ABNORMAL RECOVERY AND RECURRENCE OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE EXACERBATIONS

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Author's Declaration

I, Alex Mackay confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis. In particular I am grateful for contributions from the following individuals, in alphabetical order, in the specific areas described:

Dr Gavin Donaldson assisted with the statistical analysis of the data presented in Chapter 4.

Dr Davinder Garcha and Dr Siobhan George tested sputum samples for Bacterial and Rhinovirus PCR, the results of which were included in Chapter 6.
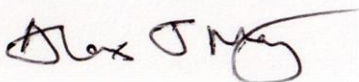
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Acknowledgements

Abstract

This thesis examines chronic obstructive pulmonary disease (COPD) exacerbation recovery in depth. This is an important topic to study since prolonged recovery and recurrence are common, severe and poorly understood events.

The methodology involved patient reported outcome (PRO) tools and objective cough monitoring to examine symptomatic changes during exacerbations, and also the measurement of systemic and airway biomarkers during COPD exacerbations to identify patients at risk of non-recovery and recurrence. Finally, this thesis also included a randomised placebo-controlled trial to investigate if Roflumilast can improve exacerbation recovery.

This thesis showed that PROs provide reliable measurements of exacerbation severity. Furthermore, cough frequency increased acutely from baseline levels at exacerbation and fell during subsequent recovery. The biomarker work demonstrated that increased systemic inflammation at exacerbation onset predicts non-recovery and that faster resolution of neutrophilic inflammation is associated with shorter recovery. Roflumilast did not accelerate reduction of sputum neutrophils from exacerbation onset to 2 weeks post exacerbation, but did improve lung function recovery when given on top of standard therapy.

This thesis demonstrates that PROs can be used in clinical practice to evaluate exacerbation recovery, and in trials of acute exacerbation therapies. It also provides evidence that targeting inflammation may improve recovery time. Future research should seek to define the use of Roflumilast as part of a personalised strategy that treats patients according to exacerbation phenotype.

<u>Awards arising from this thesis</u>

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- September 2012   GSK Sparrows Symposium winner at ERS

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Publications arising from this thesis

- **Detection and Severity Grading of COPD Exacerbations Using The Exacerbations of Chronic Obstructive Pulmonary Disease Tool (EXACT).** Mackay AJ, Donaldson GC, Patel AR, Singh R, Kowlessar B, Wedzicha JA. European Respiratory Journal. 2014 March;43(3):735-44.

- **Usefulness of the Chronic Obstructive Pulmonary Disease Assessment Test to evaluate severity of COPD exacerbations.** Mackay AJ, Donaldson GC, Patel AR, Jones PW, Hurst JR, Wedzicha JA. American Journal of Respiratory and Critical Care Medicine. 2012 June 1;185(11):1218-24.

Manuscripts in Preparation

- **Effect of roflumilast 500 μg tablets once daily at acute COPD exacerbations treated with standard therapy of oral steroids and antibiotics: A randomised, double-blind, placebo-controlled, parallel-group trial.** Mackay AJ, Donaldson GC, Patel AR, Singh R, Rapsford R, Goehrin UM, Wedzicha JA.

- **Utility of Objective Cough Monitoring in Stable COPD and at Exacerbation**. Mackay AJ, Donaldson GC, Singh R, Holt K, Belvisi MG, Smith JA, Wedzicha JA.

Presented abstracts arising from this thesis

- **Mackay AJ,** Donaldson GC, Singh R, Holt K, Belvisi MG, Smith JA, Wedzicha JA. Utility of Objective Cough Monitoring in Stable COPD and at Exacerbation. *American Thoracic Society* (2015).

- **Mackay AJ**, Donaldson GC, Kowlessar B, Patel ARC, R.Singh, Wedzicha JA. Baseline Characteristics Of The EXAcerbations Of Chronic Obstructive Pulmonary Disease Tool (EXACT). *American Thoracic Society* (2013).

- **Mackay AJ**, Donaldson GC, Kowlessar B, Patel ARC, R.Singh, Wedzicha JA. Relationship Between COPD Exacerbations Detected Using Symptom Diary Cards And The EXAcerbations Of Chronic Obstructive Pulmonary Disease Tool (EXACT). *American Thoracic Society* (2013).

- **Mackay AJ**, Donaldson GC, Kowlessar B, Patel ARC, R.Singh, Wedzicha JA. Utility Of The EXAcerbations of Chronic Obstructive Pulmonary Disease Tool (EXACT) To Evaluate Severity Of COPD Exacerbations. *American Thoracic Society* (2013).

- **Mackay AJ**, Donaldson GC, Patel ARC, R.Singh, Hurst JR, Wedzicha JA. Combined assessment of the impact of COPD using the COPD Assessment Test™ (CAT), spirometry and exacerbation history. *European Respiratory Society* (2012).

- **Mackay AJ**, Patel ARC, Donaldson GC, Hurst JR, Wedzicha JA. The COPD Assessment Test^{TM} (CAT) can detect changes in health status during COPD exacerbations. *American Thoracic Society* (2011).

- **Mackay AJ**, Patel ARC, Donaldson GC, Hurst JR, Wedzicha JA. Utility of the COPD Assessment Test^{TM} (CAT) in stable COPD. *American Thoracic Society* (2011).

Abbreviations

a2M   Alpha-2-Macroglobulin

ATS   American Thoracic Society

cAMP   Cyclic adenosine monophosphate

BMI   Body Mass Index

CD   Cluster of Differentation

COPD   Chronic Obstructive Pulmonary Disease

CC16   Clara cell secretory protein-16

CAT   The COPD Assessment Test

CCD   Charge-coupled device

CRP   C-Reactive Protein

CXCR   CXC chemokine receptor

DTT   Dithiothreitol

ELISA   Enzyme-Linked Immunosorbent Assay

ERS   European Respiratory Society

EXACT   Exacerbations of Chronic Obstructive Pulmonary Disease Tool

FDA   Food and Drug Administration

FEV_1 Forced Expiratory Volume in one second

FVC   Forced Vital Capacity

g   gram

GOLD   Global initiative for chronic Obstructive Lung Disease

HCU   HealthCare Utilisation

HIV	Human Immunodeficiency Virus
HRP	streptavidin-horseradish peroxidase
HRV	Human Rhinovirus
IFN	Interferon
IP-10	Interferon gamma-inducible protein 10
IL	Interleukin
IMP	Investigational Medicinal Product
IQR	Inter-Quartile Range
l	litre
MCID	Minimal Clinically Important Difference
mcg	microgram
mg	milligram
ml	millilitre
MMP	Matrix metalloproteinase
MPO	Myeloperoxidase
MRC	Medical Research Council
NE	Neutrophil Elastase
n	number
ng	nanogram
NHYA	New York Heart Association
PaO$_2$	arterial Partial Pressure of Oxygen
PARC	Pulmonary and activation-regulated chemokine
PBS	Phosphate-Buffered Saline
PDE4	Phosphodiesterase 4

PCR	Polymerase Chain Reaction
PEF	Peak Expiratory Flow
PEFR	Peak Expiratory Flow Rate
pg	picogram
PLGF	Placental growth factor
PPM	Potentially Pathogenic Micro-organism
PRO	Patient Reported Outcome
r	Pearson correlation co-efficient
sRAGE	soluble Receptor for Advanced Glycation End-products
rho	Spearman rank correlation co-efficient
SAA	Serum Amyloid A
SD	Standard Deviation
SGRQ	St. George's Respiratory Questionnaire
TIMP	Tissue Inhibitor of MetalloProteinase
TREAT	Treatment with Roflumilast at ExAcerbaTion
TNF-α	Tumour Necrosis Factor-alpha
UK	United Kingdom
US	United States
^{O}C	degrees Celsius
μl	microlitre
μm	micrometre
Δ	Delta (change in)

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INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD), is a common preventable, treatable condition characterised by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases (1). It is a critically important disease that is a leading cause of death worldwide (2). The World Health Organisation estimates 80 million people worldwide have COPD (3). Approximately 15 million people in the USA (4) and over 3 million people in the UK are estimated to have COPD (5).

COPD is associated with episodic periods of symptom deterioration termed exacerbations. Exacerbations are amongst the commonest causes of medical admission to hospital (6) and the rate at which they occur appears to reflect an independent susceptibility phenotype (7). They are also important events in the natural history of COPD that drive lung function decline (8, 9), increased risk of cardiovascular events (10) and are responsible for much of the morbidity (11) and mortality (12) associated with this highly prevalent condition.

Exacerbations can be severe and prolonged events, with recovery incomplete in a significant proportion of exacerbations (13). Hospital admissions account for over 1 million bed days per year in the UK and 90 day mortality following admission is over 15% (14). Mean length of hospital stay is over 8 days with 40% of patients staying more than 1 week and 15% remaining inpatients for more than 14 days (14). Furthermore, approximately 25% of exacerbations are followed by a second recurrent event within 8 weeks, and approximately one third of exacerbations are recurrent exacerbations (15). This places a huge burden upon healthcare resources with approximately one third of patients being readmitted within 3 months of their initial discharge (14).

Despite this, exacerbation recovery and recurrence are poorly understood and novel therapies to reduce exacerbation length and recurrence rates are lacking. This thesis will examine exacerbation recovery in depth, to elucidate symptomatic changes during exacerbations to enable improved monitoring and assessment during recovery. It will also study systemic and airway biomarkers during COPD exacerbations to identify patients at risk of non-recovery and recurrence. Finally, this thesis will investigate a new approach to acute exacerbation treatment to improve these critical clinical outcomes.

1.1   Use of Patient Reported Outcomes to Monitor COPD Exacerbation Recovery

An exacerbation of COPD is an acute worsening of respiratory symptoms, accompanied by a variable degree of physiological deterioration (13). The critical Anthonisen study defined exacerbations symptomatically with a particular focus on the symptoms of increased dyspnoea, sputum volume and sputum purulence (16). However, most pharmaceutical studies have used a healthcare utilisation (HCU) definition based on sustained worsening of the patient's condition from the stable state that requires a change in regular medication (17), which frequently take the form of unscheduled physician visits, the use of antibiotics or oral steroids at exacerbation, and hospital admission. This is reflected in the Global Initiative for chronic Obstructive Lung Disease (GOLD) strategy which defines an exacerbation as "an acute event characterised by a worsening of the patient's respiratory symptoms that is beyond normal day-to-day variations and leads to a change in medication" (1).

An HCU definition of exacerbations has significant limitations. Healthcare use in COPD varies depending on access, resulting in a lack of generalisability across different healthcare systems. Most importantly, up to two-thirds of COPD exacerbations are not reported to health-care professionals and are either self-treated or untreated and may not be captured by such definitions. Unreported exacerbations are important events to recognise and quantify. Previous studies have shown that up to two-thirds of all exacerbations are not reported to health care professionals for evaluation and treatment (11, 18, 19). These events impair health-related quality of life (11, 18, 19) and failure to report exacerbations is also associated with increased risk of emergency hospitalisation (20).

Symptom diary cards are capable of capturing all exacerbation events to yield an accurate exacerbation frequency but most are not sensitive enough to assess severity of these events. Assessment of exacerbation severity is an important outcome measure and most clinical trials of preventative therapy to date have used therapy and hospitalisation rates to gauge this parameter: mild if increases in regular inhaled medication are needed, moderate if courses of steroids or antibiotics are administered, and severe if the patient requires hospital admission. The use of therapy to define exacerbation severity is also compromised by failure to capture unreported exacerbations, as well as differential prescribing guidelines and thresholds between clinicians and healthcare systems.

Partially in response to these limitations, the Food and Drug Administration (FDA) recommended the development of patient reported outcome (PRO) instruments to measure treatment benefit in medical product clinical trials, including symptom-based methods for standardizing the severity of reported and unreported exacerbations (21). The FDA defines a PRO as "any report of the status of a patient's health condition that comes directly from the patient, without interpretation of the patient's response by a clinician or anyone else. The outcome can be measured in absolute terms (e.g., severity of a *symptom*, *sign*, or state of a disease) or as a change from a previous measure". The standard assessment of an exacerbation involves the reporting of symptoms which are known directly by the patient and clinical assessments are based on patient description to the clinician. It would be more reliable to gather reports directly from patients and thus PROs are ideally suited to the assessment of COPD exacerbations. This thesis will explore the use of patient reported outcomes to quantify COPD exacerbation symptom severity, specifically examining the COPD Assessment Test[TM] (CAT) and the Exacerbations of Chronic Obstructive Pulmonary Disease Tool (EXACT).

The COPD Assessment Test[TM] (CAT)

The COPD Assessment Test[TM] (CAT) was developed to enhance communication between patients and healthcare professionals by providing a standardised, patient-centred assessment tool that quantifies information on daily symptoms, exercise limitation and other COPD manifestations. Detailed questionnaires such as the St George's Respiratory Questionnaire (SGRQ) are well validated but are not practical for use outside of a research setting due to the length of the instrument and the complexity of scoring algorithms. Therefore the CAT was designed to provide valuable information in the form of a simple, brief PRO that could be routinely used in clinical practice (22).

Initially 21 draft items were generated following interviews and focus groups with COPD patients, community physicians and respiratory physicians (23). These were systematically reduced to 8-items following detailed study (22) to cover the following aspects of COPD:

CAT items

1. Cough
2. Sputum (phlegm)
3. Chest tightness
4. Breathlessness
5. Activity
6. Confidence
7. Sleep
8. Energy

Each item is graded 0-5 so that the total tool provides a score out of 40 to indicate disease impact, without the need for complex calculation (**Figure 3.3**). Higher scores indicate more severe disease. Initial studies demonstrated that the tool is simple for patients to complete and that CAT scores correlate closely with health-related quality of life as measured by the St. George's Respiratory Questionnaire (SGRQ) when patients are stable (22). It was also shown that in the stable state, CAT scores are not affected by low levels of comorbidity (24), but are responsive to pulmonary rehabilitation and able to distinguish different levels of response (25).

Preliminary studies showed that CAT scores were higher in patients at exacerbation compared to scores obtained in the stable state (22, 24). **Chapter 4.1** describes the first study examining the utility of the CAT to evaluate HCU COPD exacerbation severity and model recovery.

The Exacerbations of Chronic Obstructive Pulmonary Disease Tool (EXACT)

The Exacerbations of Chronic Obstructive Pulmonary Disease Tool (EXACT) is a daily symptom diary developed to capture frequency, severity, and duration of exacerbations in clinical trials of COPD (26). The initial development of the EXACT involved focus groups and interviews with 83 patients with COPD and a recent history of exacerbations, and 2 expert panel meetings to inform the development of instrument content and structure. Patients were asked to describe their exacerbation experiences, including key symptoms, progression, and recovery. Additionally, cues for self-diagnosis and triggers to self-manage or seek assistance from healthcare professionals were elicited (27). These results were then reviewed by physicians and experts in questionnaire development to generate a preliminary framework consisting of 23 items **(Figure 1.1)**:
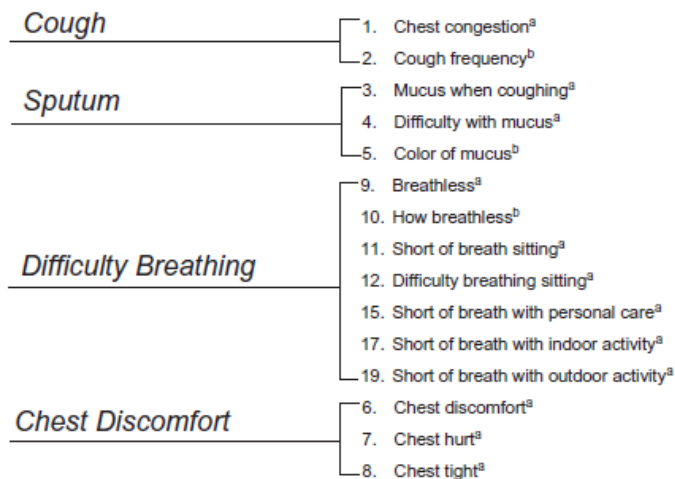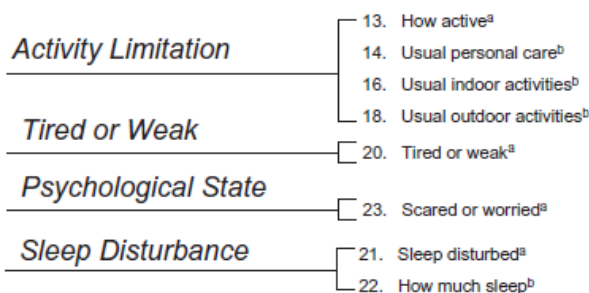
Figure 1.1. Preliminary Conceptual Framework for the EXACT. Reproduced from (24).

Following this a large observational study comprising 410 patients with COPD (188 stable; 222 at exacerbation) in the US was performed to collect data for item reduction and to assess the tool's measurement properties (26). The item-reduction process was conducted in the stable cohort and resulted in a final tool consisting of 14 questions (**Figure 3.5**) that address the same themes as the CAT but with multiple questions assessing some items:

1. Cough-two questions

2. Sputum (phlegm)-two questions

3. Chest tightness--two questions

4. Breathlessness--two questions

5. Activity-three questions

6. Confidence-one question

7. Sleep-one question

8. Energy-one question

EXACT scores are calculated from the responses to these 14 questions (further details available in methods 3.2.2). Scores range from 0 to 100, with higher scores indicating more severe symptoms. Validation studies showed that the EXACT is a reliable tool to assess symptom severity and found that EXACT scores were significantly elevated at exacerbation compared to patients in the stable state (26). As a result, the authors proposed thresholds for exacerbation detection and severity based on rises in EXACT score so that the tool might be used as an outcome measure in pharmaceutical trials of novel COPD medications (**Figure 1.2**).

Exacerbation
Acute sustained worsening beyond
day-to-day variability

Frequency
Number of
occurrences within a
given time period

Severity
Magnitude at a point
in time or over time

Severity
Magnitude at a point
in time or over time

EXACT
Total
Score

Day-to-day variability

Duration
Period of time to a
specified target

Duration

Time
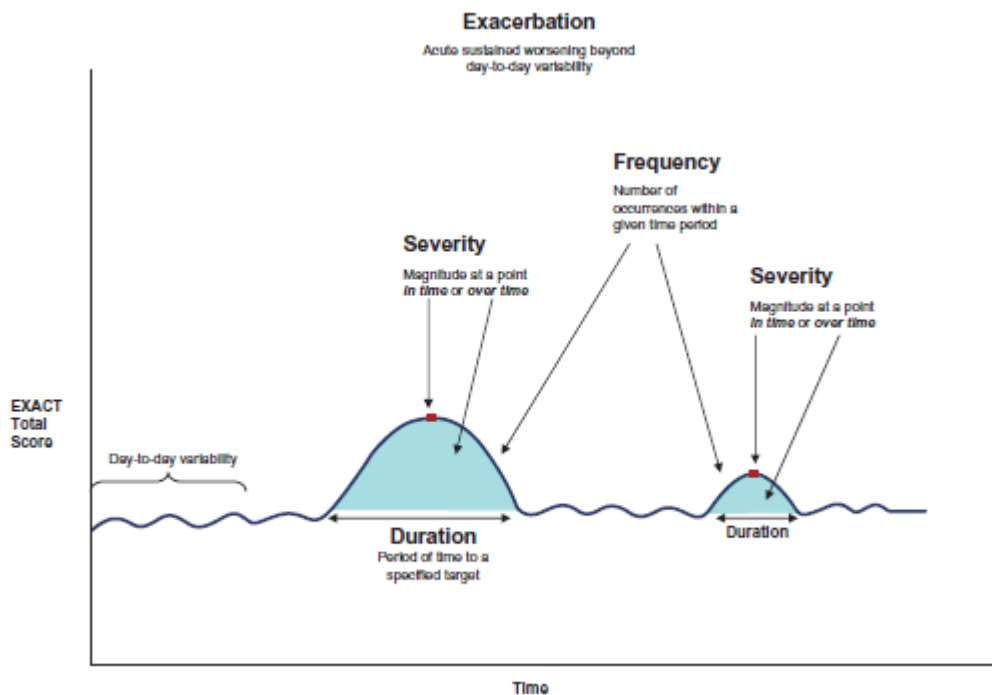
Figure 1.2. Proposed Utility of the EXACT. Reproduced from (24).

However, no external validation of the ability of the EXACT to detect or assess the severity of exacerbations was performed. **Chapter 4.2** describes the first study to assess ability of the EXACT to accurately assess the severity of both reported and unreported COPD exacerbations, defined by the London COPD cohort diary card.

1.2   Monitoring COPD Exacerbation Recovery using Objective Cough Frequency Measurements

Cough (either with or without sputum) is one of the principle symptoms of COPD, along with dyspnoea, and is one of the key domains assessed in both the CAT and EXACT PROs. Cough has a high impact on the lives of COPD patients, being reported in approximately 60% of patients, with almost 30% of patients rating their cough as moderately to extremely severe (28). Epidemiological studies have found that the reported presence of chronic cough and mucus hypersecretion may be linked to accelerated decline in lung function (29), more frequent exacerbations (30), and hospitalizations (31) in COPD patients. However, previous work has shown that subjective measures of cough and cough reflex sensitivity are only moderately correlated to objective measures of cough (32), and have insufficient predictive value for understanding the determinants of cough. The inconsistency between subjective and objective measures is probably because patients rate cough severity on more than just frequency. The length of coughing bouts, forcefulness of the cough, severity of accompanying breathlessness and the extent to which daily activities and sleep are disrupted are likely to be key determinants of self-reported severity (33).

To overcome these issues, colleagues at the University of South Manchester led by Professor Jacklyn Smith have developed a system for making sound recordings over 24 hours in ambulatory patients, which allows the objective quantification of coughing (Vitalojak; Vitalograph Ltd., Buckingham, UK) (34). In a recent publication, this group showed that in the stable state, current healthy smokers and COPD ex-smokers have similar cough frequencies, both significantly greater than healthy nonsmokers. Amongst COPD patients, current smoking had an additive effect, giving COPD current smokers double the cough

frequency of those with either factor alone. Prior studies have shown that chronic expectoration is associated with increased neutrophilic inflammation (35) and in this paper, the degree of neutrophilic airway inflammation in COPD patients as measured in sputum samples in the stable state was positively related to cough frequency, independent of the influence of current smoking. In those patients who provided sputum samples, over a third of the variation in cough rates was explained by the degree of neutrophilic airway inflammation and current smoking (34).

