1 50–80% (%)	121 (55%)	67 (60%)	54 (49%)
Mean FEV_1 percent predicted (SD)	52.5 (15.9)	54.4 (15.8)	50.6 (15.8)
Mean FEV_1/FVC (SD)	0.45 (0.13)	0.47 (0.12)	0.43 (0.13)
Median oxygen saturation [IQR]	95 [94, 97]	96 [94, 97]	95 [93, 97]
Number with chronic bronchitis (%)	137 (62%)	67 (60%)	70% (64%)
Median number of treated exacerbations in prior 12 months [IQR]	2 [1, 4]	3 [1, 4]	2 [1, 4]
Number with ≥2 exacerbations in prior 12 months (%)	158 (71%)	82 (73%)	76 (69%)
Mean CAT Score (SD)	21.1 (8)	21.4 (8.4)	20.8 (7.7)
Mean SGRQ Total (SD)	51.7 (19.4)	51.3 (19.5)	52.2 (19.4)
Mean SGRQ Symptoms (SD)	56.7 (23.4)	56.5 (24.8)	56.9 (21.9)
Mean SGRQ Activity (SD)	69.1 (22.1)	68.5 (21.4)	69.7 (22.8)

Mean SGRQ Impacts (SD)	40.1 (20.7)	39.7 (20.7)	40.5 (20.7)
Number prescribed inhaled corticosteroid (%)	180 (81%)	89 (79%)	91 (83%)
Number prescribed triple Therapy – LAMA, LABA with ICS (%)	144 (65%)	74 (66%)	70 (64%)
Number prescribed long-term oral prednisolone (%)	4 (2%)	0	4 (4%)
Mean CRP (SD), mg/L	4 (6)	3 (5)	5 (6)
Median Eosinophil count [IQR], 10^9/L	0.20 [0.10, 0.30]	0.20 [0.10, 0.30]	0.20 [0.10, 0.30]
Number with eosinophil count ≥0.3 x 10^9/L (%)	59 (27%)	30 (27%)	29 (26%)

403 **Table 2: Adverse events and median time on study according study arm:**

404 Specific adverse event categories are included where at least 1% of subjects experienced that type of
405 adverse event. Specific events are additionally included (shown in italics) if at least 5% of subjects had
406 that adverse event.

407

	Placebo group	Doxycycline group
Total adverse events	**270**	**250**
Serious adverse events	**48**	**45**
Non-serious adverse events	**222**	**205**
Cardiac disorders	11	13
Ear and labyrinth disorders	0	5
Eye disorders	5	2
Gastrointestinal disorders	25	39
Diarrhoea	*3*	*3*
Dyspepsia and gastritis	*5*	*12*
Nausea and vomiting	*1*	*6*
Hepatobiliary disorders	3	3
Infections	55	28
Candida infection	*3*	*2*
Dental infection	*7*	*3*
Ear infection	*5*	*4*
Urine infection	*4*	*2*
Bacterial infection not specified	*19*	*11*
Injury or poisoning disorder	16	24
Metabolic or nutrition disorder	13	11
Musculoskeletal disorder	33	29
Neoplasm	3	7
Nervous system disorder	21	9

Headache	*3*	*2*
Renal or urinary disorders	4	7
Respiratory disorders	11	10
Skin or cutaneous disorders	14	10
Rash non specified	*4*	*2*
Eczema or dermatitis	*1*	*4*
Surgical or medical procedures	25	20
Vascular disorders	2	3
Other	29	30
Median time (days) on study of all participants randomised into each study arm (IQR)	400(21)	398 (20)

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Figure 2: Kaplan Meier plot of time to first exacerbation split between doxycycline and placebo groups (See supplement for graph stratified by site).