Exacerbations are symptomatic deteriorations in respiratory symptoms and cough plays a prominent role in these events. Recovery from exacerbations is often prolonged and cough may be a protracted feature of some events (13). Exacerbations are also characterised by increased neutrophilic airways inflammation (36, 37) and frequent exacerbators have higher neutrophilic inflammation than infrequent exacerbators in the stable state (38). Given the relationship between cough and inflammation, objective cough monitoring may be a useful tool in the setting of acute exacerbations of COPD. Thus in collaboration with the University of South Manchester I have examined the utility of objective cough monitoring to differentiate between stable COPD and exacerbation and whether cough frequency can distinguish patient exacerbation frequency phenotype in the stable state. We also examined the utility of cough monitoring as an objective means of determining the severity of an exacerbation, and investigated if different exacerbation phenotypes have different cough responses at exacerbation and during recovery. This work is described in **Chapter 5**.

1.3   Systemic and Airway biomarkers to evaluate COPD exacerbation recovery and recurrence

The increased cough and sputum seen at exacerbation is associated with increased inflammation. In studies of COPD patients presenting to primary-care physicians, increasing sputum purulence was associated with increasing sputum neutrophilia, and systemic inflammation as measured by serum C-reactive protein was also higher in exacerbations associated with purulent rather than mucoid sputum (39). The increased inflammation seen in this work appeared to be related to the presence of bacteria.

Exacerbation Aetiology

In the same study by Stockley and colleagues (39), positive bacterial cultures were obtained from 84% of patients if sputum was purulent at presentation but only 38% if the sputum was mucoid. Moreover, the median bacterial load for positive purulent culture samples was significantly higher than for mucoid samples. When the same patients were re-examined in the stable state following antibiotics, sputum colour improved significantly in the group who presented with purulent sputum. The presence of green (purulent) sputum was 94.4% sensitive and 77.0% specific for a high bacterial load ($>10^7$ colony forming units (cfu)/mL) (39). Both the prevalence of potentially pathogenic microorganisms (PPM), and airway bacterial load in sputum have been shown to increase from stable state to exacerbation, the most frequently isolated organisms being *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae* (40). These studies suggest a causative role for increasing bacterial load at acute exacerbations.

Strain changes may also play an important role in the aetiology of COPD exacerbations. In a prospective study, Sethi and colleagues hypothesised that acquisition of a new bacterial strain would be associated with an exacerbation of COPD and so collected sputum samples from 81 outpatients with moderate to severe COPD on a monthly basis and at exacerbation. Molecular typing of sputum showed that isolation of a new strain of a pathogen (*Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae*) was associated with a significant increase in the risk of exacerbation. These findings were proposed as a mechanism to explain recurrent bacterial exacerbations of COPD, the authors speculating that following a first exacerbation, patients develop a protective immune response that is strain specific. Therefore, acquisition of a different strain from the same bacterial species may still lead to a second exacerbation (41). However, not all exacerbations were associated with strain change, and not all strain changes were associated with exacerbation.

Rhinovirus

Respiratory viral infections are detected in up to 60% of exacerbations using polymerase chain reaction (PCR) techniques, with rhinovirus being the most common species identified (42, 43). Experimental rhinovirus infection models also provide evidence of a direct causal relationship between respiratory virus infection and acute exacerbations of COPD (44). When COPD patients are infected with rhinovirus they develop increased respiratory symptoms, fall in peak expiratory flow (PEF), and increased blood and airway inflammation. A temporal relationship is observed between virus detection in the respiratory tract and the onset of symptoms and airflow obstruction, and virus clearance is followed by clinical recovery.

Furthermore, peak sputum virus load correlates positively with peak serum C-reactive protein (CRP) concentration and sputum inflammatory markers (neutrophils, interleukin (IL)-6, IL-8, neutrophil elastase (NE) and tumour necrosis factor (TNF)-α). Indeed, naturally occurring viral infections appear to be more severe events than non-viral exacerbations since exacerbations associated with dyspnoea and cold symptoms (a surrogate for rhinovirus infection) at onset are associated with larger falls in peak flow, prolonged recovery times and higher levels of airway inflammatory markers (IL-6) (13, 38).

Interaction of Bacteria and Viruses

Experimental rhinovirus models have also provided evidence of potential synergy between bacterial and viral infections (45, 46). COPD patients infected with rhinovirus exhibit subsequent secondary bacterial infection as evidenced by increased bacterial load and disruption of the respiratory microbiome. A limitation of this experimental rhinovirus model is that the patients included had only moderate COPD and the exacerbations induced did not require additional systemic therapy.

Recent work in the London COPD cohort examined coinfection in naturally occurring exacerbations which all required treatment with additional systemic therapy (47). Patients with moderate to severe COPD who had known rhinovirus infection at exacerbation were examined closely during their recovery phase. In samples without bacteria detected at exacerbation, bacterial load increased by day 14 with 73% of negative samples becoming positive for bacteria by day 14. HRV prevalence and load increased at COPD exacerbation,

and resolved during recovery. Studies have also shown that exacerbations associated with both human rhinovirus and *Haemophilus influenzae* exhibit a greater bacterial load and inflammation than those without both pathogens (48). These data confirm that rhinovirus infection at naturally-occurring COPD exacerbations is commonly associated with secondary bacterial infection and greater airway inflammation, thus providing another possible mechanism for exacerbation recurrence.

Eosinophilic Inflammation at exacerbation

As described above exacerbations are triggered mainly by respiratory viruses and bacteria, which infect the lower airway and increase airway inflammation (49). Typically, the rise in inflammation seen at exacerbation has been considered predominantly neutrophilic (36, 37, 50, 51). However more recently there has been considerable interest in eosinophilic inflammation, which had previously been thought more confined to asthmatic patients. Bafadhel and colleagues used cluster analysis to propose 4 distinct biological clusters at exacerbation: bacterial-, viral-, or eosinophilic-predominant, and a fourth associated with limited changes in the inflammatory profile termed "pauciinflammatory" (52). In this work, 28% of events were associated with sputum eosinophilia which might be identified using a peripheral blood eosinophil count of 2%. This threshold had a sensitivity of 90% and specificity of 60% for identifying a sputum eosinophilia of greater than 3% at exacerbation. Following on from this work, it has been proposed that the use of corticosteroids both in the stable state, in the form of inhaled corticosteroid-containing combination therapy (53, 54), and in terms of systemic therapy at exacerbation (55), might have the greatest benefit in patients with peripheral blood eosinophil levels above this threshold. However, to date prospective studies based on blood eosinophil profiling are limited and previous studies have

not examined the relationship of blood eosinophil levels to exacerbation recovery and recurrence.

Inflammation during exacerbation recovery

Not all exacerbations recover and patients are susceptible to another or recurrent exacerbation after an index event. In the London COPD cohort, in 23% of patients, symptoms did not recover to baseline by day 35 and 22% of patients had a recurrent exacerbation within 50 days of the first (index) exacerbation (56).

Monitoring the temporal profile of inflammatory mediators during recovery from an acute exacerbation is challenging. Previous studies of inflammatory markers measured at exacerbation and during recovery have shown elevated levels of systemic inflammatory markers such as CRP, IL-6 and IL-8, which fall rapidly with treatment (56-59). In hospitalised exacerbations, changes in CRP levels (between measurements taken at intensive care unit admission and discharge) correlated with the duration of mechanical ventilation and severity of dyspnoea on the Medical Research Council (MRC) scale (60).

Persistent inflammation appears to be associated with worse clinical outcomes. Patients with prolonged recovery have persistently higher levels of serum CRP during the recovery period (56). Previous studies have suggested that the period 14 days after exacerbation onset may be a critical time, with those patients with a recurrent exacerbation having a significantly higher CRP level 14 days after the index exacerbation, compared to those who did not have a recurrence (56).

It is unclear why airway and systemic inflammation remain persistently high in some patients treated appropriately at an initial exacerbation. As discussed above, it is possible that there is persistence of the initial infective agent, acquisition of a new organism or bacterial type (41), or an interaction between pathogens present in the airways in the stable state (61) and also when these pathogens act as exacerbation triggers. However, it had previously not been possible to accurately identify which COPD patients are likely to have a prolonged recovery or recurrent exacerbation based on their biochemical characteristics at exacerbation onset, or which features of the index exacerbation predict recurrence (56, 59).

To investigate whether increased inflammatory markers at exacerbation can inform on these key clinical outcomes, we have intensively sampled patients in the London COPD cohort at exacerbation, during their recovery and also captured subsequent recurrent events. Samples were tested for high value biomarkers which were selected based on their biological/mechanistic plausibility to inform on exacerbation recovery and recurrence. Past studies examining these biomarkers are summarised in **Appendix 1**. This has enabled me to examine whether elevated systemic and airway biomarkers at exacerbation onset predict abnormal exacerbation recovery and recurrent exacerbations within 8 weeks after an index event. This work is described in **Chapter 6**.

1.4   Treatment with Roflumilast at ExAcerbaTion (TREAT)

Existing exacerbation treatments have limited efficacy to reduce the increased inflammation seen at exacerbation, improve recovery and reduce recurrence. Current standard therapy of moderate to severe exacerbations include increased bronchodilators, oral corticosteroids and if exacerbations are associated with increasing sputum volume and/or purulence, antibiotics (49). Bronchodilators relieve dyspnoea and airflow obstruction during exacerbations (62) and short-acting inhaled β_2 agonists are usually the preferred bronchodilators for the initial treatment of COPD exacerbations (1).

Antibiotics

There is considerable evidence (as described above) to support the role of bacteria in COPD exacerbation aetiology and most guidelines highlight that antibiotics are beneficial in selected patients (1). Purulent sputum is a reasonable surrogate of bacterial infection (39) and routine antibiotic use is normally advised only in the context of exacerbations associated with an increase in sputum purulence (1). Much of the evidence for these recommendations stems from the seminal study by Anthonisen and colleagues that provided strong evidence that antibiotics had a significant effect on PEFR and led to earlier resolution of symptoms (16). Type 1 exacerbations (those associated with increased sputum volume, sputum purulence and dyspnoea) benefited the most with resolution of symptoms in 63% of the antibiotic treated exacerbations and 43% of the placebo group. However, patients with type 3 exacerbations (who met just one of the three cardinal symptoms) did not show significant benefit.

Studies have also assessed the benefits of stratifying antibiotic use according to exacerbation severity, determined by therapy. Antibiotics have been shown to increase clinical cure rate and prolong time to next exacerbation in mild to moderate exacerbations (those requiring increased bronchodilators, oral antibiotics and/or steroids) (63). A recent Cochrane review found that antibiotics had no statistically significant effect on mortality and length of hospital stay in inpatients (64). However, in COPD exacerbations requiring mechanical ventilation, oral ofloxacin reduced in-hospital mortality, duration of hospital stay, length of mechanical ventilation and reduced the need for additional courses of antibiotics (65). Therefore, in addition to exacerbations associated with increased sputum purulence, antibiotics are recommended in 'severe' exacerbations requiring mechanical ventilation (1).

The choice of antibiotics remains uncertain, predominantly due to methodological limitations hampering comparison of studies examining different antibiotics. At present, most guidelines suggest initial empirical treatment should be in the form of an aminopenicillin, a macrolide or a tetracycline, taking into account guidance from local microbiologists and in the light of local resistance patterns (66). In hospitalized patients, sputum should be sent for culture at exacerbation if purulent, and the appropriateness of therapy checked against sensitivities when available. In those patients at high risk of *Pseudomonas aeruginosa*, fluoroquinolones should be considered.

Unfortunately, antibiotic resistance is become increasingly prevalent in both hospitals and the community and many in the respiratory community question the wisdom of antibiotics for acute exacerbations of COPD. Respiratory viruses are detected in more than 50% of exacerbations and are a major infectious trigger (42) which will not respond to antibiotics.

Additionally, antibiotic therapy is associated with increased side effects, particularly gastro-intestinal, such as diarrhoea. To offer a definitive answer verdict on this issue, a large randomised double-blind trial is currently underway to demonstrate non-inferiority of placebo against adequate antibiotic treatment in addition to standardized treatment, with the hypothesis that antibiotics are not needed for COPD exacerbations (67).

Systemic Corticosteroids

Multiple studies have found significant short-term benefits of corticosteroids in the treatment of COPD exacerbations. Corticosteroids lead to improvements in FEV_1 in the first three to five days of treatment (68-70) and Arterial partial pressure of oxygen (PaO_2) in the first 72 hours in comparison to placebo (70, 71). Corticosteroids have also been shown to reduce hospitalisation length (68, 69), and the likelihood of treatment failure (72). However, treatment of exacerbations with corticosteroids has not been shown to improve mortality (72) and recent studies have suggested that the benefit of corticosteroid therapy at exacerbation is limited to patients with an eosinophilic inflammatory profile, and that in fact use of corticosteroids in non-eosinophilic exacerbations may impair recovery (55).

Controversy also exists regarding the optimal dose and duration of treatment for acute exacerbations, often owing to the heterogeneity of treatment regimens in different clinical trials (73). There is no clear benefit of intravenous therapy over oral preparations and most guidelines recommend a dose of 30-40mg oral prednisone per day for a duration of 7-14 days (1, 66). There is no advantage in prolonged courses of therapy (69) and shorter courses of therapy reduce the risk of adverse effects, with tapering of dose not required for most patients

(74). The commonest reported adverse effect of corticosteroid therapy is hyperglycaemia, particularly in patients with pre-existing diabetes mellitus (69).

Self Management

Rapid recognition of exacerbation symptoms and earlier treatment improves recovery and reduces the risk of hospitalisation (20). Self-management plans are designed to enable patients to respond appropriately to the first signs of an exacerbation without leading to overtreatment of minor symptom variations. Patients at high risk of exacerbations are therefore often provided with a course of "rescue" antibiotics and corticosteroids to keep at home and instructed to commence oral corticosteroid therapy if their increased dyspnoea interferes with activities of daily living, either independently or after seeking advice from a health-care professional. Antibiotics may be started in response to increased sputum volume and/or purulence and bronchodilator therapy increased to control symptoms (66). Such interventions have been shown to reduce admission rates (75, 76), however not all patients are suitable for these strategies. COPD patients are frequently elderly and may have cognitive difficulties limiting their ability to self manage, particularly when acutely unwell. Furthermore, patients suffer from repeated exacerbations (7, 11) and may need repeated courses of corticosteroids, exposing patients to significant side effects including cataracts, osteoporosis and suppression of the immune system (77). Therefore there is a risk that self-management of exacerbations may lead to uncontrolled and unnecessary corticosteroid use with all its associated complications. Furthermore, corticosteroid resistance is common in COPD patients (78). Crucially, 27% of patients fail treatment with combined antibiotic and corticosteroid therapy by 30 days, and treatment failure rates rise to 37% by 90 days (69, 79).

Thus, new therapies for management of acute exacerbations are urgently needed, in particular specifically targeting the increased inflammation seen at exacerbation.

TNFα antagonists

Unfortunately, to date attempts to use alternative agents to treat exacerbations have been unsuccessful. As described above, exacerbations are inflammatory events, associated with increased sputum concentrations of Tumour Necrosis Factor-alpha (TNFα) (36). TNFα upregulates adhesion molecules and facilitates migration of leucocytes into the bronchial mucosa during AECOPD, stimulates neutrophil degranulation and superoxide production (80).

Etanercept binds to soluble TNF and blocks the interaction of TNF with cell surface TNF receptors. Therefore, the drug competitively inhibits TNF binding to cell surface TNF II receptors, rendering TNF biologically inactive (81). Observational data suggested that use of Etanercept in patients with both rheumatoid arthritis and COPD reduced hospitalisation risk (82). Subsequently, Aaron and colleagues conducted a randomised placebo controlled trial to establish if etanercept improved lung function and decreased treatment failure rates compared corticosteroids (83). Both groups received oral antibiotics and bronchodilators as part of standard therapy. Unfortunately, Etanercept failed to demonstrate any improved clinical efficacy compared to standard treatment with prednisone with respect to change in FEV_1 from baseline. Furthermore, the non-statistically significant differences observed in the trial tended to favour the prednisolone treated group, the relative risk of 90-day treatment failure being greater (by 25%) in those randomised to Etanercept.

CXCR2 antagonists

As outlined previously, neutrophils are one of the key inflammatory cells in COPD, and a recent trial examined whether targeting neutrophilic inflammatory pathways using the CXC receptor antagonist MK-7123 could improve lung function in stable COPD patients (84). CXC chemokines recruit neutrophils to the lungs. These factors are recognized by receptors (CXCR1 and CXCR2) on neutrophils (85, 86). MK-7123 is a CXC receptor antagonist with high affinity for CXCR2, the receptor responsible for neutrophil trafficking (87). MK-7123 had previously been shown to reduce neutrophilia in animal (87, 88) and human (89) models of pulmonary inflammation.

Therefore the investigators performed a 6-month, randomized, double-blind, placebo-controlled trial, comparing three doses of MK-7123 with placebo in patients with moderate to severe COPD already receiving standard therapy (84). After 6 months, the highest dose of MK-7123, 50 mg daily, increased post-bronchodilator FEV_1 significantly relative to placebo. The effects of the two lower doses were not significant. Furthermore, compared with placebo, treatment with MK-7123 50 mg resulted in a significant reduction of sputum neutrophils at 3 months and a trend toward reduction at 6 months.

However, use of the higher effective dose resulted in severe serum neutropaenia, and 18% of subjects treated with the highest dose had to be withdrawn from the trial. These side effects therefore preclude current use of this agent in clinical practice. Nevertheless, this study adds further weight to the argument that targeting neutrophilic inflammatory pathways can lead to clinical benefit in COPD patients.

Roflumilast

Roflumilast is a phosphodiesterase 4 (PDE4) inhibitor, a non-steroid, anti-inflammatory agent designed to target both the systemic and pulmonary inflammation associated with COPD. The mechanism of action is the inhibition of PDE4, the major cyclic adenosine monophosphate (cAMP)-metabolising enzyme in structural and inflammatory cells important in the pathogenesis of COPD. Inhibition of PDE4 increases intracellular cAMP and typically leads to an anti-inflammatory effect (90).
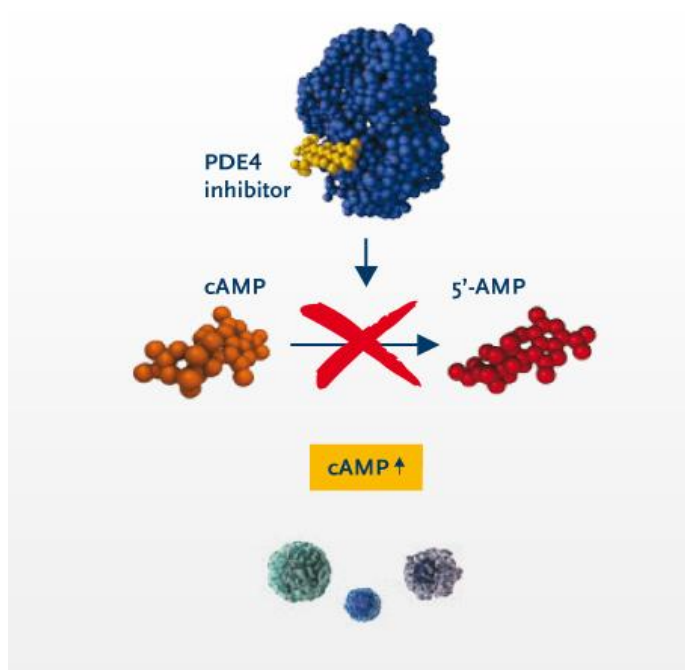
Figure 1.3. Effect of PDE4 inhibition on inflammatory cell activity. Reproduced from (70).

In vivo, roflumilast reduces tobacco smoke-induced lung inflammation, mucociliary malfunction, lung fibrotic and emphysematous remodelling, oxidative stress, pulmonary vascular remodelling and pulmonary hypertension (91). In vitro, roflumilast N-oxide has been demonstrated to affect the functions of many cell types, including neutrophils, monocytes/macrophages, CD4+ and CD8+ T-cells, endothelial cells, epithelial cells, smooth muscle cells and fibroblasts (91). In particular, roflumilast and roflumilast N-oxide potently inhibit neutrophil recruitment, chemotaxis and adhesion (91).

COPD exacerbations are associated with an increased neutrophilic inflammation (38) which is relatively insensitive to corticosteroids. In contrast to corticosteroids, roflumilast has been shown to reduce neutrophilic airway inflammation in COPD patients (92). This suppression of inflammation has been shown to improve key clinical outcomes in trials of COPD patients.

In two double-blind, multicentre studies, compared with placebo, roflumilast was found to consistently improve lung function when taken daily for 24 weeks in patients treated with salmeterol, and in those treated with tiotropium (93). Furthermore, in two placebo-controlled, double-blind, multicentre trials including COPD patients with severe airflow limitation, bronchitic symptoms, and a history of exacerbations, roflumilast reduced the rate of moderate (corticosteroid treated) but not severe (hospitalised) exacerbations. Although in this study patients were not on maximal preventative therapy, and were only taking longacting $\beta2$ agonists since those taking inhaled corticosteroids and longacting anticholinergic drugs were not allowed during the study (94). More recently, roflumilast has been shown to reduce rates of moderate (oral antibiotic and/or corticosteroid treated) exacerbations and hospital admissions in patients with severe COPD, chronic bronchitis and a history of frequent exacerbations, taking triple preventative therapy consisting of a fixed inhaled corticosteroid plus longacting $\beta2$ agonist combination, and background tiotropium (95).

Additionally, roflumilast has been shown to reduce endotoxin-induced inflammation in healthy volunteers (96). Bronchoalveolar lavage following segmental endotoxin challenge after 28 days treatment found that the influx of total cells was 36% lower in the subjects receiving roflumilast compared to placebo. The influx of neutrophils and eosinophils of roflumilast-treated subjects was also 39% and 74% lower than with placebo, respectively. Thus, roflumilast should be beneficial in the acute treatment of COPD exacerbations.

Therefore in the final section of this thesis I shall describe the TREAT (**T**reatment with **R**oflumilast at **ExA**cerba**T**ion) study: a novel, proof-of-mechanism trial to investigate if roflumilast can reduce exacerbation severity. Specifically in this study I examined whether roflumilast can ameliorate neutrophilic airways inflammation during exacerbations. Sputum neutrophils increase at exacerbation and are related to exacerbation severity, as measured by reduction in lung function, regardless of the presence of bacterial or viral infection (97). Furthermore, sputum neutrophil measurements can be safely and successfully obtained from COPD patients (98, 99), are reproducible (98) and therefore have been proposed as an effective biomarker to assess the effectiveness of novel therapies in early phase COPD trials (100), particularly those drugs that target neutrophils in the airways, such as Roflumilast (92).

In addition, during this trial we have examined the potential benefit of roflumilast on the recovery period of exacerbations, measuring the length and the severity of exacerbations using patient diaries and the CAT and EXACT patient reported outcome tools. The results of this study are described in depth in **Chapter 7**.

2

HYPOTHESIS AND AIMS

The introduction to this thesis has highlighted the current paucity of tools to assess exacerbation severity and the lack of treatments to improve clinical outcomes following exacerbation. To address these important unmet needs this thesis aimed to address the following general hypotheses:

1) Patient symptoms can be used to accurately monitor exacerbation severity and recovery

2) Inflammation is a key driver and indicator of exacerbation intensity, measurements of which can be used to predict important clinical outcomes

3) Reduction of inflammation at exacerbation by novel pharmacotherapy will decrease exacerbation severity.

Specifically, we sought to address the following key questions:

- Can patient reported outcomes, specifically the CAT and EXACT questionnaires, be used to monitor COPD exacerbation severity and recovery? (Chapter 4)

- What is the relationship between exacerbations diagnosed using validated London COPD cohort diary cards and physician review, and symptom-defined events captured by the EXACT patient reported outcome? (Chapter 4)

- Is objective cough monitoring an accurate means of determining the severity of an exacerbation and can this method identify patients at risk of prolonged recovery and recurrence? (Chapter 5)

- Can airway and systemic inflammatory biomarkers measured at onset or during the recovery phase of a COPD exacerbation predict prolonged recovery and recurrence? (Chapter 6)

The final part of this thesis describes a randomised, placebo controlled trial to test the hypothesis that Roflumilast reduces neutrophilic inflammation during exacerbations of COPD when added to the usual care of these patients. This study serves as a proof-of-mechanism trial to examine whether roflumilast can improve clinical recovery following COPD exacerbations. In addition, the potential benefit of roflumilast to reduce the length and severity of exacerbations has been assessed using patient diaries and the CAT and EXACT PROs (Chapter 7).

The methodology employed to investigate these questions is the subject of the following chapter.

3

METHODOLOGY

3.1   Subject recruitment

Patients enrolled in the MRC (Patient Research Cohorts Initiative) London COPD cohort were recruited for this study. The patients form part of a rolling cohort of approximately 200 patients used to prospectively investigate the mechanisms of COPD exacerbations (11). Patient recruitment was performed from both primary and secondary care settings to ensure a range of disease severity. Patients were allowed to leave the study at any point following recruitment and to ensure confidentiality, each subject was assigned with a unique study number. Ethical approval for the study was granted from the Royal Free Hospital research ethics committee (Ref. 09/H0720/8) and all patients gave written informed consent.

Inclusion criteria

Patients were included if the post-bronchodilator forced expiratory volume in 1 second (FEV_1) was ≤100% predicted from age, height, and sex and FEV_1/forced vital capacity (FVC) ratio was <0.7, in keeping with GOLD grades I-IV (101).

Exclusion criteria

Patients were excluded if they had a history of asthma, primary bronchiectasis or any other significant respiratory diseases. Patients unable to complete the daily symptoms diary cards (described below) were also excluded. Specific additional exclusion criteria were also specified for the TREAT randomised controlled trial (see methods section 3.5).

Clinical measurements

Clinical measurements were performed by either by myself, or by the research nurse and clinical research fellows who are part of the London COPD research team.

Spirometry

Spirometry was performed on study participants at all clinic visits. FEV_1 and FVC were measured in accordance with ATS/ERS guidelines using a Vitalograph Gold Standard spirometer (Vitalograph Ltd, Maids Moreton, UK) except for during the TREAT trial when patients used a FlowScreen®CT (ERT®, Philadelphia, PA, USA). Values were taken as the best of three reproducible attempts, and are expressed either as absolute volume or as a percentage predicted based on sex, weight and height (using Quanjer tables).

Health assessment questionnaires

At annual review, the St George's Respiratory Questionnaire (SGRQ) (102), the Medical Research Council (MRC) dyspnea score, and the patient reported outcome (PRO) questionnaires; the COPD Assessment Test^{TM} (CAT) and the Exacerbations of Chronic Obstructive Pulmonary Disease Tool (EXACT) were completed. For specific studies these PROs were also completed at all other clinic visits, including 3-monthly stable visits, at exacerbation and exacerbation-follow-up. Further details of the use of these patient reported outcome is detailed in section 3.2.

Diary cards

At recruitment, patients were taught how to record on daily symptom diary cards, and provided with written instructions for filling these in (**Figure 3.1** and **Figure 3.2**).

NAME

Study Number

THE LONDON COPD STUDY

Jan 2011

WORSENING SYMPTOMS? CALL US

NEXT APPOINTMENT / /11 . am

DATE	1 sat	2 sun	3 mon	4 tues	5 wed	6 thurs	7 Fri	8 Sat	9 Sun	10 Mon	11 Tues
Peak Flow											
CHANGE in Symptoms											
CHANGE in Treatment											
Hours out of the home											

DATE	12 wed	13 thur	14 Fri	15 Sat	16 Sun	17 Mon	18 Tues	19 Wed	20 thur	21 Fri	22 Sat
Peak Flow											
CHANGE in Symptoms											
CHANGE in Treatment											
Hours out of the home											

DATE	23 Sun	24 Mon	25 Tue	26 Wed	27 Thurs	28 fri	29 Sat	30 Sun	31 Mon
Peak Flow									
CHANGE in Symptoms									
CHANGE in Treatment									
Hours out of the home									

Figure 3.1. Front of Symptom Diary Card

Instructions for filling in the DIARY CARDS

EVERY DAY…

1. After taking morning medications record the best of 3 attempts at the PEAK FLOW blowing test in the box on the sheet.
2. Please record any **WORSENING** of symptoms **ABOVE YOUR USUAL** daily level. The symptoms we are interested in are listed below, just put the appropriate letter in the box on the sheet. Continue recording until the symptom has gone away or got back to the level you consider 'normal'.

Letter	Symptom
A	increased BREATHLESSNESS.
B1	increased SPUTUM COLOUR.
B2	increased SPUTUM AMOUNT.
C	a COLD (such as a runny or blocked nose).
D	increased WHEEZE or CHEST TIGHTNESS.
E1	SORE THROAT.
E2	increased COUGH.
F	FEVER.

If you experience a worsening in any of these symptoms please phone us to arrange an assessment visit, and do this BEFORE starting any antibiotic or steroid tablets. The phone number is ✱✱✱✱✱✱✱✱✱✱✱✱.

Anant or Alex will have the phone and we can usually arrange to see you later the same day.

Please phone if you are not sure what to write down or you have any questions.

3. Please record any **CHANGE** to your usual treatment for as many days as it applies. Again, just put the appropriate letter in the box on the sheet.

Letter	Treatment
H	I am in Hospital.
I	I am taking more than usual INHALED STEROID (red / brown/purple)
R	I needed to take extra RELIEVER (blue / green / grey / nebuliser). **HOW MANY PUFFS?** Write, eg 'R3' for 3 puffs, 'R2' for 2 etc
S	I am taking STEROID (Prednisolone) TABLETS. **HOW MANY TABLETS?** Write, eg 'S6' for 6 tablets, 'S5' for 5 etc
X	I am taking ANTIBIOTIC TABLETS. **PLEASE RECORD WHICH** (write the name on the diary card).

4. Finally, please estimate the time that you were out of your own home on the previous day.

Figure 3.2. Back of Symptom Diary Card including completion instructions.

Patients were asked to record their daily morning post-bronchodilator peak expiratory flow rate (PEFR), recording the best of three attempts (using a Mini-Wright peak flow meter, Clement Clarke International Ltd, Harlow, UK). In addition, there were asked to record any increase in their usual respiratory symptoms using a letter-annotated system (Table 1), and hours spent outside the home. Patients were instructed not record any symptoms that they normally experience when they are well or stable, but only to document any perceived increase in symptoms over their normal, stable condition.

Letter	Major or Minor	Symptom/Symptom Change
A	Major	Increased breathlessness
B1	Major	Increased sputum colour
B2	Major	Increased sputum amount
C	Minor	A cold (e.g. runny or blocked nose)
D	Minor	Increased wheeze/chest tightness
E1	Minor	Sore throat
E2	Minor	Increased cough
F	Minor	Fever

Table 3.1. Letter-annotated system to record any increase in patients' usual respiratory symptoms, either major or minor, on daily diary cards.

Example: if a patient has symptoms of a cold, 'C' should be recorded on the diary card. If on the subsequent day the patient has both cold and increased breathlessness symptoms, then 'C' and 'A' should be recorded.

Patients were asked to contact the study team, via a dedicated mobile telephone number, if they experienced a worsening in any symptoms to arrange a clinic assessment visit, and to do this before starting any antibiotic or steroid tablets.

Diary card definition of stable state

Stable state was defined as those patients without evidence of symptom-defined exacerbations in the preceding 4 weeks and the subsequent 2 weeks post-clinic visit.

Diary card definition of exacerbations

An exacerbation was defined using previously validated symptomatic criteria (11) as an increase for two consecutive days in respiratory symptoms, with at least one major symptom (dyspnoea, sputum purulence or sputum volume) plus either another major or a minor symptom (wheeze, cold, sore throat, and cough), the first of which was defined as the day of onset of the exacerbation. Symptom counts were obtained by summating each increased respiratory symptom recorded on diary cards per day.

Exacerbation duration was defined as the number of days after onset that worsening symptoms persisted. The last day of recorded worsening symptoms before two consecutive symptom-free days defined the end of the exacerbation. Exacerbation recovery was not determinable if patients failed to record diary card symptoms or continuously recorded symptoms for more than 99 days after onset.

Exacerbations were classified as 'physician reported exacerbations' – those seen by the study clinical team or the patient's general practitioner and 'unreported exacerbations' – those unseen by physicians but recorded on diary cards.

Recording of exacerbation treatment on diary cards

Patients with any worsening in their daily symptoms were asked to record any changes to their usual treatment, using a letter-annotated system, as shown below.

Letter	Treatment
H	Admitted to hospital
I	Increased use of inhaled corticosteroids (red/brown/purple inhalers)
R	Increased reliever medication and no. of puffs
S	Use of steroid tablets and no. taken
X	Use of antibiotic tablets and name

Table 3.2. Letter-annotated system to record any changes to patients' usual treatment on daily diary cards.

<u>**Clinic visits schedule**</u>

Recruitment and annual review

At recruitment and annual review, a full medical and smoking history was obtained, and clinical examination performed, including measurement of oxygen saturations (PureSAT®, Nonin Medical Inc, Plymouth, MN, USA). Body mass index (BMI) was calculated from

height and weight. Completion of health assessment questionnaires were requested. Comorbid diagnoses were established using clinical history and examination findings, supported where appropriate with a review of the available medical records. Medication history was reviewed and where necessary, treatment optimised. Patients were taught, or re-educated, on completing daily symptom diary cards. Spirometry was performed and sputum and venous blood samples were collected.

Stable visits

Following recruitment, patients were regularly seen at 3-monthly intervals when stable. Diary cards, medical history and concurrent medication were reviewed to ensure the patient was stable before spirometry was performed. Patients were requested to complete health questionnaires, and sputum and venous blood samples were collected.

Exacerbation visits

Patients contacted the study team for review if they experienced any worsening of their respiratory symptoms and prior to commencing any additional systemic therapy. Patients were only included if they presented within 7 days of exacerbation symptom onset and had not yet taken any systemic therapy, such as antibiotics or oral corticosteroids prior to assessment. Exacerbations were treated according to the prevailing guidelines and clinical judgment with increased inhaled therapy, antibiotics and/or oral steroids. When patients attended for an exacerbation, medical assessment was performed by one of the clinical research team. Spirometry was performed and patients were requested to complete the

relevant questionnaires. Sputum and venous blood samples were taken prior to commencing exacerbation treatment.

Exacerbation follow-up visits

Patients were reviewed in clinic at day 3, one week, two weeks and five weeks following presentation for exacerbation. Clinical assessment was performed, and diary cards were reviewed to determine exacerbation end date, and if ongoing symptoms were present, together with clinical history, to determine whether re-treatment was clinically recommended. Spirometry was performed, patients were requested to complete the relevant health questionnaires, and sputum and venous blood samples were taken.

Calculation of exacerbation frequency

Exacerbation frequency was calculated for each patient using diary card data obtained between recruitment and onset of the sub-study. For recently recruited patients with less than one year diary data, exacerbation frequency was based on the number of exacerbations the patient recalled for the year prior to recruitment. Previous work has shown a good correlation between the number of exacerbations recorded on diary cards and the number of exacerbations remembered by the patient over the same 1 year period (103) and has shown that exacerbation frequency represents a stable patient phenotype (7).

3.2   Patient Reported Outcomes

3.2.1 COPD Assessment Test (CAT)

The COPD Assessment Test[TM] (CAT) is a validated 8-item questionnaire. Each item is graded on a six-point scale from zero ("no symptoms") to five ("very severe symptoms"). It is short and simple for patients to complete, providing a score out of 40 to indicate disease impact, without the need for complex calculation (**Figure 3.3**). Permission to use the CAT questionnaire was obtained from GlaxoSmithKline (GlaxoSmithKline plc, Middlesex, UK).

Your name: _____   Today's date: _____   **CAT**
COPD Assessment Test

How is your COPD? Take the COPD Assessment Test™ (CAT)

This questionnaire will help you and your healthcare professional measure the impact COPD (Chronic Obstructive Pulmonary Disease) is having on your wellbeing and daily life. Your answers, and test score, can be used by you and your healthcare professional to help improve the management of your COPD and get the greatest benefit from treatment.

For each item below, place a mark (X) in the box that best describes you currently. Be sure to only select one response for each question.

Example: I am very happy   (0) (✗) (2) (3) (4) (5)   I am very sad

SCORE

I never cough	(0) (1) (2) (3) (4) (5)	I cough all the time	☐
I have no phlegm (mucus) in my chest at all	(0) (1) (2) (3) (4) (5)	My chest is completely full of phlegm (mucus)	☐
My chest does not feel tight at all	(0) (1) (2) (3) (4) (5)	My chest feels very tight	☐
When I walk up a hill or one flight of stairs I am not breathless	(0) (1) (2) (3) (4) (5)	When I walk up a hill or one flight of stairs I am very breathless	☐
I am not limited doing any activities at home	(0) (1) (2) (3) (4) (5)	I am very limited doing activities at home	☐
I am confident leaving my home despite my lung condition	(0) (1) (2) (3) (4) (5)	I am not at all confident leaving my home because of my lung condition	☐
I sleep soundly	(0) (1) (2) (3) (4) (5)	I don't sleep soundly because of my lung condition	☐
I have lots of energy	(0) (1) (2) (3) (4) (5)	I have no energy at all	☐

TOTAL SCORE   ☐

Figure 3.3. The COPD Assessment Test (CAT)

CAT administration

For the study described in **Chapter 4.1**, patients completed the CAT at least once under supervision in clinic and then at home, based on their symptoms experienced on the day of completion. Patients completed at least one CAT questionnaire in the stable, baseline state. Baseline occurred more than 35 days post- and 21 days pre- exacerbation onset. If unavailable pre-index exacerbation, CAT scores during periods of stability post-exacerbation were used to provide a baseline. No differences were seen between baseline scores obtained pre-index exacerbation and baseline scores post-exacerbation. Repeat scores were averaged to give a baseline CAT score. CAT questionnaires were also administered during exacerbation between April 2010 and June 2011. The exacerbation CAT score took place within 7 days of the symptomatic onset of the exacerbation as judged by diary cards, was completed prior to starting therapy and was recorded on the day treatment commenced. These were mandatory study criteria.

A subgroup of patients also completed CAT scores on a daily basis during their recovery. For the recovery subgroup, the first exacerbation was selected for analysis provided the patient had fully completed the questionnaire on at least 21 of 35 days post onset. CAT Recovery was the time taken from exacerbation onset for the CAT score to return to baseline value (**Figure 3.4**).
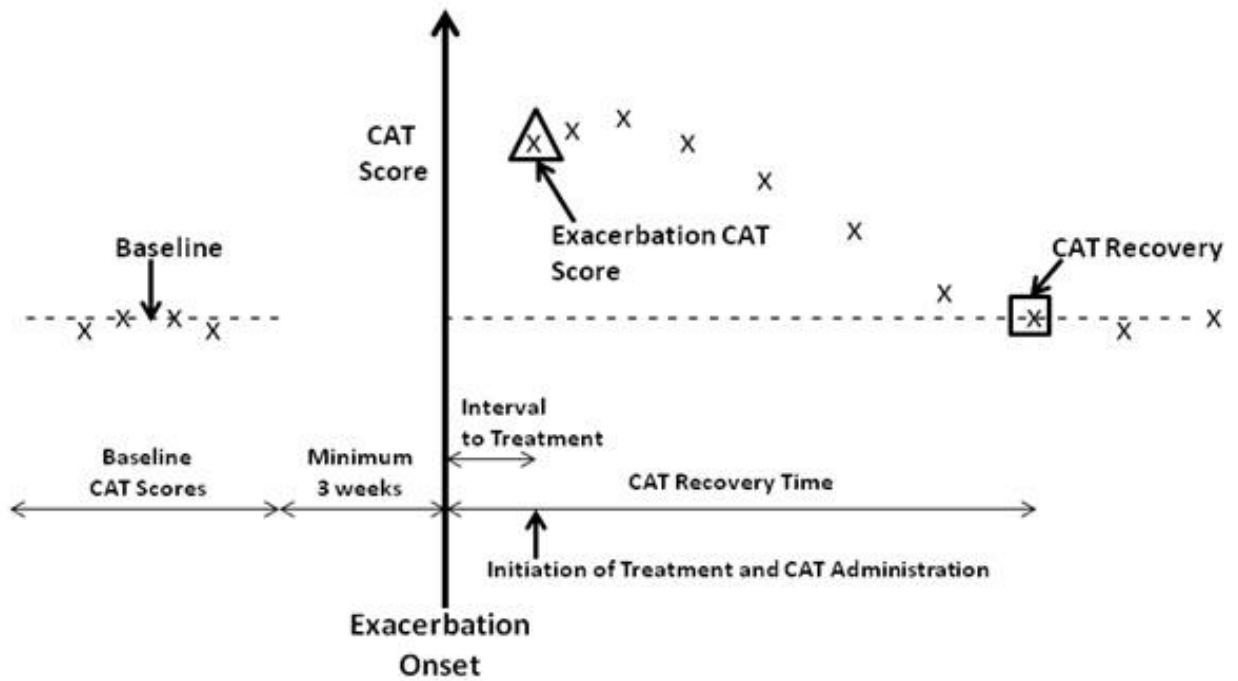
Figure 3.4. Schematic timeline of CAT scores (x) recorded during exacerbation. Exacerbation CAT score (△) was the CAT score recorded on the day treatment commenced. CAT Recovery □ was the time taken from exacerbation onset for the CAT score to return to baseline value. This figure is for illustrative purposes only and does not indicate real data.

3.2.2 Exacerbations of Chronic Pulmonary Disease Tool (EXACT)

The EXACT consists of 14-items (**Figure 3.5**). To calculate the EXACT score, responses for each of the 14 questions are summed to yield a "raw summed score". Each "raw summed score" is converted to a 0 to 100 score using a simple conversion table, with higher scores indicating more severe symptoms. Permission to use the EXACT questionnaire was obtained from United BioSource Company (UBC, Bethesda, MD, USA).

Your name:	Today's date:

EXACT DIARY

Please complete this diary *every evening*, just before you go to bed. Please check the box ☐ that best describes your experience *today*. If you have any questions or problems, please call 07762038662. Thank you!!

1. Did your chest feel congested today?
Not at all.. ☐
Slightly .... ☐
Moderately .... ☐
Severely .... ☐
Extremely.. ☐

2. How often did you cough today?
Not at all .... ☐
Rarely .... ☐
Occasionally..................................... ☐
Frequently .... ☐
Almost constantly .... ☐

3. How much mucus (phlegm) did you bring up when coughing today?
None at all .... ☐
A little .... ☐
Some .... ☐
A great deal .... ☐
A very great deal .... ☐

4. How difficult was it to bring up mucus (phlegm) today?
Not at all .... ☐
Slightly .... ☐
Moderately.. ☐
Quite a bit .... ☐
Extremely .... ☐

5. Did you have chest discomfort today?
Not at all .... ☐
Slight .... ☐
Moderate... ☐
Severe .... ☐
Extreme .... ☐

6. Did your chest feel tight today?
Not at all... ☐
Slightly .... ☐
Moderately .... ☐
Severely .... ☐
Extremely .... ☐

7. Were you breathless today?
Not at all... ☐
Slightly .... ☐
Moderately .... ☐
Severely .... ☐
Extremely .... ☐

8. Describe how breathless you were today:
Unaware of breathlessness.................. ☐
Breathless during strenuous activity...... ☐
Breathless during light activity............... ☐
Breathless when washing or dressing.... ☐
Present when resting.......................... ☐

9. Were you short of breath today when performing your usual personal care activities like washing or dressing?
Not at all .... ☐
Slightly .... ☐
Moderately ..... ☐
Severely ....☐
Extremely .... ☐
Too breathless to do these☐

10. Were you short of breath today when performing your usual indoor activities like cleaning or household work?
Not at all......... ☐
Slightly .... ☐
Moderately ..... ☐
Severely ....☐
Extremely .... ☐
Too breathless to do these☐

11. Were you short of breath today when performing your usual activities outside the home such as yard work or errands?
Not at all ....☐
Slightly ....☐
Moderately ......☐
Severely ....☐
Extremely .... ☐
Too breathless to do these☐

12. Were you tired or weak today?
Not at all .... ☐
Slightly ....☐
Moderately ......☐
Severely...........☐
Extremely........ ☐

13. Last night, was your sleep disturbed?
Not at all .... ☐
Slightly ....☐
Moderately ......☐
Severely ....☐
Extremely .... ☐

14. How scared or worried were you about your lung problems today?
Not at all .... ☐
Slightly ....☐
Moderately...... ☐
Severely ....☐
Extremely .... ☐

Please complete every question

Figure 3.5. The Exacerbations of Chronic Pulmonary Disease Tool (EXACT)

EXACT administration

For the study described in **Chapter 4.2**, patients completed a paper version of the EXACT at least once under supervision in clinic and were instructed to complete the EXACT diary each evening before bedtime, based on their symptoms experienced that day. Patients prospectively completed the EXACT on a daily-basis when stable and continued long-term to enable capture of the exacerbation prodrome, the onset of the event, its nadir and recovery. Patients in the analysis completed at least one EXACT at both the symptomatic onset of an exacerbation and during a baseline period -14 to -8 days before onset. The median number of EXACT questionnaires completed per person was 196 (IQR 106-311).