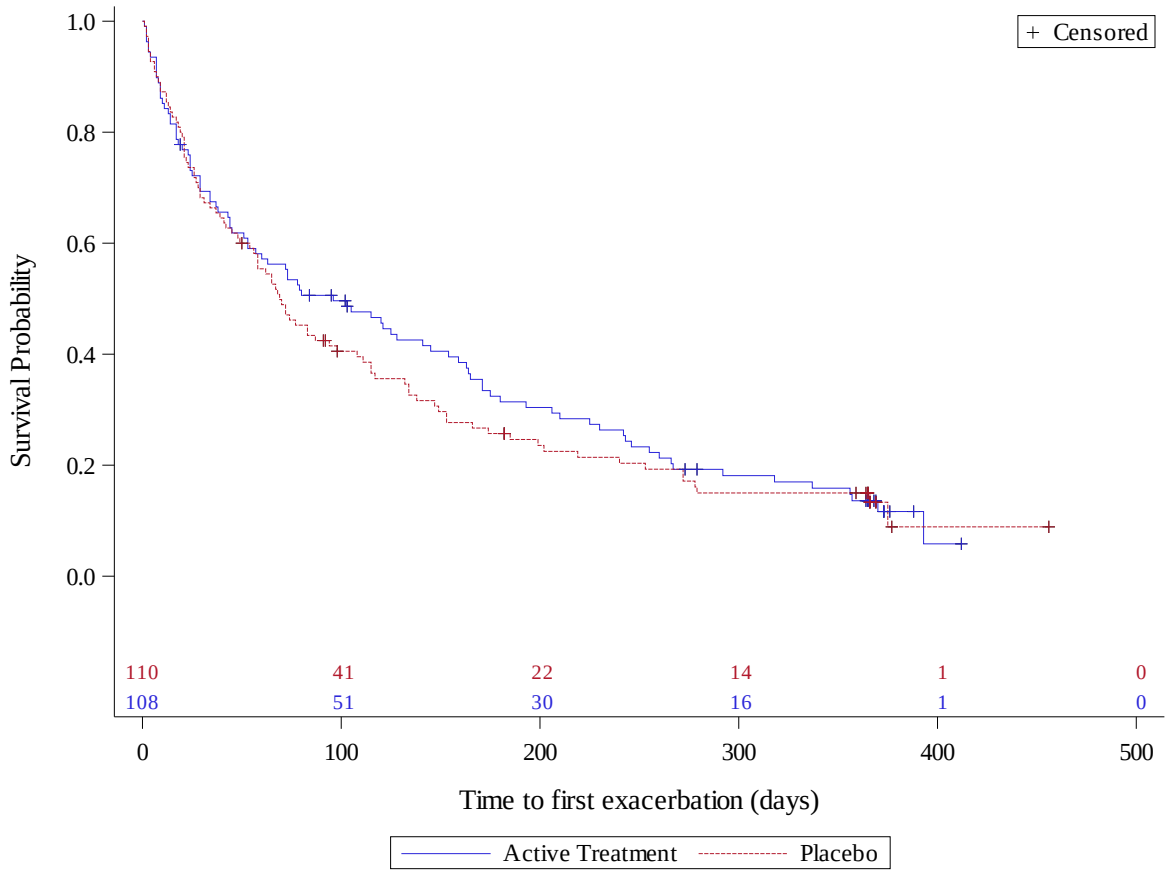
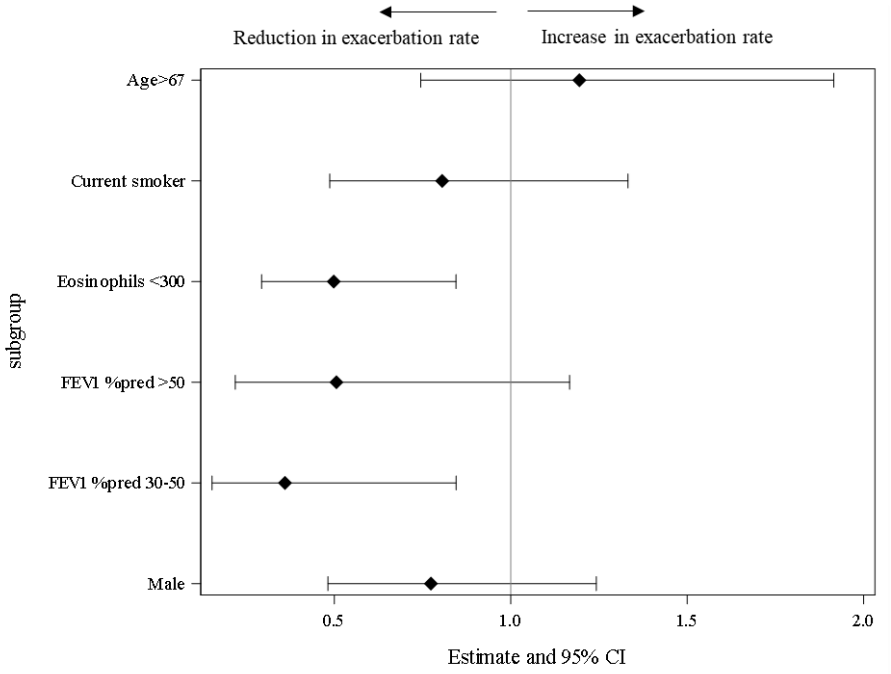


Figure 3: The interaction of co-variates with the effect of doxycycline (A: Forest plot; B: tabulated indicating the relevant comparator for each comparison). Models are adjusted for baseline exacerbation rate in the previous year. Doxycycline reduced exacerbation rate to a greater degree in those with severe COPD (FEV_1 30-50% predicted) relative to very severe COPD (FEV_1 <30% predicted) (RR 0.36 95%CI 0.15 to 0.85, P=0.019). Doxycycline reduced exacerbation rate to a greater degree in those with eosinophil count <300 cells/μL relative to those with eosinophil count ≥300 cells/μL (RR 0.50 95%CI 0.29 to 0.84, P=0.01).

A:



B:

Variable	Relative Risk	95% confidence interval	p-value
Age			
<67	Reference		
>67	1.19	0.75, 1.92	0.46
Current smoker			
No	Reference		
Yes	0.81	0.48, 1.33	0.40
Eosinophils			
≥300 cells/μL	Reference		
<300 cells/μL	0.50	0.29, 0.84	0.01
FEV_1 %pred			
<30	Reference		
30-50	0.36	0.15, 0.85	0.019
>50	0.51	0.22, 1.17	0.11
Sex			
Female	Reference		
Male	0.77	0.48, 1.24	0.29

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