EXACT Scoring Definitions

Each patient's baseline value was represented by the mean value recorded during the stable state (median 7 days (IQR 7-7) of data recorded during baseline period). As specified in the User Manual, EXACT events were defined as an increase of 12 points above the patient's mean baseline for 2 consecutive days **OR** an increase of 9 points above patient mean baseline for 3 consecutive days. The presence of either constitutes onset of an event. For the purposes of this study, EXACT recovery was defined as the time taken from exacerbation onset for the EXACT score to return to baseline value.

Exacerbation Severity

Total net EXACT recovery score of an exacerbation was created to give a novel measure of exacerbation severity which reflects the overall symptomatic intensity of an exacerbation. Total net EXACT recovery score of an exacerbation was calculated as the sum of daily mean change from baseline EXACT scores from exacerbation onset to symptomatic resolution as judged by London COPD cohort diary cards (**Figure 3.6**).

Figure 3.6. Illustration of calculation of Total Net EXACT Recovery Score. Daily mean changes from baseline EXACT scores are represented by dashed arrows and Total Net Recovery Score is represented by the grey shaded area.
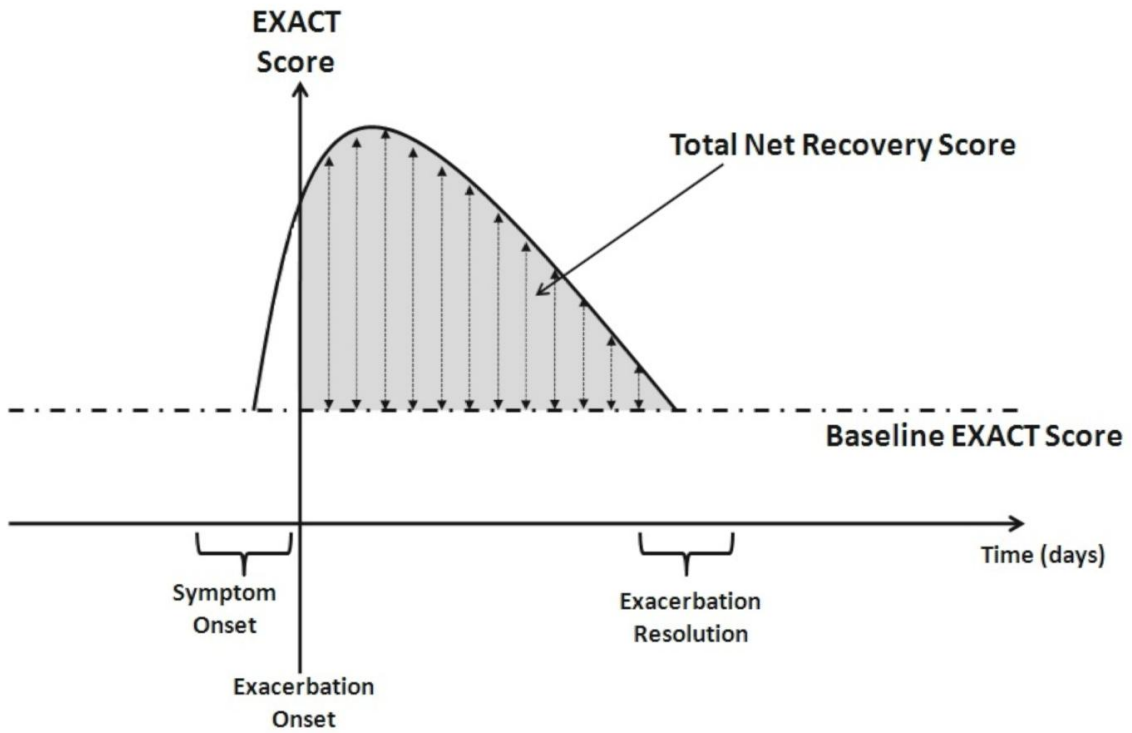
3.3   Monitoring COPD Exacerbation Recovery using Objective Cough Frequency Measurements

Cough monitoring

Ambulatory cough sound monitoring was performed using the VitaloJAK model 7100 cough monitor (**Figure 3.7**, Vitalograph, UK). This a custom-built device attached to a lapel microphone and a chest wall sensor positioned over the sternum (**Figure 3.8**). Devices were supplied by the University of South Manchester under the supervision of Dr Jaclyn Smith. I was trained in the use of this device and its accompanying software by staff at the University of Manchester.

Figure 3.7: VitaloJAK Cough Monitor   **Figure 3.8:** Placement of cough sensor
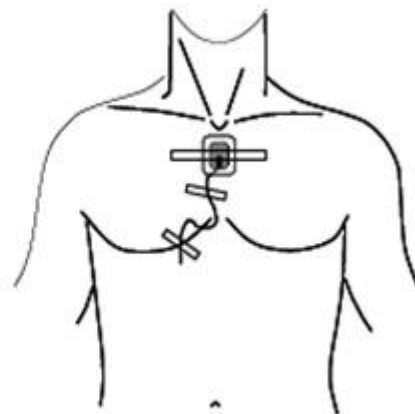
Cough monitors were placed on patients at clinic visits and worn for the immediate 24 hours after each visit. Recordings stored on data cards within the device were transferred securely via Filezilla data transfer systems to a separate computer system. Subsequently, recordings were processed by validated software that removes silences and background noise but retains the coughs present (104). The resulting compressed version of the recording was listened to by staff at the University of South Manchester, who were specifically trained to identify and count coughs, and tags placed on each explosive cough sound. University of South

Manchester in house software extracts the tags to generate all the coughs from the original 24 hour recording then creates a standard hourly report. To ensure consistency, the same counter tagged all recordings for one patient. Furthermore, 10% of each cough counters work is quality control checked by a senior member of staff, using pre-defined acceptable limits of agreement based on 95% confidence intervals from a collection of previous data.

3.4 Systemic and Airway biomarkers to evaluate COPD exacerbation recovery and recurrence

Classification of Exacerbations

Exacerbations are categorized into three types: ''isolated,'' ''initial,'' and ''recurrent''. An isolated exacerbation was not followed within 8 weeks by any other exacerbation. An initial exacerbation was an exacerbation that was followed within 8 weeks of onset by the onset of a second exacerbation. A recurrent exacerbation was an exacerbation that had an onset within 8 weeks of a preceding exacerbation. All exacerbations were separated by at least 5 days on which no symptoms were recorded (**Figure 3.9**)(15).
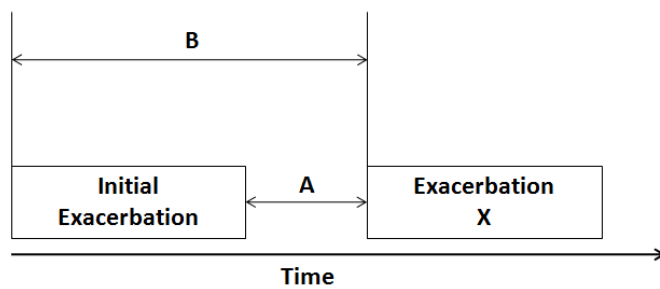
Figure 3.9. Classifications of Exacerbations. Exacerbation X is a ''recurrent'' exacerbation if onset is within 8 weeks of onset of the index exacerbation (B $\leq$ 56 days). Recovery is defined as $\geq$5 symptom-free days (A).

Recovery Visits

Patients were asked to attend recovery monitoring visits at Days 3 ($\pm$1), 7 ($\pm$2), 14 ($\pm$2), 35 ($\pm$3) after the initial exacerbation visit. Recurrent exacerbations occurring within 56 days after exacerbation onset were assessed and sampled in the same manner as for an index exacerbation, as described above.

Sputum sampling and processing

Patients were asked to spontaneously expectorate sputum samples into a sterile pot, after rinsing their mouth. Sputum samples were kept at 4°C for no longer than 2 hours prior to further processing to prevent RNA degradation. Sputum plugs were separated from any saliva macroscopically. Sputum plugs were homogenised with Dulbecco's phosphate buffered saline (D-PBS), as previously published (48, 105). Selected sputum plugs were transferred into an empty, pre-weighed polypropylene centrifuge tube. The weight of the sputum was calculated (weight of tube and sputum − weight of the empty centrifuge tube). Eight volumes x sputum weight (in grams) of D-PBS was added, and the sputum dispersed by repeated gentle aspiration into a plastic pipette.

Approximately 0.5ml of glass beads (Glass Balls 2.5-3.5mm. VWR International Ltd Cat No. 33212 4G) was added and the tube was vortexed for 15 seconds (Whirlimixer IKA-Vibrax-VXR, Scientific & Chemical Supplies Ltd.) and then subsequently for 15 minutes on a bench rocker (Voltex Mixer, Bench rocker (variable speed) Scientific & Chemical Supplies Ltd.). The centrifuge tube was re-vortexed for further 15 seconds. A 500μl aliquot of the homogenized sputum samples was stored in 1.5ml Eppendorf tubes and frozen at -80°C for later batch analysis for qPCR.

The remaining homogenized sputum was filtered through a nylon filter mesh (Plastok (Mesh and Filtration) Ltd, Merseyside, UK), and centrifuged at 790g for 10 minutes with the brakes off (Benchtop refrigerated centrifuge. Hettich Universal 380 R. Wolf Laboratories Ltd.). 500μl aliquots of the sputum supernatant were stored in 1.5ml Eppendorf tubes, taking care

not to disturb the cell pellet. The aliquots were frozen at -80°C for later batch analysis for sputum biomarkers.

Quantitative PCR for typical bacteria and Human Rhinovirus

Multiplex bacterial polymerase chain reaction (PCR) was performed for the most prevalent bacterial pathogens in exacerbated COPD: *Haemophilus influenzae, Streptococcus pneumoniae*, and *Moraxella catarrhalis* (40) by the Centre for Clinical Microbiology, University College London. The lower limit of detection for this technique was 1×10^4 colony-forming units per millilitre. Reverse transcriptase PCR for human rhinovirus, the most prevalent virus at COPD exacerbation, was also performed (47). The sensitivity of this technique was also high with a lower limit of detection of 10.23 plaque-forming units per millilitre.

Blood sampling and processing

Full Blood Count

3ml of whole blood was collected from study participants by venepuncture in Vacutainer® EDTA tubes for total white blood cell count and differential neutrophil and eosinophil counts.

Serum for CRP

3.5ml of whole blood was collected from study participants by venepuncture in Vacutainer® SST tubes, containing spray-coated silica and a polymer gel for serum separation. Serum C-reactive protein (CRP) was measured using Modular Analytics E 170 Module (Roche, Burgess Hill, UK). The lower limit of detection was 1mg/l.

Plasma for fibrinogen

2.7ml of whole blood was collected by venepuncture in Vacutainer® Citrate tubes containing 3.2% buffered sodium citrate solution. Plasma fibrinogen was measured using the Clauss method (IL ACL Top Coagulation Analyzer, Lexington, MA, USA).

Serum for Cytokines

Venous blood was collected into a sterile vacutainer, centrifuged, and the serum stored at -80°C until batch analysis.

Cytokine Measurement

Levels of cytokines in serum (excluding those listed above) and sputum were measured using the Aushon multiplex immunoassay platform (Aushon BioSystems, Billerica, MA). The Aushon platform is a quantitative multiplexed sandwich ELISA containing up to 12 different capture antibodies spotted on the bottom of a 96-well polystyrene microtiter plate. Each antibody captures a specific protein present in the standards and samples added to the plate. The bound proteins are then detected with a biotinylated detection antibody, followed by the addition of streptavidin-horseradish peroxidase (HRP) and lastly, a chemiluminescent substrate. The luminescent signal produced from the HRP-catalyzed oxidation of the substrate is measured by imaging the plate using the Aushon Cirascan Imaging System, which is a cooled charge-coupled device (CCD) camera. The amount of luminescent signal produced is proportional to the amount of each protein present in the sample. Concentrations are interpolated from a standard curve. Aushon Bio systems were funded by Novartis Pharmaceuticals UK Ltd for this project.

Cytokine Selection

The following biomarkers were measured using the Aushon multiplex immunoassay platform:

Serum Biomarkers: IL-6, IL-8, IP-10, PLGF, TNFα, MMP-1, MMP-8, MMP-9, sRAGE, PARC, α2-macroglobulin, CC16, SAA

Sputum Biomarkers: IFNγ, IL-18, IL-1β, IL-5, IL-6, TNFα, IL-8, MMP-1, MMP-8, MMP-9, MPO, TIMP-1, α2-macroglobulin

3.5   Treatment with Roflumilast at ExAcerbaTion (TREAT)

The Treatment with Roflumilast at ExAcerbaTion (TREAT) study was a novel, proof-of-mechanism trial to investigate if roflumilast can reduce exacerbation severity. It was conducted as a randomised, double-blind, parallel-group, single centre, phase II trial.

Inclusion Criteria

All patients were recruited from the London COPD patient cohort at exacerbation (see methods section 3.1 for exacerbation definition). Patients were eligible for trial inclusion if presenting with an exacerbation with predominantly bronchitic symptoms, characterised by increased sputum volume or a change in sputum colour. This selection criterion was used because Roflumilast has been shown to be most effective in bronchitic patients during maintenance therapy trials. Patients were randomised 1:1 to either roflumilast 500 μg (standard maintenance therapy dose) or placebo, once daily for 4 weeks. As discussed in the introduction, 8 weeks post an initial exacerbation is a high risk period for exacerbation recurrence (15), and excess inflammation 14 days post exacerbation is associated with both non-recovery and recurrence (56). Therefore a 4 week period post exacerbation onset was chosen to both reduce exacerbation non-recovery and recurrence, whilst minimising exposure to adverse effects.

To measure the efficacy of Roflumilast in separate exacerbations within the same patient, repeated exacerbations from the same patient were included if the second event occurred at least 3 months after end of the initial 8 week exacerbation follow-up period. Therefore 2 groups were available for analysis:

1. Initial Approach-including just one exacerbation per patient

2. Extended Approach-repeat exacerbations within the same patient were also included

All patients continued their standard maintenance COPD therapy (except theophyllines, see below) and additionally received standard treatment for COPD exacerbations (oral corticosteroids for 10 days and antibiotics for 7 days), therefore the placebo treatment did not bear any additional risk and was thus ethically justified. All COPD severity stages were included, except for patients who developed type 2 respiratory failure during the exacerbation.

Exclusion Criteria

- Diagnosis of asthma and/or other relevant lung disease

- Recurrent exacerbations (within 8 weeks of a preceding exacerbation)

- Treatment of current exacerbation with oral corticosteroids and/or antibiotics already started at enrolment

- Treatment with PDE4 inhibitors within 3 months prior to Visit V0

- Contraindications to sputum collection or inability to obtain valid sputum sample for analysis

- Respiratory failure or hospitalisation required when presenting at Visit V0

- Severe psychiatric or neurological disorders

- History of depression associated with suicidal ideation or behaviour

- Congestive heart failure (NHYA Grade IV), haemodynamically significant cardiac arrhythmias or heart valve deformations

- Immunological diseases or known HIV infection

- Liver impairment Child-Pugh B &C and/or active viral hepatitis

- Severe infectious diseases

- Any diagnosis of a malignant disease (other than basal or cutaneous squamous cell carcinoma) within 5 years before trial start

- Alcohol or drug abuse within the past year

- Suspected hypersensitivity to the IMP or its ingredients

- Female patients of childbearing potential, not using and not willing to continue using a medically reliable method of contraception for the entire trial duration, such as oral, injectable, or implantable contraceptives, or intrauterine contraceptive devices, unless they are surgically sterilised/hysterectomised or post-menopausal >1 year

- Pregnancy, breast feeding, planned oocyte donation or oocyte implantation

- Planned donation of germ cells, blood, organs or bone marrow during the course of the trial

- Participation in another trial (use of investigational product) within 30 days of Visit V0

- Suspected inability or unwillingness to comply with trial procedures

- Suffering from any concomitant disease that might interfere with trial procedures or evaluations

- Employee at the trial site, relative or spouse of the investigator

- Use of disallowed concomitant medication:
  - Maintenance therapy with oral and parenteral corticosteroids Oral or transdermal β2-agonists
  - Theophylline containing products
  - Lipoxygenase inhibitors
  - Leukotriene antagonists

    o   Use of immunosuppressive medications within 4 weeks prior to enrolment (e.g. cyclosporine, methotrexate, tumour necrosis factor alpha receptor inhibitors or antibodies, gold, azathioprine)

    o   PDE4 inhibitors (with the exception of the IMP provided by the sponsor)

Clinical Trial Overview

Clinic visits were performed at Days 1 (=V0, inclusion and randomisation), 7 (Visit V1), 14 (Visit V2), 28 (Visit V3) and 56 (Follow-up Visit VFU) (**Figure 3.10**). During the entire trial period patients completed daily London COPD cohort symptom diary cards on which they recorded their morning post-medication peak expiratory flow (PEF) and any increase in their daily respiratory symptoms. At clinic visits pulmonary function tests were performed, and sputum and blood collected to assess airway and systemic inflammatory biomarkers.
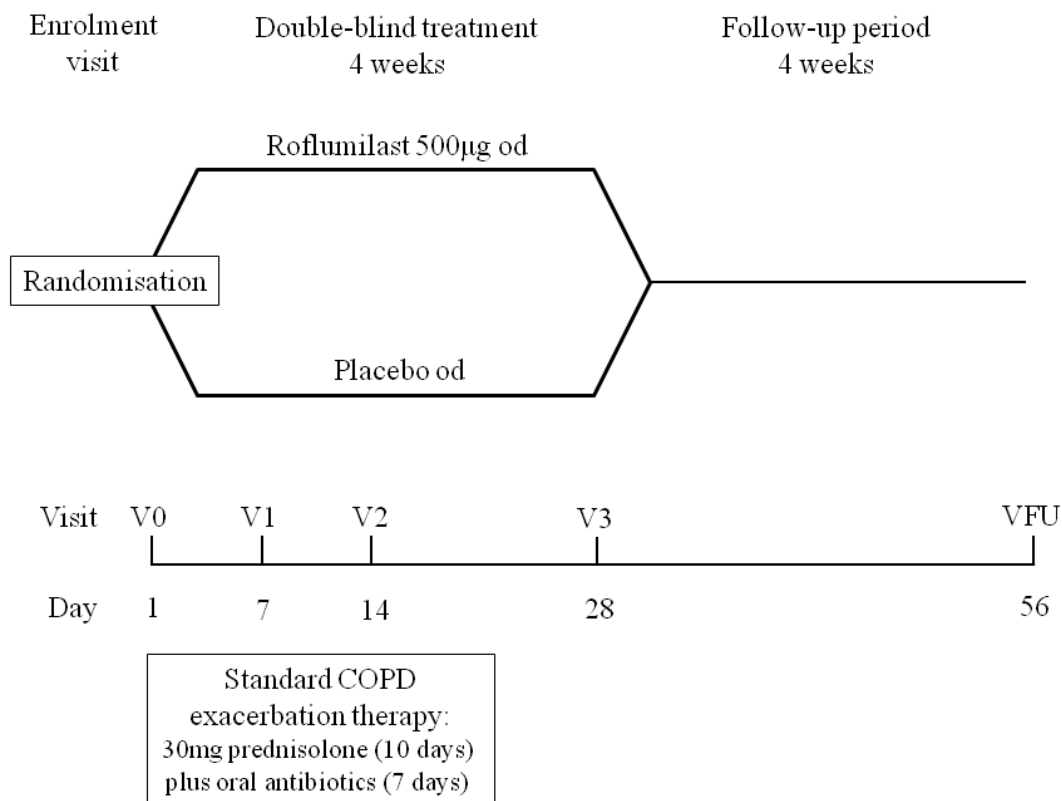
Figure 3.10. Clinical trial overview.

Blinding

During the double-blind treatment period and until database hard lock, all parties involved in the trial (patients, investigators, site personnel, monitors, sponsor) were blinded. Roflumilast and placebo tablets were of identical appearance, shape and colour (both yellow, triangular tablets embossed with a 'D') and had identical labelling and packaging.

Sputum induction and processing

Sputum induction was performed by inhalation of 5ml 3% (v/v) saline aerosol performed using an ultrasonic nebuliser (output approximately 2ml/min), in three, five-minute cycles (106). After the initial 5 minutes, FEV_1, and if clinically indicated, oxygen saturations were re-measured. If FEV_1 fell by <10%, the nebulisation was continued with 3% (v/v) saline. If FEV_1 fell by >20%, or if the participant experienced any distressing symptoms, the process was discontinued. Study participants were allowed to expectorate sputum at any point during the induction process. Prior to expectoration, patients were requested to blow their nose and rinse their mouth out with water before attempting sputum expectoration.

Samples were examined within 2 hours of collection. Sputum plugs were separated from contaminating saliva by macroscopic examination and selected portions of the sputum processed with dithiothreitol (DTT) for measurements of cell counts. For measurements of inflammatory markers and bacterial and viral quantitative polymerase chain reaction (PCR), sputum samples were processed without DTT (as per methods section 3.4).

Total and differential cell counts

Total cell count (absolute number of nonsquamous cells per gram of the original sputum sample) and cell viability was determined with a Neubauer haemocytometer and the trypan blue exclusion method. For differential cell counts, aliquots of a cell suspension prepared from the sputum sample were used to prepare cytospin slides. Cytospin slides were stained with Diff-Quik to obtain differential cell counts by counting 400 nonsquamous cells per slide (107). Absolute cell numbers were calculated as (% cell x total cell count)/sputum weight.

Measurement of biomarkers in sputum

Interleukin (IL)-6, IL-8, neutrophil elastase (NE) and myeloperoxidase (MPO) were quantified by commercial sandwich enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Abingdon, UK; BioVendor, Heidelberg, Germany).

Blood sampling for and determination of biomarkers

Peripheral venous blood (up to 25 mL) was collected, serum separated and stored in appropriate aliquots at -80C until batch analysis (as per methods section 3.4) (56). Serum IL-6 and IL-1β was quantified using commercial sandwich ELISAs (R&D Systems, Abingdon, UK). Serum CRP was measured using Modular Analytics E 170 Module (Roche, Burgess Hill, UK). The Clauss method was used to process plasma fibrinogen.

ELISAs

Serum and sputum supernatants were thawed and processed according to the manufacturer's instructions to measure biomarker levels using high sensitivity ELISA kits (R&D Systems, Abingdon, UK; BioVendor, Heidelberg, Germany).

Supernatants were diluted in as necessary (IL-6: no dilution, IL-8: 1:100, IL-1β: no dilution, MPO: 1:100, NE: 1:100). Assay diluents then samples or standards were added to the appropriate microplate wells, covered and left at room temperature for 2 hours (the MPO plate was placed onto a horizontal microplate shaker set at 500±50 rpm; NE was incubated for only 1 hour whilst shaking at 350rpm), before being washed three times (for IL-1β), or four times (for IL-6, IL-8, MPO and NE) in wash buffer.

The appropriate detection antibody was added to all wells, (200μl for IL-1β, IL-6 and MPO conjugate; 100μl for IL-8 conjugate; 150μl for NE conjugate), and the plates were re-covered and incubated for a further 1 hour (for IL-8; IL-1β; NE-on shaker) or 2 hours (IL-6, MPO-on shaker). Plates were washed three or four times as above, and 200μl of the substrate solution was added to each well (equal volumes of Colour reagent A (H_2O_2) and Colour Reagent B (Tetramethylbenzidine) mixed directly before addition to the plates). The plates were covered and incubated in the dark for 20 minutes (IL-6, IL-β, NE) or 30 minutes (IL-8 and MPO), following which, the reaction was stopped by the addition of 50μl/well Stop solution (1M H_2SO_4).

The OD of each well was determined using a spectrophotometer (BioTek Gen5 v2.0 All-In-One Microplate Reader Software), read at 450nm with wavelength correction set at 570nm to correct for any optical imperfections in the plate. Concentrations of cytokines were derived from extrapolation from standard curve graphs generated from the spectrophotometer (**Figure 3.11**). The lower limit of detection for the ELISA assays were 0.7 pg/ml, 3.5 pg/ml, <1.0 pg/ml, 0.014 ng/ml and 0.2ng/ml for IL-6, IL-8, IL-1β, MPO and NE respectively.
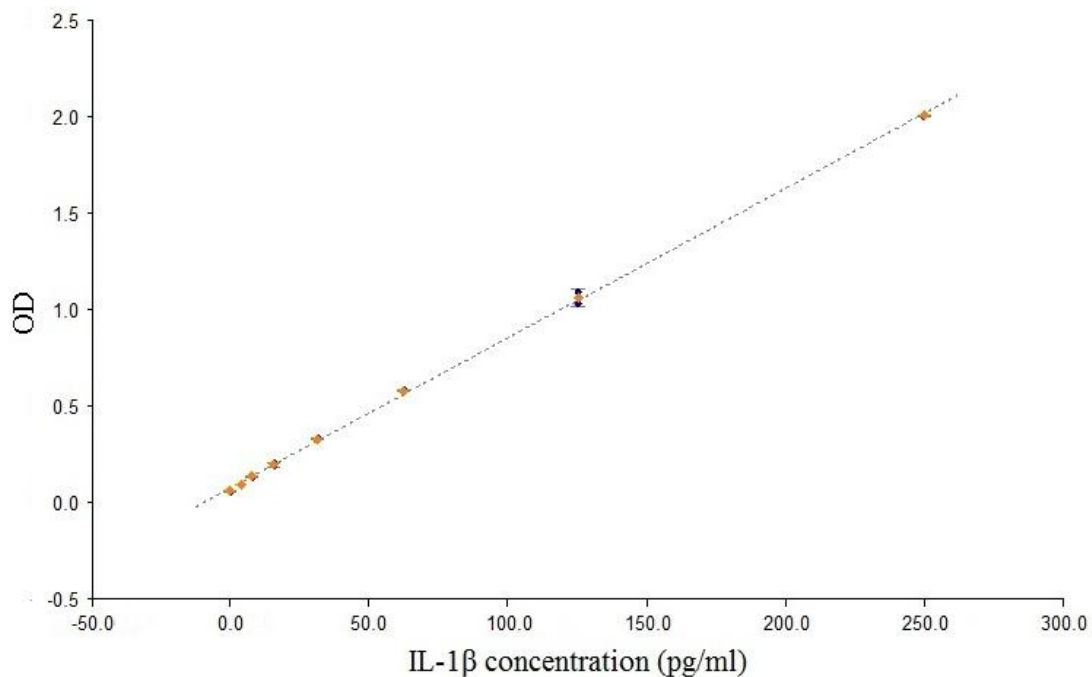
Figure 3.11. Example of standard curve generated using spectrophotometer programme, used to determine unknown concentrations of IL-1β in cell supernatants. The absorbance was read at λ450nm. Similar standard curve graphs were generated for IL-6, IL-8, neutrophil elastase and MPO.

Spirometry

Spirometry for pulmonary function testing was performed according to the recommendations of the American Thoracic Society - European Respiratory Society consensus guidelines on pulmonary function testing (108) using a FlowScreen®CT (ERT®, Philadelphia, PA, USA). Spirometry was performed after patients have taken their standard COPD medications. FEV1 and FVC was chosen as the largest value from different efforts. These values could come from different test curves. FEV1/FVC was calculated from the largest FEV1 and largest FVC.

Patient-reported outcome measures

Patients completed London COPD cohort diary cards, the CAT (©GSK) as well as the EXACT-PRO (©UBC) questionnaires on a daily basis throughout the 2 month trial period.

Study Endpoints

The primary endpoint was change in sputum neutrophil counts from Visit V0 to V2, 14 days after randomisation. The time to normalisation of neutrophil counts after an acute exacerbation is unknown. However, it was assumed that it will parallel symptom recovery after an acute exacerbation, which was reported to be 5 days (median) with an interquartile range (IQR) of 2 to 10 days (15). Based on this recovery time a 14-day period was considered adequate. As data was also being collected 1 week prior to Day 14 and 2 weeks after Day 14, the double-blind treatment period covered 4 weeks from the start of the exacerbation.

Secondary endpoints included the assessment of change from visit V0 to each post randomisation visit for sputum markers (total and differential cell counts (absolute and

percentage): neutrophils, macrophages, eosinophils, lymphocytes; IL-6, IL-8, MPO, neutrophil elastase) and blood biomarkers (change from Visit V0 to each post-randomisation visit for the blood concentration of: CRP, fibrinogen, IL-6, and IL-1β).

Additional secondary endpoints included spirometric assessments (change from Visit V0 to each post-randomisation visit for the following: FEV_1, FVC, FEV1/FVC percentage), and exacerbation length as judged by daily symptom diary cards. Patient reported outcomes assessments were also important secondary endpoints, including changes on a weekly basis for the CAT and EXACT-PRO questionnaires, and total symptom burden, measured by Area under Curve of changes from V0 (exacerbation presentation, Day1) to V3 (4 weeks) and VFU (8 weeks) in daily CAT and EXACT scores .

Safety

In addition to the above endpoints safety was assessed by comparing rates of adverse events, in particular changes in body weight, between both treatment groups.

Registration and Funding

This study is registered with ClinicalTrials.gov, number NCT01473758 and was funded by Takeda.

3.6   Statistical Analysis

The statistical techniques used are detailed in the methods section of each individual chapter.

4

USE OF PATIENT REPORTED OUTCOMES TO MONITOR COPD EXACERBATION RECOVERY

This chapter assesses the utility of the COPD Assessment Test^{TM} (CAT) and the Exacerbations of Chronic Obstructive Pulmonary Disease Tool (EXACT) to monitor COPD exacerbation recovery. The data presented has been published in the American Journal of Respiratory and Critical Care Medicine and the European Respiratory Journal respectively, and was presented at the American Thoracic Society.

4.1 Utility of the COPD Assessment Test™ (CAT) to evaluate severity of COPD exacerbations in Clinical Practice

4.1.1 Introduction

COPD exacerbations are characterized by a worsening of respiratory symptoms from the usual stable state, especially dyspneoa, increased sputum volume and purulence. Changes in exacerbation symptoms relate to exacerbation recovery time (13), which is an index of exacerbation severity. In addition to exacerbation length, exacerbation severity influences acute treatment (13), drives hospital admission and also mortality (12).

Patient diary cards are direct measures of exacerbation symptoms that provide accurate information regarding the commencement and resolution of exacerbations (13). They can detect exacerbations that are both reported and unreported to health care professionals thus allowing accurate determination of exacerbation frequency (11). However, currently there is no standardised, objective method for assessing symptom severity at exacerbation that has been universally accepted and available for use in both routine clinical practice and clinical trials.

The COPD Assessment Test™ (CAT) is a validated 8-item questionnaire It has excellent measurement properties (22) and is short and simple for patients to complete, providing a score out of 40 to indicate disease impact, without the need for complex calculation (**Figure 3.3**). Initial studies have shown that the CAT correlates closely with health-related quality of life as measured by the St. George's Respiratory Questionnaire (SGRQ) when patients are stable (22), and is responsive to pulmonary rehabilitation (25).

4.1.2 Aim

We hypothesised that elevated CAT scores at COPD exacerbation relate to exacerbation severity as measured by exacerbation length, lung function impairment and systemic inflammation. Furthermore, we hypothesised that CAT scores can be used to model recovery. Therefore, well characterized patients were prospectively assessed using the CAT in the baseline stable state, at exacerbation presentation and thereafter for five weeks during the recovery period.

4.1.3 Methods

Full methodology for this chapter is described in methods section 3.2.1. This study involved 161 COPD patients enrolled in the London COPD cohort between 1st January 2009 and 1st June 2011. Exacerbation frequency was calculated for each patient using diary card data obtained between January 2009 and June 2011. Patients completed at least one CAT questionnaire in the stable, baseline state. CAT questionnaires were also administered during exacerbation between April 2010 and June 2011. The exacerbation CAT score took place within 7 days of exacerbation onset as judged by diary cards. A subgroup of patients also completed CAT scores on a daily basis during their recovery (**Figure 3.4**).

Exacerbations were treated according to guidelines. CAT scores played no role in treatment decisions. At exacerbation, blood samples were taken (for serum CRP and Fibrinogen) and spirometry performed prior to commencing exacerbation treatment.

Statistical analysis

Data were analysed with STATA 8.2 (Stata Corporation, Texas, USA). Normally distributed data were expressed as mean and standard deviation (SD) and skewed data as median and interquartile range (IQR). Comparisons were made by paired Student t-test or Wilcoxon signed-rank test. The relationship between exacerbation frequency and baseline CAT scores was examined with a negative binomial regression model, whilst Poisson regression was used to model exacerbation recovery and CAT scores. Cross-sectional regression models were used to analyze the relationship between inflammatory markers during exacerbation and CAT score as allowance could be made for repeated measures on the same patient.

4.1.4 Results

Patient Characteristics

161 COPD patients completed at least one CAT questionnaire when stable (exacerbation free). Their baseline characteristics are reported in **Table 4.1** alongside 75 patients who were assessed using the CAT at exacerbation and the 52 of these who completed the CAT questionnaire daily during exacerbation recovery. The patients had moderate to severe disease with a mean FEV_1 % predicted of 50.3% (range 14.0-79.7%). Patients in whom CAT was assessed at exacerbation had significantly higher exacerbation frequencies (p<0.001) but differed in no other respect.

Patients completed the CAT successfully when stable and when acutely unwell during an exacerbation. In total, 6404 out of 6514 questionnaires (98.3%) were completed fully. There was no significant difference in the percentages fully completed at baseline, 3496 of 3561 (98.2%) compared to those at exacerbation onset and during recovery, 2908 of 2953 (98.5%; p=0.35).

Table 4.1.Clinical characteristics of patients in the baseline, exacerbation and recovery analyses

‡ Comparison between 75 patients with exacerbation CAT scores at exacerbation and 86 patients in whom an exacerbation was not examined.

[†] Comparison between 52 patients with exacerbation CAT scores during recovery and 109 patients in whom a recovery time course was not examined.

	Baseline n=161	Exacerbation n=75	p-value‡	Recovery Subgroup n=52	p-value[†]
	Mean±SD	Mean±SD		Mean±SD	
Age (years)	71.3±9.4	71.3±8.4	0.99	71.3±7.9	0.96
FEV_1 (L)	1.22±0.47	1.14±0.41	0.10	1.14±0.4	0.12
FEV_1 (% predicted)	50.3±16.9	47.9±16.3	0.10	47.1±16.6	0.10
FVC (L)	2.59±0.78	2.51±0.79	0.09	2.52±0.9	0.45
FEV_1 / FVC ratio (%)	47.4±12.6	46.5±13.0	0.38	46.2±13.1	0.40
Smoking pack years	53.5±38.9	52.2±39.9	0.62	50.6±37.3	0.53
SpO_2 (%)	94.7±2.0	94.6±2.1	0.52	94.4±2.1	0.10
BMI (kg/m^2)	26.4±5.6	26.6±5.4	0.56	27.0±5.1	0.31
	Median (IQR)	Median (IQR)		Median (IQR)	
Exacerbation frequency	1.95 (0.90-3.00)	2.73 (1.70-4.10)	<0.01	3.00 (2.20-4.40)	<0.01
	N (%)	N (%)		N (%)	
Male gender	97 (60)	43 (57)	0.48	31 (60)	0.91
Current Smokers	49 (31)	20 (27)	0.36	11 (22)	0.08

Use of CAT at Baseline

Baseline CAT and exacerbation frequency

The 161 patients had a mean baseline CAT score of 18.1 (SD 7.45). Patients with more frequent exacerbations had higher CAT scores (negative binomial regression coefficient = 0.018 exacerbations per year per CAT unit increment, 95% CI 0.001-0.036; p=0.048). Frequent exacerbators (≥2 exacerbations per year, n=80) had a mean CAT score of 19.5 (SD 6.6) compared to infrequent exacerbators (<2 exacerbations per year, n=81) whose mean CAT score was 16.8 (SD 8.0; p=0.025, **Figure 4.1**). Thus, there was an average 2.7 point difference in CAT score between the frequent and infrequent exacerbators.
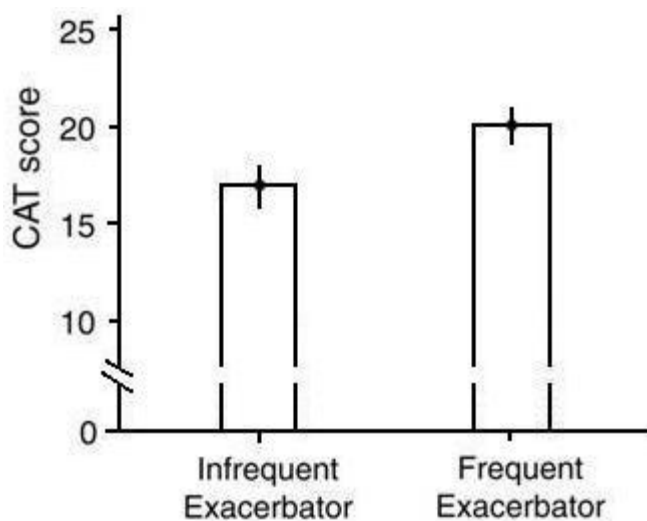
Figure 4.1. Mean baseline CAT scores between frequent and infrequent exacerbators (161 patients). Vertical lines represent standard errors.

Relationship between CAT score and systemic inflammatory markers in the baseline state

At baseline, serum CRP was measured on the same day as a CAT was completed in 318 blood samples obtained from 150 separate patients and plasma fibrinogen in 282 blood samples from 144 patients. There was a significant relationship between systemic inflammation, as measured by $\log_{10}$ fibrinogen, and CAT score on the day of baseline sampling, regression coefficient= 0.0014 (95% CI 0.0001-0.0027; p=0.035, **Figure 4.2**), R^2=0.024 using random-effects GLS regression. However, there was no statistically significant relationship between $\log_{10}$ CRP and CAT scores, regression coefficient = 0.0059 (95% CI -0.0016-0.0133; p=0.122).
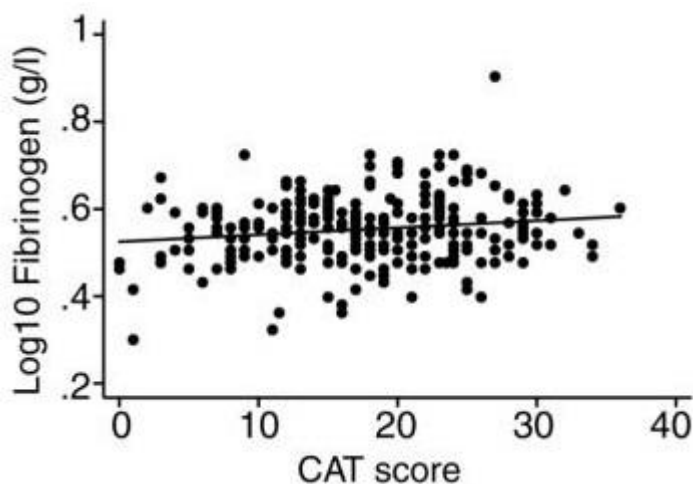
Figure 4.2. Relationship between $\log_{10}$ fibrinogen and CAT score at baseline (282 samples from 144 patients).

No difference in baseline CAT scores was seen between patients with or without potentially confounding comorbidities (congestive heart failure, renal failure, obesity or sleep disordered breathing, **Table 4.2**), confirming previous work that CAT scores appear unaffected by low levels of comorbidity (18).

Table 4.2. Effect of Comorbidities on Baseline CAT Score

	CAT Score Comorbidity Absent	CAT Score Comorbidity Present	p-value (unpaired t-test)
	Mean ± SD	Mean ± SD	
Congestive heart failure	18.2 ± 7.6 (n=143)	18.1 ± 6.8 (n=16)	0.968
Ischemic heart disease	17.9 ± 7.6 (n=130)	19.4 ± 7.2 (n=29)	0.328
Any cardiovascular disease (excluding hypertension)	17.8 ± 8.0 (n=102)	18.9 ± 6.6 (n=57)	0.396
Hypertension	17.7 ± 8.4 (n=73)	18.6 ± 6.7 (n=86)	0.469
Obstructive sleep apnoea	18.0 ± 7.5 (n=153)	22.9 ± 5.4 (n=6)	0.119
Obesity (body mass index >30kg/m^2)	18.0 ± 7.7 (n=122)	18.7 ± 6.4 (n=36)	0.655
Chronic kidney disease (estimated glomerular filtration rate <60ml/min)	18.5 ± 7.6 (n=126)	16.5 ± 6.2 (n=29)	0.205
Severe chronic kidney disease (estimated glomerular filtration rate <30ml/min)	18.1 ± 7.4 (n=152)	21.0 ± 4.5 (n=3)	0.500

Use of CAT at exacerbation

The CAT was completed at 152 treated exacerbations by 75 patients. The median interval from diary card exacerbation onset to the day of treatment was 2 days (IQR 1-4). **Figure 4.3** shows that the CAT score rose from an average baseline value of 19.4 (SD 6.8) to 24.1 (SD 7.3; $p<0.001$) at exacerbation.
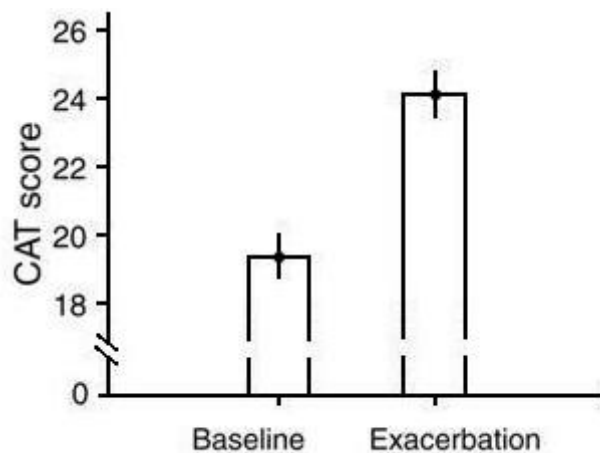
Figure 4.3. Mean CAT scores at baseline and exacerbation for 152 exacerbations (75 patients). Vertical lines represent standard errors.

The magnitude in rise of CAT score from baseline to exacerbation was not affected by patient baseline characteristics. Patients whose change in CAT score at exacerbation was on average greater or equal to 2 units displayed no significant difference in age (73.2 vs 70.3 years; $p=0.13$), $FEV_1\%$ predicted (47.6 vs 47.3 %; $p=0.94$) or exacerbation frequency (2.73 vs 2.48; $p=0.586$) from those with smaller changes in CAT score.

Results were also unaffected by the timing of the baseline. In 98 exacerbations where a baseline CAT score was available prior to the index exacerbation, the CAT score rose from an average baseline value of 19.0 to 24.3 ($p<0.001$) at exacerbation. In the main analysis, in 152 exacerbations using baselines obtained during stable periods that occurred prior to or following exacerbations, the CAT score rose from an average baseline value of 19.4 to 24.1 ($p<0.001$) at exacerbation.

Exacerbation characteristics

The symptomatic characteristics of exacerbations did not affect the magnitude of CAT rise at exacerbation, except those few exacerbations characterized by the presence of the 3 symptoms of dyspnea, cold and sore throat only, which had significantly increased rises in CAT scores at exacerbation compared to those without (n=8, mean 11.5, SD 8.2 vs n=144, mean 4.4, SD 6.3, p=0.003). Exacerbations associated with symptoms of both increased sputum volume and purulence did not display a significantly increased change in CAT score compared to those without (n=61, mean 5.3, SD 6.7 vs n=91, mean 4.4, SD 6.5, p=0.392). This may be a due to the absence of a specific question assessing sputum purulence in the CAT.

Peak CAT scores

CAT scores rose further following exacerbation onset to reach a maximum, peak CAT score. CAT scores in the recovery subgroup increased from a mean baseline score of 18.3 (SD 7.5) to a mean peak score of 26.5 (SD 7.1; p<0.001, **Figure 4.4**).
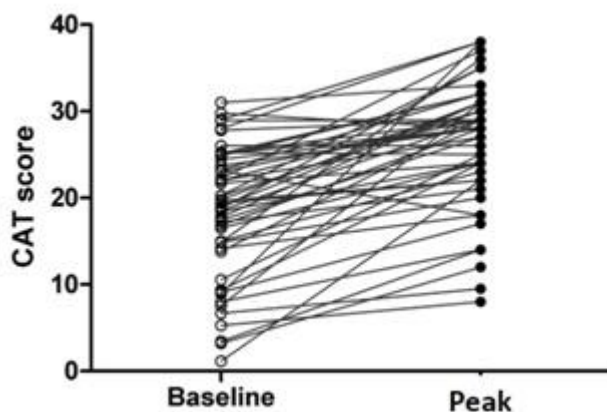
Figure 4.4. Change in CAT scores from baseline to peak exacerbation value for 52 exacerbations (52 patients).

Effect of Exacerbation Treatment

Whilst patients within the London COPD cohort complete daily symptom diary cards which allows detection of exacerbations that are unreported to healthcare professionals and untreated with extra medication (7), all 152 exacerbations included in the analyses were treated. The vast majority of exacerbations were treated with systemic treatment following clinical review by a member of the research team; 103 exacerbations were treated with antibiotics and oral corticosteroids, 22 with antibiotics alone, and 7 with oral steroids alone. Just 20 patients increased inhaled therapy (bronchodilators and/or inhaled corticosteroids) alone without systemic treatment. Increases of more than 2 units in CAT score were associated with a greater likelihood of treatment with antibiotics (88.7% vs 70.4%; p=0.004) but not oral steroids (75.5% vs 66.7%; p=0.243).

The main analysis was repeated using a strict health care utilisation (HCU) definition of an exacerbation, based on physician review and increased systemic treatment. 132 exacerbations fitted this criterion. The mean change in CAT score from baseline to HCU exacerbation was 5.2 units (SD 6.7, n=132). Mean change in CAT score from baseline to exacerbation for patients who received increased inhaled therapy alone was 2.0 (4.9), although this was based on just 20 exacerbations. Further work is required to further explore the relationships between changes in CAT at exacerbation and choice of exacerbation treatment.

Effect of exacerbation frequency phenotype on CAT change at exacerbation

It does not appear that the exacerbation data from 75 patients who experienced 152 exacerbations was affected by a few patients with frequent exacerbations. In analyses which examined the average change from baseline to exacerbation for each of the 75 patients, the mean change was 4.98 units, compared to 4.70 in the main results for 152 exacerbations.

Repeatability during exacerbations

Of the 75 patients included in the main exacerbation analysis, 38 underwent at least one further subsequent exacerbation. No difference was seen in the magnitude of change from baseline to exacerbation between their first and second exacerbation (mean 5.2 vs 5.3, p=0.924). 19 of the 52 recovery subgroup patients recorded CAT scores during a second exacerbation. There was no difference in peak CAT score between first and second exacerbation (mean 27.0 (SD 7.86) versus 26.4 (8.5); p=0.687, **Figure 4.5**) or in the change from baseline to peak score (6.7 (SD 4.9) versus 6.1 (5.1); p=0.687).
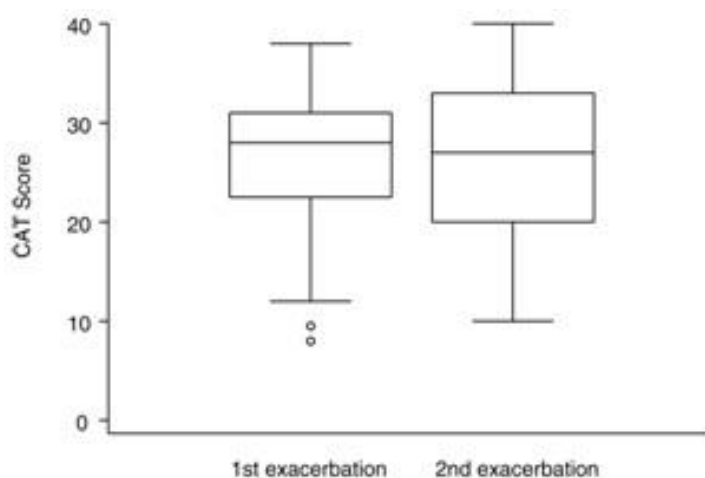
Figure 4.5. Repeatability of CAT changes at separate exacerbations (19 patients).

Relationship between CAT score and systemic inflammatory markers at exacerbation

CAT scores at exacerbation were significantly related to concurrent levels of systemic inflammatory markers. At exacerbation, serum CRP was measured on the same day as a CAT was completed in 114 exacerbations and plasma fibrinogen in 111 exacerbations. After $\log_{10}$ transformation, both inflammatory markers were significantly related to the CAT score recorded at exacerbation, and with allowance for repeated measures in the same patient, $\log_{10}$CRP increased by 0.028 (95% CI 0.013-0.043; p<0.001) and $\log_{10}$fibrinogen by 0.003 (95% CI 0.001-0.005; p=0.015), per unit increase in CAT score. Change in CAT score from baseline to exacerbation onset was significantly related to change in CRP (rho=0.26; p=0.008) but not to change in fibrinogen (rho=0.09, p=0.351) from baseline to exacerbation.

Lung function changes and CAT scores at exacerbation

CAT scores were significantly related to contemporaneous spirometry, as measured by FEV_1. At exacerbation, spirometry was performed on the same day as a CAT was completed in 112 exacerbations. Mean paired FEV_1 measured at baseline was 1.12 L (SD 0.44) and 1.01 L at exacerbation (SD 0.44) (p<0.001). Rises in the CAT score recorded at exacerbation were significantly associated with falls in FEV_1 at exacerbation (rho=-0.20, p=0.032).

Time Course of CAT scores during exacerbation recovery

52 different patients completed the CAT questionnaire on at least 21 of 35 days during the recovery phase following an exacerbation. All of these 52 exacerbations were treated; 41 with antibiotics and oral steroids, 5 with antibiotics alone, 3 with oral steroids alone, and 3 were treated with increased inhaled therapy alone. **Figure 4.6** shows the time course of the CAT scores, PEFR and diary card symptom counts.
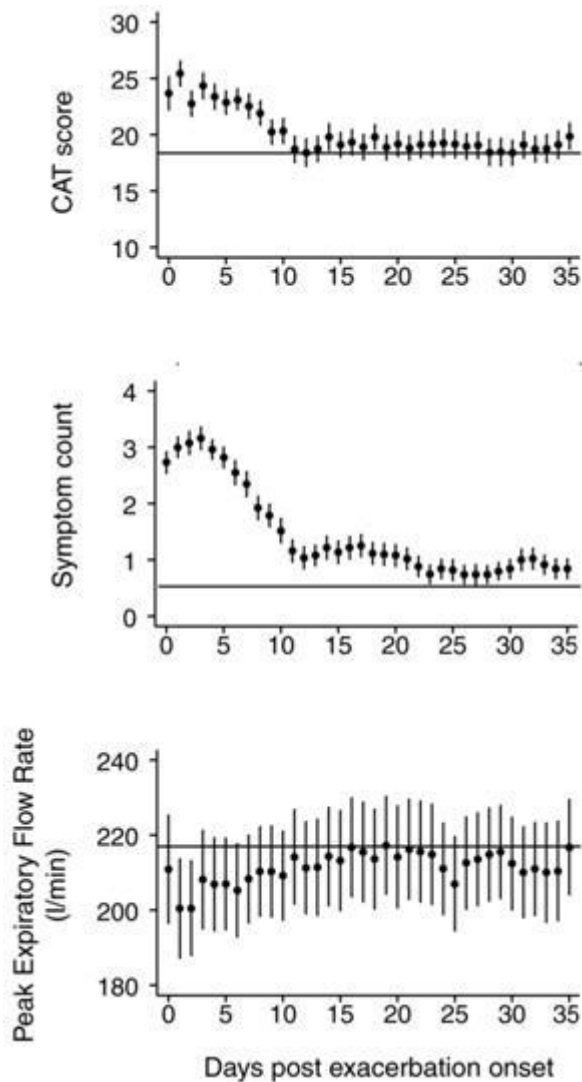
Figure 4.6 Time course of CAT scores, PEFR and diary card symptom counts during exacerbation recovery (52 patients). Vertical bars represent standard errors. Horizontal lines indicate mean baseline scores.

Symptom counts were obtained by summating each increased respiratory symptom recorded on the London cohort diary cards per day. The mean baseline symptom count (denoted by a horizontal line) lies at greater than zero because patients may record sporadic increases in 1 or more symptoms but not reach the definition of an exacerbation (increase for two consecutive days in respiratory symptoms, with at least one major symptom (dyspnea, sputum purulence or sputum volume) plus either another major or a minor symptom (wheeze, cold, sore throat, and cough)). This phenomenon of patients experiencing sporadic increases in respiratory symptoms following the end of an exacerbation is also the reason for the slight elevation of symptom counts above baseline at 12 days (recovery time). The last day of recorded worsening symptoms before two consecutive symptom-free days defined the end of the exacerbation.

Relationship between CAT score and symptom recovery

CAT scores reflected symptomatic recovery following exacerbations. Amongst the 52 episodes, the median recovery time as judged by symptom diary cards was 12 days (IQR 9-23, n=47) and this was significantly related (rho=0.42; p=0.012) to the time taken for the CAT score to return to baseline (median 11 days, IQR 4.5-17, n=40).

4.1.5 Discussion

This novel study prospectively assessed the utility of the CAT to evaluate exacerbation severity in COPD patients. At exacerbation, CAT scores were significantly elevated from paired baseline values and this work uniquely demonstrated that CAT scores reflect exacerbation severity as measured by exacerbation length and reduction in lung function. A weak relationship was also found between systemic inflammatory markers and CAT scores at exacerbation. Furthermore, this work demonstrated that baseline CAT scores are significantly elevated in stable COPD patients with a history of frequent exacerbations.

The CAT is a validated health status questionnaire that is free to use and can be administered without prior permission for research purposes and by individual practitioners (http://www.catestonline.org). Previous studies have shown that the instrument can be successfully administered in both primary (109) and secondary care settings (110), and is responsive to a course of pulmonary rehabilitation and able to distinguish different levels of response (110). Additionally, CAT scores exhibit little variability across countries; they are not influenced by age or sex but reflect disease severity in the stable state as determined by Global Initiative for chronic Obstructive Lung Disease (GOLD) spirometric staging, MRC dyspnoea score, SGRQ and clinician-judged severity (22, 109).

This study complements this existing work by demonstrating that the CAT can be used as a score of the multi-dimensional nature of COPD exacerbation severity. At present the assessment of symptom severity at exacerbation and during recovery is subjective in nature, with no established scoring system in clinical practice. Exacerbation therapy is currently determined by a subjective physician assessment of exacerbation severity and so an objective

tool to determine exacerbation severity will fulfill an important unmet need. This has particular relevance as patients are increasingly seen by healthcare professionals in the community, often within their own homes, without the benefit of objective measures of exacerbation severity such as accurate spirometry or systemic inflammatory markers. PEFR is a cheap, reliable and easy way for patients to assess lung function on a daily basis. Previous studies have shown that PEFR decreases to a small extent but significantly at exacerbation onset and can be a useful tool to indicate exacerbation recovery in population studies. However the changes are not large enough to use PEFR at an individual level for exacerbation detection and monitoring (13).

The CAT provides an objective quantification of the impact of symptoms that is acceptable to patients and can be easily completed at exacerbation and during recovery. Systemic inflammation, as measured by plasma fibrinogen and serum CRP increases at exacerbation (37, 111, 112), and this study has demonstrated a weak relationship between CAT scores at exacerbation and systemic inflammatory markers. Inflammatory changes at COPD exacerbations are also related to clinical non-recovery and recurrent exacerbations within 50 days (56). Recovery time is an index of exacerbation severity (13) and for the first time this study has evaluated use of the CAT during exacerbation recovery. CAT scores reflect recovery following exacerbations; the time taken for scores to return to baseline being significantly related to recovery time as judged by symptom diary cards. Additionally, at exacerbation, CAT scores are significantly but modestly related to contemporaneous lung function impairment, as measured by FEV_1, consistent with previous data examining the relationship between baseline CAT scores and FEV_1 (109). Thus CAT scores provide an easily quantifiable, overall score of exacerbation severity and may be useful in studies evaluating interventions for the management of acute exacerbations.

When measured in the stable state CAT scores are highly correlated to concurrent SGRQ measurements (22). However, this study has shown a divergence between the behavior of the CAT and SGRQ during exacerbation recovery. Following a study of exacerbations of chronic bronchitis, whilst an early improvement is seen in SGRQ scores when measured 4 weeks after an index event, improvements can also slowly continue for several months (113). In this study CAT scores have returned to baseline levels more rapidly. This may be a result of the daily use of the instrument in this study and the response system in the CAT, which is based on categories of difference between two extreme statements about the same COPD impact. In contrast, the SGRQ has predominantly dichotomous yes/no responses and is administered at intervals. Thus, although the CAT can reliably assess exacerbation severity, daily CAT readings may over-estimate the speed of recovery of health status post-exacerbation.

This study has also added to previous data examining the use of the CAT in the baseline stable state by examining the relationship between baseline CAT scores and exacerbation frequency. Patients with a history of frequent exacerbations have worse quality of life (11), increased risk of hospitalisation (114) and greater mortality (12). Frequent exacerbators also exhibit faster decline in lung function (8) and may have worse functional status, as measured by time outdoors (115). In this study baseline CAT scores relate to exacerbation frequency. When used in the stable state, scores were significantly elevated in frequent exacerbators, defined by two or more exacerbations per year, compared to infrequent exacerbators. Also, baseline CAT scores were weakly but significantly related to concurrent fibrinogen levels. Plasma fibrinogen levels are elevated in stable patients with COPD (112) and that increased systemic inflammation, as measured by fibrinogen, in stable COPD patients over time is directly linked to disease progression, as defined by lung function decline (27). Further work

is required to explore whether CAT scores may potentially be a useful marker of disease progression over time in COPD.

The CAT is a potentially useful, widely applicable tool which can aid assessment of exacerbation severity. The instrument can be easily and rapidly completed in many healthcare settings and could potentially be integrated into care bundles of COPD patients without additional cost. Patient recognition of exacerbation symptoms and prompt treatment improves exacerbation recovery and reduces the risk of hospitalisation in COPD patients (20). Further evaluation is now required of the CAT within exacerbation management strategies to assess utility of the tool within clinical practice.

4.1.6 Conclusion

In conclusion, the CAT provides a reliable score of exacerbation severity. CAT scores increase at exacerbation and reflect exacerbation severity as determined by lung function and exacerbation length. A weak relationship was also found between systemic inflammatory markers and CAT scores at exacerbation. Thus, the CAT is a valuable instrument to enhance and standardise COPD exacerbation assessment. Incorporating this questionnaire into assessment strategies may aid health care professionals to determine the severity of exacerbations, particularly in situations where access to other objective measures of severity is limited.

This work formed the basis an original publication published in the American Journal of Respiratory Critical Care Medicine:

Usefulness of the Chronic Obstructive Pulmonary Disease Assessment Test to evaluate severity of COPD exacerbations.

Mackay AJ, Donaldson GC, Patel AR, Jones PW, Hurst JR, Wedzicha JA.

American Journal of Respiratory and Critical Care Medicine. 2012 June 1;185(11):1218-24.

doi: 10.1164/rccm.201110-1843OC.

4.2 Performance of the Exacerbations of Chronic Pulmonary Disease Tool (EXACT) in detection and severity grading of COPD exacerbations

4.2.1 Introduction

Chronic obstructive pulmonary disease (COPD) is associated with episodes of symptomatic deterioration termed exacerbations (49). COPD exacerbations are amongst the commonest causes of medical admission to hospital (6). Patients with frequent exacerbations (7) have accelerated lung function decline (8, 9), worse quality of life (11), are at increased risk of cardiovascular events (10) and have greater mortality (12).

Assessment of exacerbation severity is an important outcome measure in COPD and most clinical trials of preventive therapy to date have used a dichotomous approach to assigning severity levels, with outpatient drug therapy (antibiotics and/or steroids) categorized as "moderate" and hospitalisations labelled "severe". These outcome measures are limited by inaccurate reporting, lack of generalisability across different healthcare systems and their failure to capture unreported exacerbations. Unreported exacerbations are common (11, 18, 19) and important events, associated with worsening quality of life (11, 18) and increased risk of subsequent hospitalisation (20).

Exacerbation symptoms systematically recorded on daily diary cards accurately detect both reported and unreported exacerbations (11, 13), but most symptom diary cards are not sensitive enough to assess severity of these events. The FDA's patient-reported outcome (PRO) guidance document (21) offers recommendations for developing PRO instruments for use in medical product development, including symptom-based methods for standardising the severity of reported and unreported exacerbations.

The Exacerbations of Chronic Obstructive Pulmonary Disease Tool (EXACT) is a PRO daily symptom diary developed to capture frequency, severity, and duration of exacerbations in clinical trials of COPD (26). Scores range from 0 to 100, with higher scores indicating more severe symptoms. To date, no published data have examined the relationship between EXACT scores and pulmonary function or markers of inflammation during exacerbations. Furthermore, the EXACT was designed to quantify the evaluation of exacerbations of COPD. Specifically, the intent was to capture unreported events, using a threshold-based definition of sustained symptomatic worsening, and provide a standardised metric for evaluating the severity of unreported and reported, healthcare utilisation (HCU) events. However, in published papers thus far, no data has been reported examining the relationship between symptom-defined events captured by the EXACT, exacerbations detected by London COPD cohort symptom diary cards, and HCU events.

4.2.2 Aim

We hypothesised that the EXACT can accurately assess the severity of both reported and unreported COPD exacerbations, defined by the London COPD cohort diary card, as measured by exacerbation length, lung function impairment and systemic inflammation. Furthermore, we sought to examine the relationship between exacerbations diagnosed using validated London COPD cohort diary cards (8, 11, 13) and physician review, and symptom-defined events captured by the EXACT. Therefore, patients in the London COPD cohort prospectively completed the EXACT at baseline and during exacerbation.

4.2.3 Methods

Full methodology for this chapter is described in methods section 3.2.2. This study involved 58 COPD patients enrolled in the London COPD cohort between January 2010 and April 2012. Exacerbation frequency was calculated for each patient using diary card data obtained between 1/4/2010 and 1/4/2012.

Patients completed a paper version of the EXACT at least once under supervision in clinic and were instructed to complete the EXACT diary each evening before bedtime, based on their symptoms experienced that day. Patients prospectively completed the EXACT on a daily-basis when stable and continued long-term to enable capture of the exacerbation prodrome, the onset of the event, its nadir and recovery (**Figure 4.7**). Exacerbations were treated according to guidelines. EXACT scores played no role in treatment decisions. At exacerbation, blood samples were taken (for serum CRP and Fibrinogen) and spirometry performed prior to commencing exacerbation treatment.

Statistical analysis

Data were analysed with STATA 8.2 (Stata Corporation, Texas, USA). Normally distributed data were expressed as mean and standard deviation (SD) and skewed data as median and interquartile range (IQR). Comparisons were made by paired Student t-test or Wilcoxon signed-rank test. The relationship between exacerbation frequency, determined using London COPD cohort diary cards, and baseline EXACT scores was examined with a negative binomial regression model, whilst Poisson regression was used to model exacerbation recovery and EXACT scores. Cross-sectional regression models were used to analyse the relationship between CRP levels during exacerbation and EXACT score as allowance could be made for repeated measures on the same patient.

4.2.4 Results

Patient Characteristics

Full baseline clinical characteristics of the 58 patients included in this analysis are reported in

Table 4.3. Patients had moderate to severe COPD with a mean FEV_1 % predicted of 48.6%.

	Mean±SD
Age (years)	70.2±8.2
FEV_1 (l)	1.22±0.56
FEV_1 (% predicted)	48.6±18.1
FEV_1/ FVC (%)	46.6±14.2
Smoking pack years	54.0±42.1
SpO_2 (%)	94.6±2.0
BMI (kg/m2)	26.8±5.7
	Median (IQR)
Diary card Exacerbation Frequency (per patient per year)	2.88 (1.92-4.43)
	n=
Male, n (%)	36 (62.1)
Current Smokers, n (%)	11 (19.0)
Comorbidity present, n (%)	47 (81.0)
Congestive heart failure, n (%)	6 (10.3)
Ischemic heart disease, n (%)	13 (22.4)
Hypertension, n (%)	32 (55.2)
Diabetes Mellitus, n (%)	4 (6.9)
Patients receiving maintenance inhaled corticosteroids, n (%)	52 (89.6)

Table 4.3. Clinical characteristics of the 58 patients at time of recruitment to the cohort.

Baseline EXACT scores were significantly related to disease severity. These baseline EXACT scores in 58 patients were significantly correlated with London COPD cohort diary card exacerbation frequency (rho=0.38, p=0.003), FEV_1 (rho=-0.32, p=0.015) and FEV_1% predicted (rho=-0.30, p=0.020).

Stability of baseline EXACT scores over time

53 patients had two 7-day baseline periods distinct from the pre-exacerbation baseline, on average 278 days apart (**Figure 4.7**).

Figure 4.7. Schematic timeline of EXACT scores (x) recorded when stable and during exacerbation. This figure is for illustrative purposes and does not indicate real data.
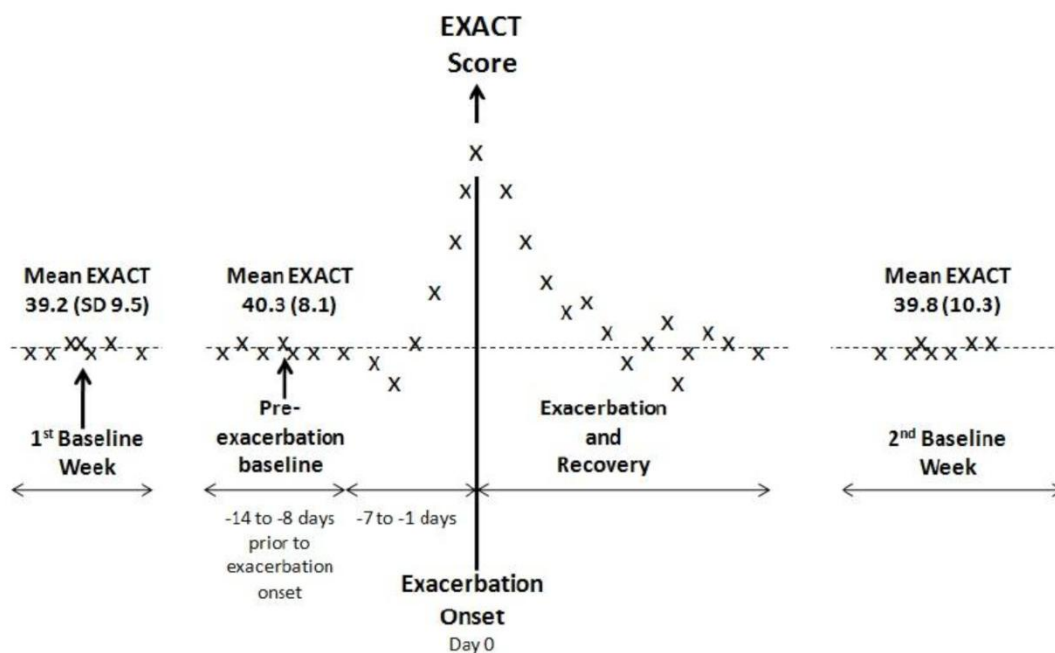
Overall EXACT scores were stable over time, with no significant difference found between EXACT scores recorded between the first 7-day baseline and the last 7-day baseline (mean 39.2 (SD 9.5) vs. 39.8 (10.3), p=0.117). However, in a small but important proportion of

patients, baseline scores did vary over time. EXACT scores rose between the 1st and 2nd baseline periods by more than 12 points in 3 patients and by more than 9 points in 5 patients. EXACT scores fell between baseline periods by more than 12 points in 3 patients and by 9 points in 3 patients (**Figure 4.8**).
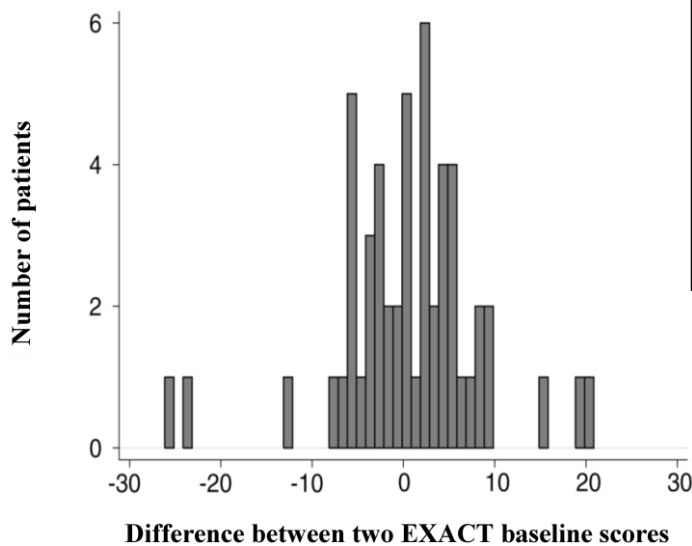
Figure 4.8. Variability in mean EXACT scores between two separate seven day baseline periods (53 patients).

Exacerbation Studies

58 patients completed the EXACT during 128 London COPD cohort diary card exacerbations. 85 of 128 (66.4%) were treated with systemic therapy; 82 (64.1%) with antibiotics and 64 (50.0%) with oral corticosteroids. There were no hospitalised exacerbations.

The baseline mean EXACT score averaged over 7 days (-14 to -8 days before exacerbation onset) was 42.6 (SD 8.6). This increased to 48.0 (8.6) at London COPD cohort diary card exacerbation onset, 5.4 (7.1) above baseline (p<0.001), representing a 13% rise. EXACT scores increased further to a maximum score of 54.1 (8.9) or 11.4 (7.6) above baseline (p<0.001), during the 2 weeks following exacerbation onset (**Figure 4.9**). The median time

from exacerbation onset to peak EXACT score was 3 days (IQR 1-5.5). No difference was seen in the maximum increase from onset in EXACT score between type 1 Anthonisen exacerbations (events associated with increased sputum volume, purulence and dyspnoea) and other types (n=27, 6.6 (6.3) vs. n=101, 5.9 (5.4), p=0.541).

Figure 4.9. Mean EXACT scores at baseline (□), exacerbation onset (▨), and the maximum (■) EXACT scores during the 2 weeks following exacerbation onset. Vertical lines represent standard errors.
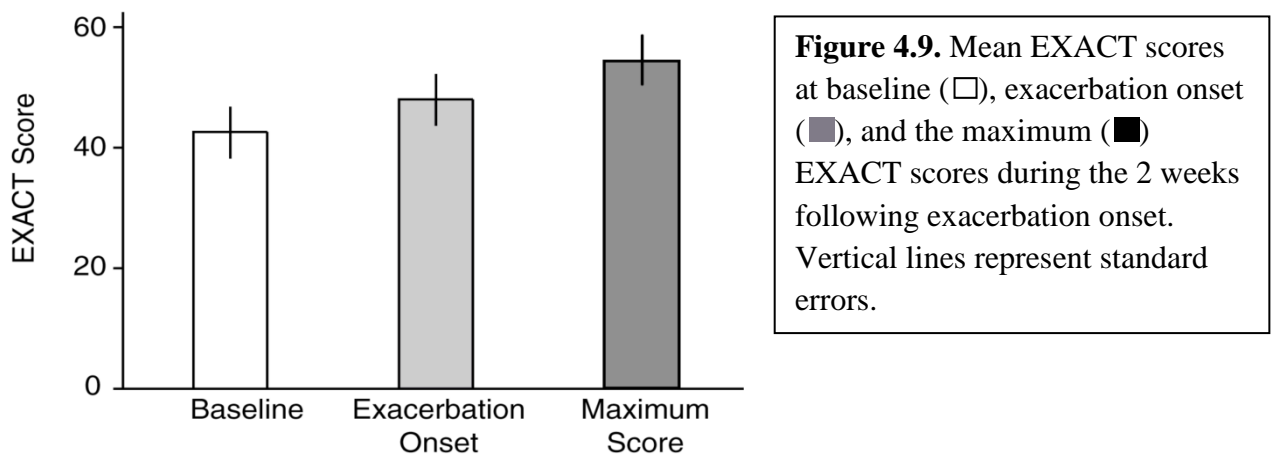
Relationship between EXACT score and systemic inflammation

EXACT score changes at exacerbation presentation were significantly related to concurrent levels of systemic inflammation. At exacerbation, $\log_{10}$CRP was related to the change in EXACT score from baseline to exacerbation (rho=0.30; p=0.041, **Figure 4.10**). The median exacerbation CRP score was 9 (IQR 4.3-16.8).
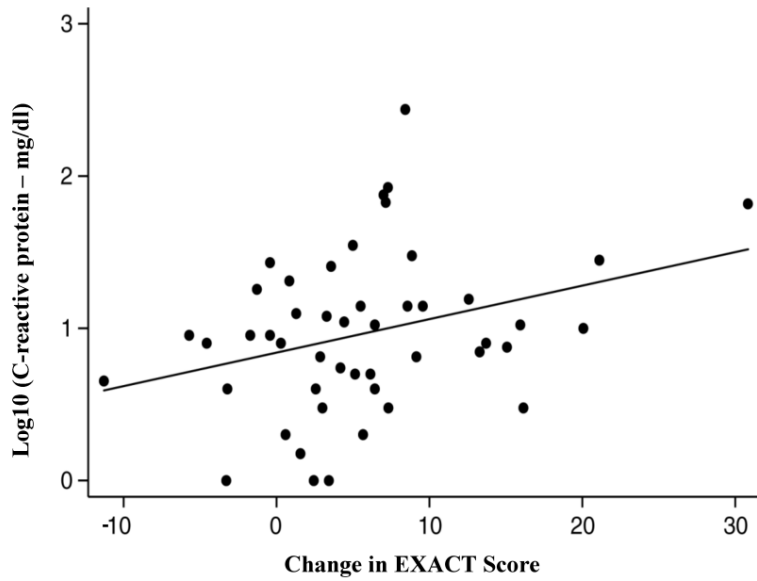
Figure 4.10. Relationship between $\log_{10}$CRP and change in EXACT scores at exacerbation (rho=0.30; p=0.041).

Evolution of EXACT scores during exacerbation recovery

EXACT scores accurately reflected exacerbation recovery. The time taken for EXACT scores to return to baseline (median 7 days, IQR 0-12; n=93) was significantly related (rho=0.44; p<0.001) to symptom recovery time as judged by London COPD cohort diary cards (median 8 days, IQR 4-12; n=114). Recovery in EXACT was also significantly correlated with lung function recovery as measured by PEFR (rho=0.32; p=0.003). Time course plots of recovery of EXACT score, London COPD cohort diary card symptom counts and peak expiratory flow rate (PEFR) generated using daily mean values of all 128 exacerbations are presented in **Figure 4.11(a), (b) and (c)** respectively.
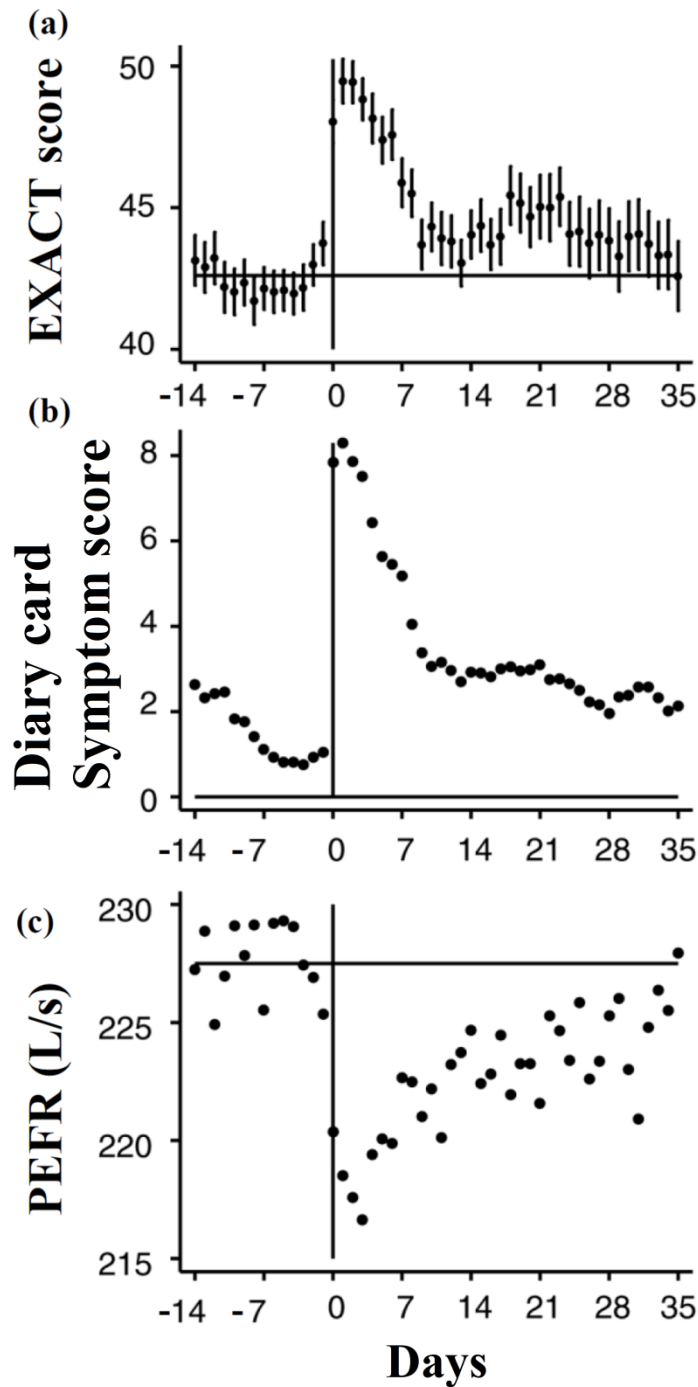
Figure 4.11. Time course plots of recovery of EXACT score, London COPD cohort diary card symptom counts and peak expiratory flow rate (PEFR) generated using daily mean values of all 128 exacerbations are presented in Figure 4.11(a), (b) and (c) respectively. Vertical bars represent standard errors. Horizontal lines indicate mean baseline scores.

Relationship between EXACT scores and exacerbation treatment

No difference was observed in exacerbation onset EXACT scores between treated (systemic therapy) and untreated (no systemic therapy) events (n=85, mean 48.2 (8.5) vs. n=43, 47.7 (9.0), p=0.762). However, maximum EXACT scores were significantly higher in treated than untreated events (n=85, mean 55.2 (9.1) vs. n=43, 51.8 (8.1), p=0.040, **Figure 4.12a**).

Time course of EXACT scores during treated and untreated exacerbations

Median time from exacerbation onset to initiation of systemic therapy for the 85 treated exacerbations was 2 days (1-4). Despite the higher maximum EXACT score following exacerbation onset, no significant difference was seen between treated and untreated exacerbations in EXACT recovery time (n=61, median 7 days (0-12) vs. n=32, 5.5 (2-12), p=0.656, **Figure 4.12b**) or total net EXACT recovery score (n=61, median 24.2 EXACT.day (0-75.3) vs. n=32, 13.1 (1.5-65.9), p=0.455).
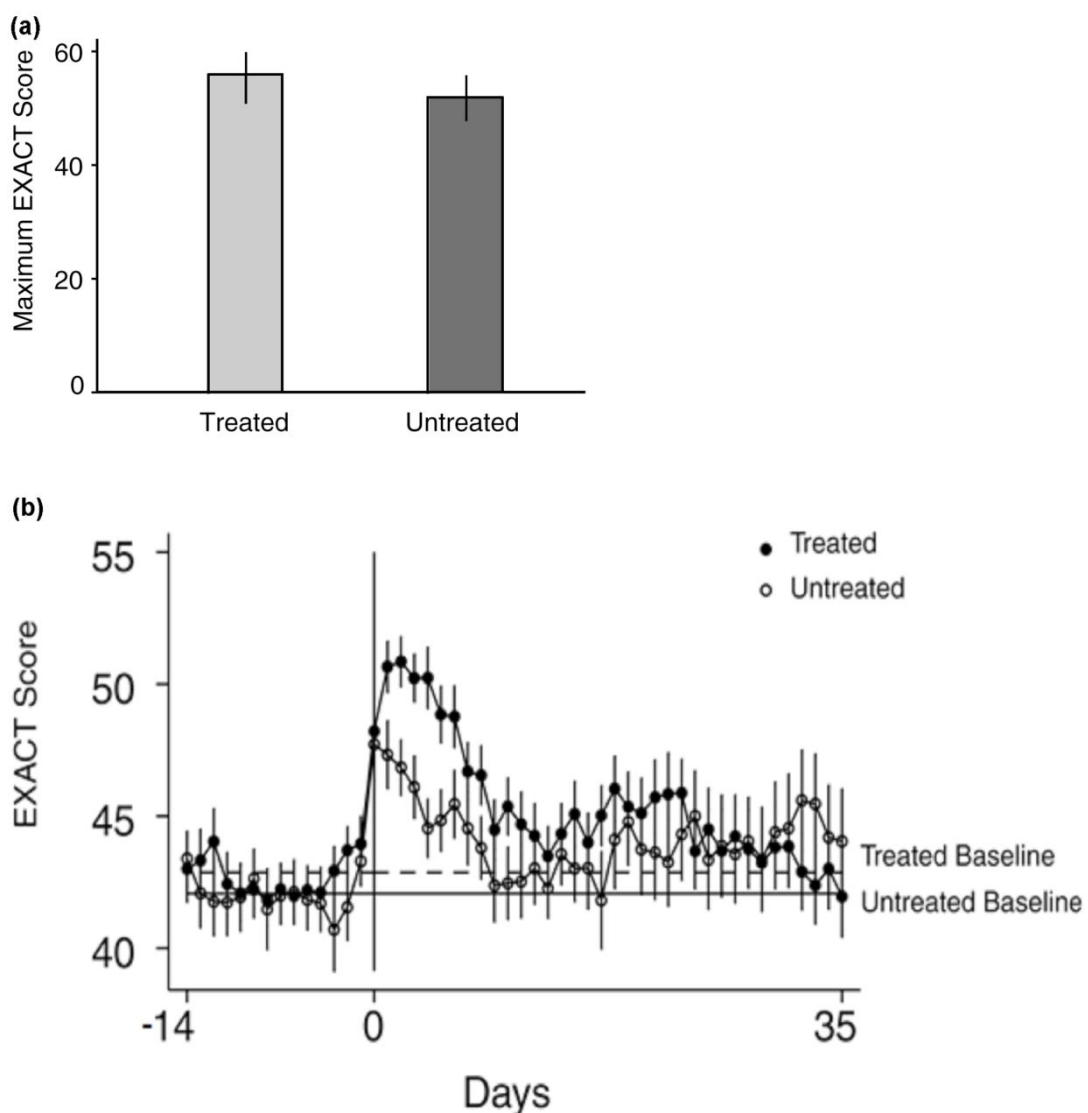
Figure 4.12. (a) Maximum EXACT scores in COPD patients treated with (▢) and without (▪) increased systemic therapy at exacerbation. Vertical lines represent standard errors. (b) Time course of EXACT scores during treated (●) and untreated (○) exacerbations. Vertical lines represent standard errors.

EXACT Concordance

Relationship between EXACT scores and London COPD cohort diary card-defined exacerbations

58 patients had 128 London COPD cohort diary card defined exacerbations, 27 of which (21.1%) attained a 12 point increase in EXACT score above baseline for 2 consecutive days. 34 of 128 (26.6%) of exacerbations attained a 9 point increase for 3 consecutive days. 57 (44.5%) of 128 exacerbations attained a 12 point increase in EXACT score above baseline at least once during the 14 day period following exacerbation onset. On average this threshold score of 12 was breached 4.4 days after symptomatic onset. 59 of 128 exacerbations (46.1%) had a 9 point increase in EXACT score above baseline at least once during the 14 day period following exacerbation onset, on average 3.7 days after symptomatic onset.

Relationship between EXACT scores and healthcare utilisation (HCU) exacerbations

85 (66.4%) of 128 London COPD cohort diary card defined exacerbations were treated with additional systemic therapy (oral antibiotics and/or corticosteroids) by the study team, consistent with a moderate severity HCU exacerbation. 22 of 85 (25.9%) HCU exacerbations attained a 12 point increase in EXACT score above baseline for 2 consecutive days. 29 of 85 (34.1%) attained a 9 point increase for 3 consecutive days. The 12 point threshold was breached at least once during the 14 day period following exacerbation onset in 43/85 (50.6%) of these HCU exacerbations and the 9 point threshold in 44/85 (51.8%).

Relationship between symptom-defined events using the EXACT and London COPD cohort diary card and HCU exacerbations

The scoring algorithm for a symptom-defined event using the EXACT is an increase of 12 points above the patient's mean baseline for 2 consecutive days or an increase of 9 points above the patient's mean baseline for 3 consecutive days. Previous analyses for the EXACT have examined the day of presentation of an HCU exacerbation ± 7 days for the presence of an EXACT event (Leidy & Murray, personal communication, 2013). In this study we analysed the period of exacerbation onset ± 7 days, as defined by London COPD cohort diary cards. 86 diary card defined exacerbations had a complete dataset of daily EXACT scores during this period. 24 (27.9%) of these exacerbations met the EXACT threshold for a symptom-defined event. Of these 86 exacerbations with a complete dataset, 52 were HCU exacerbations, 18 (34.6%) of which met the criteria for an EXACT event.

Alternative thresholds for concordance

A number of alternative thresholds were examined in an attempt to find a level that provides improved concordance compared to the 9 and 12 point thresholds. The highest sensitivity and specificity was attained using a threshold of 7.5 points for 2 consecutive days under which 43 of 85 HCU exacerbations would be consistent with this definition. This provided a sensitivity of 62.35% and specificity of 58.14% giving an area under the receiver operating characteristic curve of 0.55.

Relationship between exacerbation EXACT scores and baseline disease severity

Patients exhibited smaller rises in EXACT score at exacerbation as baseline disease severity
(judged by baseline EXACT score) increased. **Figure 4.13** shows that change between
maximum exacerbation and baseline EXACT score seen at London COPD cohort diary card
exacerbation was significantly related to baseline EXACT score (rho =-0.61, p<0.001)
allowing for repeated measures.

Figure 4.13. Relationship between baseline EXACT score and maximum rise in EXACT
score at exacerbation. Black filled dots (●) represent HCU exacerbations. White unfilled
dots (○) represent London COPD cohort diary card defined exacerbations which were not
treated with additional systemic therapy.
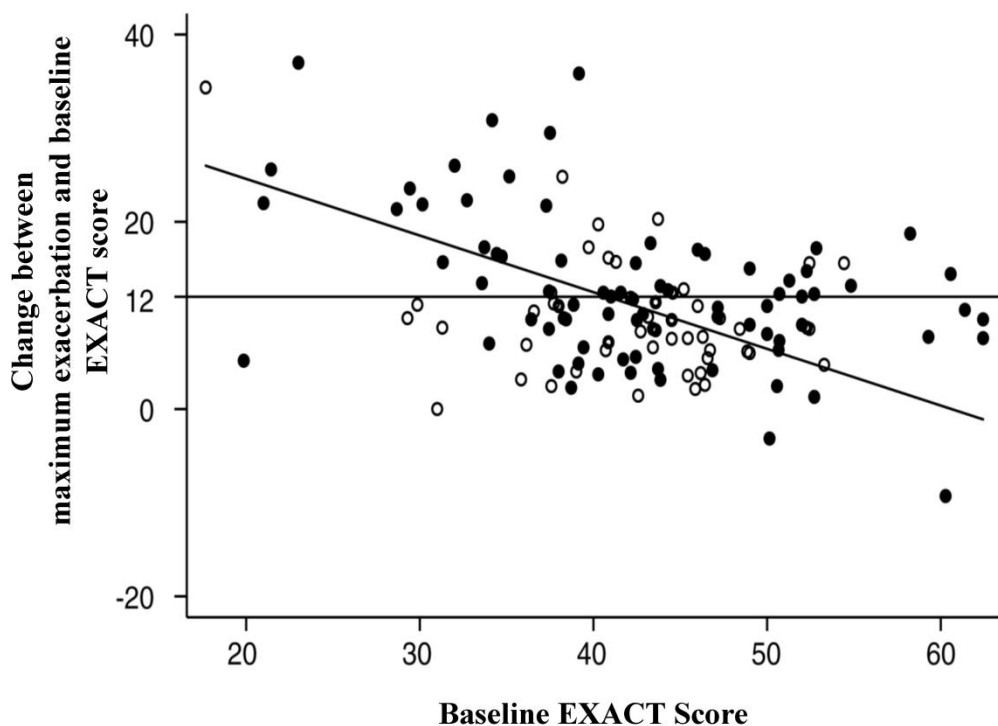
4.2.5 Discussion

This novel study is the first to independently validate the EXACT as an effective tool to assess exacerbation severity. EXACT scores increase at COPD exacerbation, the magnitude of which reflects the severity of the event in terms of treatment, systemic inflammation, airflow limitation and symptom recovery time. For the first time this work studied the EXACT against the validated London COPD cohort daily symptom diary card (8, 11, 13) and thus uniquely examined the complex relationship between symptom-defined events using the EXACT and both healthcare utilisation (HCU) exacerbations and London Cohort diary-card identified, untreated exacerbations. Approximately 50% of both diary card-defined and HCU exacerbations crossed the EXACT event threshold but only approximately one third fulfilled the criteria for an EXACT event. Thus this study has highlighted important potential limitations of the EXACT in its ability to independently identify events which were captured by physician review (HCU) or London COPD cohort diary cards. Baseline disease severity appears to play an important role in symptom reporting and physician prescribing thresholds at exacerbation.

In this study, EXACT scores at exacerbation were significantly related to systemic inflammation, as measured by concurrent levels of serum CRP. This is an important asset for a successful outcome measure of exacerbation severity since elevated systemic inflammation during exacerbations relates to both clinical non-recovery and exacerbation recurrence (56). Furthermore, the time taken for EXACT scores to return to baseline was significantly related to recovery time as judged by London COPD cohort symptom diary cards and was also modestly correlated with lung function recovery as measured by PEFR. Thus these data supports the use of the EXACT as an effective tool to measure exacerbation severity and assess recovery, particularly suited to trials of interventions to treat acute exacerbations. In

this setting, the relative proportion of patients whose EXACT scores have returned to baseline (or fallen by a predetermined magnitude) by 7 days (the median recovery time in a study evaluating recovery in over 500 exacerbations (13)) could be a valuable outcome measure.

In this work, patients completed both London COPD cohort diary cards and EXACT questionnaires prospectively to allow us to examine EXACT changes during unreported exacerbations which were not treated with increased systemic therapy. EXACT scores recorded on the day of exacerbation onset were the same for both reported and unreported events. However, while no significant difference was seen in total symptomatic burden or exacerbation length as judged by EXACT scores between either type of exacerbation, the pattern of recovery observed was different, and maximum EXACT scores recorded during the exacerbation were significantly higher in treated compared to untreated, unreported events. Furthermore, the lack of statistical significance in the total net EXACT recovery score may be due to insufficient sample size. These results demonstrate for the first time that the EXACT is responsive to both reported, HCU exacerbations, and unreported exacerbations where patients do not report symptomatic deterioration to primary care physicians or members of a research team. Identification of unreported exacerbations may be a particular advantage in pharmaceutical interventional trials which historically have experienced unexpectedly low rates of HCU exacerbations (116, 117).

The assessment of exacerbation frequency during clinical trials was an initial aim in the development of the EXACT (26, 27). To facilitate the identification of unreported exacerbations, the instrument pre-specifies a threshold for these symptom-defined events based on a persistent increase in EXACT score over 2-3 days.   In this study, the relationship

between exacerbations identified using the HCU definition, the London Cohort diary card, and the EXACT was investigated. The strength of the relationship was modest. It should be noted that patients in the London COPD cohort are instructed to report increased respiratory symptoms as recorded in their diary to ensure prompt assessment and therapy as required, and were not instructed to report based upon EXACT responses. Some studies have attempted to use EXACT scores to enhance reporting of exacerbations by remotely monitoring EXACT scores in real-time and using worsening scores to generate an alert regarding a possible exacerbation (118), however this was not the case in our study.

Both the London COPD cohort diary cards and the EXACT are responsive to a worsening of respiratory symptoms. However the EXACT requires the increase in symptoms to meet a strict numerical threshold to fulfil criteria for symptom-defined events using the EXACT, thus potentially increasing the likelihood of undercounting relative to London COPD cohort diary card defined exacerbations. Furthermore, the seminal event for the calculation of concordance of EXACT symptom-defined events is more typically the HCU exacerbation, with the date of treatment administration as the day of onset, unlike in this paper where cohort diary cards were used to define the onset of events and then examined the corresponding EXACT scores of both HCU and London COPD cohort diary card defined exacerbations.

Patients in the London COPD Cohort are specifically trained to rapidly recognise and report the increased respiratory symptoms that characterise COPD exacerbations. This may also have impacted on the relationship between symptom-defined events using the EXACT and HCU exacerbations seen in the clinic. Since our patients present early in the course of their exacerbation and receive prompt systemic treatment, this may alter the evolution of

symptoms for HCU exacerbations compared to when presentation is markedly delayed. In such circumstances, if patients do not commence therapy until late in their exacerbation, their symptoms may be of a higher intensity, and thus be more likely to cross the threshold for symptom-defined events using the EXACT. Nonetheless, despite these caveats it remains a concern that only approximately one third of HCU exacerbations within this study fulfilled the criteria for an EXACT event. The strength of the relationship between EXACT events and HCU exacerbations in our study is consistent with preliminary data from the FORWARD trial, which used the traditional physician diagnosis of exacerbations as a co-primary outcome, and the EXACT to enhance exacerbation detection, and also found marked inconsistencies between EXACT events and HCU exacerbations (118, 119).

The relationship between EXACT scores and HCU exacerbations was examined further by exploring the role of baseline COPD disease severity. At baseline, EXACT scores accurately reflected disease severity as judged by lung function impairment and London COPD cohort diary card exacerbation frequency. At exacerbation, patients exhibited smaller rises in EXACT score as baseline disease severity increased, suggesting that patients with more severe stable disease are more likely to report and receive additional systemic therapy at exacerbations associated with smaller increases in symptom intensity than patients with milder baseline disease. This result confirms that people seek care and are treated for exacerbations for a variety of reasons, including varied tolerance to symptomatic change. More severe patients may be more sensitive to change in symptoms, more frightened by smaller changes, or be better trained and therefore report their changes earlier. The London cohort is carefully trained to detect change and seek treatment early, and these results support the success of this program. These results also suggest that the EXACT thresholds for symptom-defined events are conservative, and are not overestimating the frequency of

symptom-defined events that are unreported. It remains important to count HCU events as HCU events, particularly since they remain prominent in major guidelines for the diagnosis of an exacerbation (1). However, all unreported and untreated symptom-defined events also need to be accurately detected with a diary since these events are both common (11, 18, 19) and important, contributing to impaired health status (11, 18).

The EXACT has previously been used in conjunction with a personal digital assistant (PDA) (26, 120) or smartphone (121). A potential limitation of this study is that the EXACT was administered in paper rather than electronic format. However, PROs can be reliably completed at exacerbation and during recovery in paper format (122). Furthermore, since the content of the EXACT is identical in both formats, this is unlikely to significantly alter the results obtained. Indeed, recent work in the Patient Reported Outcomes Measurement Information System (PROMIS®) initiative has confirmed that method of administration, including comparisons of PDA versus paper questionnaire, does not lead to statistically or clinically significant differences in score levels or psychometric properties of patient reported outcome tools (123). The use of a paper version of the tool also ensures that the results of the study are applicable to the widest range of COPD patients, since patients were not excluded because they were not technically capable of using an electronic instrument.

This work demonstrated that the EXACT is an effective tool to evaluate exacerbation severity in outpatient settings when the event was captured by physician review (HCU) or by London COPD cohort diary cards. Future studies should also assess the efficacy of the instrument in hospitalised patients and can use the EXACT to assess both the maximum symptomatic intensity of exacerbations and the total symptomatic burden of events.

4.2.6 Conclusion

In conclusion, the EXACT is an effective method of evaluating exacerbation severity. EXACT scores reflect severity as determined by lung function, exacerbation length and systemic inflammation. The tool is responsive to both treated and untreated exacerbations and can be effectively used in conjunction with daily symptom diary cards to provide novel outcome measures in clinical trials of acute exacerbation therapies. However, uncertainty remains regarding the effectiveness of the instrument to independently and accurately detect the onset and frequency of exacerbations, a particular concern in the study of preventative therapies for COPD exacerbations.

This work formed the basis of an original publication published in the European Respiratory Journal:

Detection and Severity Grading of COPD Exacerbations Using The Exacerbations of Chronic Obstructive Pulmonary Disease Tool (EXACT).

Mackay AJ, Donaldson GC, Patel AR, Singh R, Kowlessar B, Wedzicha JA.

European Respiratory Journal. 2014 March;43(3):735-44. doi: 10.1183/09031936.00110913.

5

MONITORING COPD EXACERBATION RECOVERY USING OBJECTIVE COUGH FREQUENCY MEASUREMENTS

The previous chapter has demonstrated that patient reported outcomes can provide important information in the assessment of exacerbation severity. Following this we wished to explore the ability of objective physiological tools to enhance exacerbation evaluation using ambulatory cough monitors.

5.1 Introduction

Cough (either with or without associated sputum expectoration) is one of the principle symptoms of COPD, along with dyspnoea. Epidemiological studies have found that the reported presence of chronic cough and mucus hypersecretion may be linked to accelerated decline in lung function (29), more frequent exacerbations (30), and hospitalisations (31) in COPD patients. However, previous work has shown that subjective measures of cough and cough reflex sensitivity are only moderately correlated to objective measures of cough (32), and have insufficient predictive value for understanding the determinants of cough.

Colleagues at the University of South Manchester have developed a system for recording sounds over 24 hours in ambulatory patients, which allows the objective quantification of coughing (Vitalojak; Vitalograph Ltd., Buckingham, UK) (34). In a recent publication, this group showed that in the stable state, current healthy smokers and COPD ex-smokers have similar cough frequencies, both significantly greater than healthy non-smokers. The degree of neutrophilic airway inflammation measured in sputum samples from COPD patients in the stable state was positively related to cough frequency, independent of the influence of current smoking. In those patients who provided sputum samples, over a third of the variation in cough rates was explained by the degree of neutrophilic airway inflammation and current smoking (34).

Exacerbations are symptomatic deteriorations in respiratory symptoms and cough plays a prominent role in these events. Recovery from exacerbations is often prolonged and cough may be a protracted feature of some events (13). Exacerbations are also characterised by increased neutrophilic airways inflammation (36, 37) and frequent exacerbators have higher neutrophilic inflammation than infrequent exacerbators in the stable state (38). A subset of

COPD patients are also associated with increased eosinophilic inflammation when stable and at exacerbation (52) and eosinophilic inflammation is known cause of chronic cough (124). Therefore objective cough monitoring may be a useful tool both in the stable state and during acute exacerbations of COPD to assess exacerbation severity and recovery.

Thus in collaboration with the University of South Manchester I examined the utility of objective cough monitoring to differentiate between stable COPD and exacerbation and whether cough frequency can distinguish patient exacerbation frequency and inflammatory phenotype in the stable state.

5.2 Aim

We hypothesised that frequent exacerbators would exhibit a higher cough frequency than infrequent exacerbators when stable. We also hypothesised that cough monitoring could serve as an objective means of determining the severity of an exacerbation, with those exacerbations with higher cough frequency at exacerbation experiencing prolonged exacerbation length and increased inflammation. Furthermore, this work examined whether cough monitoring could be a useful objective tool to assess exacerbation recovery and would identify those exacerbations at high risk of recurrence. Therefore, in this study patients were prospectively examined with an objective cough monitor in the baseline stable state, at exacerbation presentation and thereafter for five weeks during the recovery period.

5.3 Methods

Full methodology for this chapter is described in methods section 3.3. This study involved 64 COPD patients enrolled in the London COPD cohort between 1st March 2013 and 1st April 2014. Cough monitors were used to measure 24 hour cough frequency at baseline, exacerbation and during recovery. Cough monitors were placed on patients at the end of clinic visits, following completion of sputum sampling, blood sampling and spirometry. Equipment was worn for the immediate 24 hours after each visit and then returned for data download the following day.

Exacerbations were treated according to the prevailing guidelines and clinical judgment with increased inhaled therapy, antibiotics and/or oral steroids. When patients attended for an exacerbation, venous blood samples for full blood count, CRP and Fibrinogen were taken and spirometry performed prior to commencing exacerbation treatment.

Statistical Analysis

Data were analysed using IBM SPSS Statistics for Windows, Version 22.0 (Armonk, NY: IBM Corp) and GraphPad Prism 6 (La Jolla, CA, USA).Normally distributed data were expressed as mean and standard deviation (SD) and skewed data as median and interquartile range (IQR). For normally distributed data, unpaired t tests were used to assess differences between independent groups, for non-parametric data the Mann-Whitney U test was used. Paired non-parametric data was analysed using Wilcoxon matched-pairs signed rank test.

5.4 Results

Patient Characteristics

Full baseline clinical characteristics of the 64 patients included in this analysis are reported in

Table 5.1. Patients had moderate to severe COPD with a mean FEV_1 % predicted of 54.4%.

Table 5.1. Stable state clinical characteristics of the 64 patients included in the baseline analysis.

	Mean±SD
Age (years)	74.1±7.1
FEV_1 (l)	1.35±0.53
FEV_1 (% predicted)	54.5±19.6
FEV_1/ FVC (%)	47.9±13.6
Smoking pack years	52.0±32.3
SpO_2 (%)	95.1±2.2
BMI (kg/m2)	25.4±5.3
	Median (IQR)
HealthCare Utilisation Exacerbations in year prior to assessment	2.00 (1.00-3.00)
	n=
Male, n (%)	41 (64.1)
Current Smokers, n (%)	17 (26.6)
Comorbidity present, n (%)	50 (78.1)
Congestive heart failure, n (%)	4 (6.3)
Ischemic heart disease, n (%)	14 (21.9)
Hypertension, n (%)	32 (50.0)
Patients receiving ACE inhibitors, n (%)	7 (10.9)
Diabetes Mellitus, n (%)	3 (4.7)
Patients receiving maintenance inhaled corticosteroids, n (%)	51 (79.7)
Bronchitic Phenotype, n (%)	33 (51.6)

Relationship between baseline cough frequency and exacerbation status

There was no significant difference seen in 24 hour total cough frequency (coughs/hour, c/h) between frequent exacerbators (patients with 2 or more exacerbations in the year prior to assessment) and infrequent exacerbators (patients with less than 2 exacerbations in the year prior to assessment (n=38, median 5.6 (IQR 2.8-8.6) coughs/hour vs. n=26, 6.6 (2.2-15.7), p=0.702). Furthermore, there was no significant relationship between 24 hour total cough frequency and total number of exacerbations (treated and untreated events) experienced in the year prior to assessment (regression coefficient = -0.039; p=0.758). Also, no relationship was seen between exacerbation frequency and 24 hour total cough frequency when just treated, health care utilization events, were analysed (regression coefficient = -0.024; p=0.851).

There was also no difference in diurnal cough variation between frequent and infrequent exacerbators. Total cough frequency per hour whilst awake for frequent exacerbators was not significantly different than infrequent exacerbators (n=38, median 7.5 (3.1-12.3) vs. n=26, 9.3 (2.5-19.8), p=0.637). Total cough frequency per hour whilst asleep for frequent exacerbators was not significantly different than infrequent exacerbators (n=38, median 0.9 (0.2-4.8) vs. n=26, 1.6 (0.1-3.4), p=0.794).

Higher cough frequency was significantly related to worsening health-related quality of life, as measured by concurrent CAT score and SGRQ. Baseline CAT scores recorded on the day of cough recording were significantly related to $\log_{10}$ transformed 24 hour cough frequency (n=49, r=0.299, p=0.037) and SGRQ scores were also significantly related to $\log_{10}$ transformed 24 hour cough frequency (n=59, r= 0.3912, p= 0.002; **Figure 5.1**).
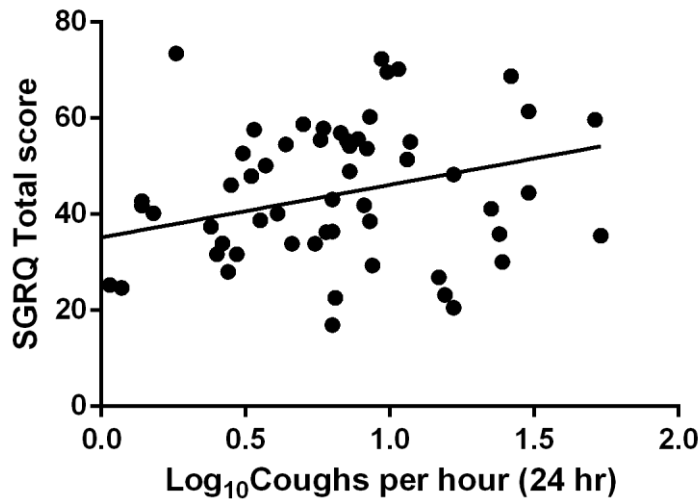
Figure 5.1. Relationship between $\log_{10}$Coughs per hour (24 hr) and SGRQ score at baseline (59 patients).

Bronchitic patients had significantly higher waking cough rates than non-bronchitic patients (n=33, mean 0.9 (SD 0.4) vs. n=31, 0.64 (0.7) $\log_{10}$cough/h, p=0.046). There was also a trend for bronchitic patients to have higher 24hour cough frequency than non-bronchitic patients (n=33, mean 0.8 (0.4) vs. n=31, 0.5 (0.7) $\log_{10}$cough/h, p=0.078). However, no significant difference was seen in sleep cough rates between bronchitic and non-bronchitic patients (n=33, mean -0.4 (1.6) vs. n=31, -0.9 (2.1) $\log_{10}$cough/h, p=0.310).

The baseline inflammatory phenotype of patients did not affect cough frequency. No significant difference was seen in 24 hour total cough frequency between eosinophilic patients (baseline blood eosinophilic percentage $\geq$ 2%) and non-eosinophilic patients (baseline blood eosinophilic percentage < 2%) (n=18, median 7.4 (IQR 3.0-10.9) coughs/hour vs. n=34, 4.8 (2.6-9.3), p=0.403).

The presence or absence of comorbidities, including self-reported gastro-oesophageal reflux, did not affect baseline cough frequency. Median 24 hour cough frequency in patients with 1 or more comorbidities was not significantly greater than in patients without comorbidities (n= 50, median 6.1 coughs/hour (IQR 2.7-11.6) vs. n=14, 5.9 (2.4-6.9), p=0.808). Furthermore,

median 24 hour cough frequency in patients with self-reported gastro-oesophageal reflux was not significantly greater than in patients without reflux symptoms (n=14, 6.7 (2.2-8.2) vs. 5.8 (2.7-12.3), p=0.691).

Exacerbation studies

Exacerbation Characteristics

17 patients performed cough recording at exacerbation presentation. All exacerbations were health-care utilisation (HCU) exacerbations, receiving additional systemic therapy (all patients received oral antibiotics and 13 of 17 (76%) patients also received oral prednisolone).

Unpaired Exacerbation Data

24-hour cough rate rose from a median baseline value of 6.0 (n=64, IQR 2.7-9.7) to 25.9 (n=17, 9.0-31.7; p<0.001, **Figure 5.2**) coughs/h at exacerbation.
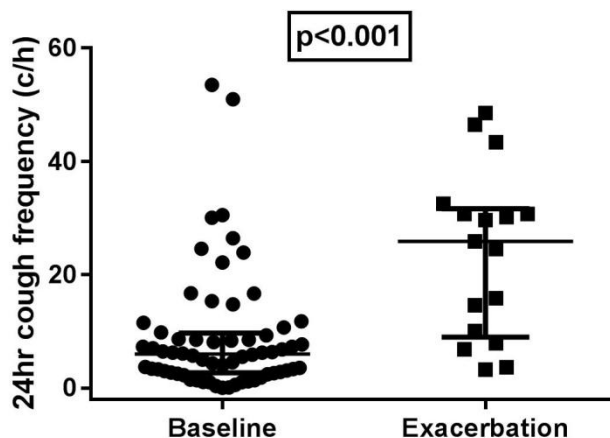
Figure 5.2. Median 24 hour cough frequency in patients at baseline (n=64) and exacerbation (n=17). Bars represent Interquartile Range.

Paired Exacerbation Data

13 patients had paired cough recordings performed at baseline and also at exacerbation onset. Exacerbation 24-hour cough rate was significantly higher than paired baseline levels: median exacerbation 24-hr cough rate 30.2 (16.2-38.0) vs. 6.5 (3.1-18.0) coughs/h, p=0.008 (**Figure 5.3**).

Figure 5.3. Change in 24hr cough frequency seen in 13 paired patients from baseline to exacerbation.
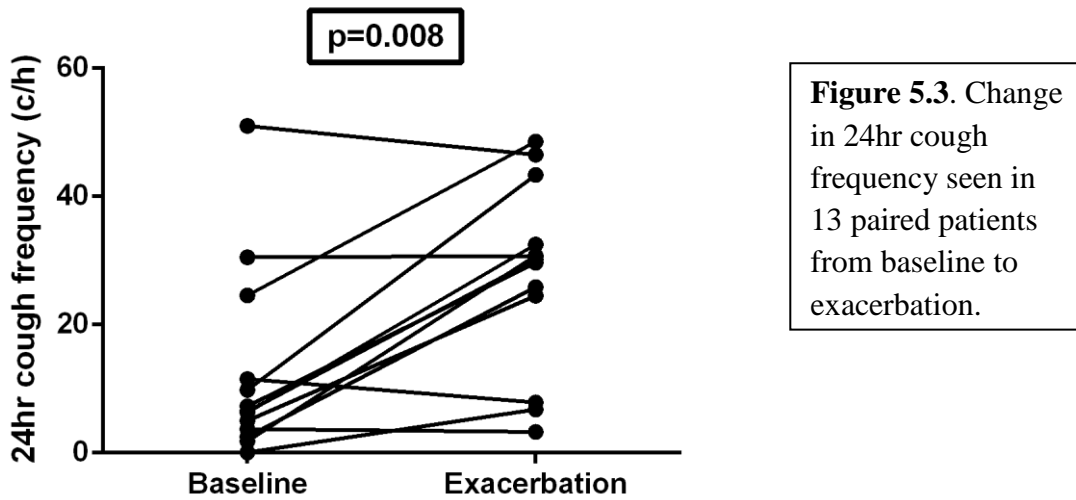
Both day and night cough rates were significantly higher at exacerbation than at baseline, with a larger relative increase in nocturnal cough rates than daytime. Day exacerbation cough rate 35.6 (17.5-45.3) vs. 9.6 (4.5-23.7) coughs/h, p= 0.011 (**Figure 5.4**).

Figure 5.4. Change in day cough frequency seen in 13 paired patients from baseline to exacerbation.
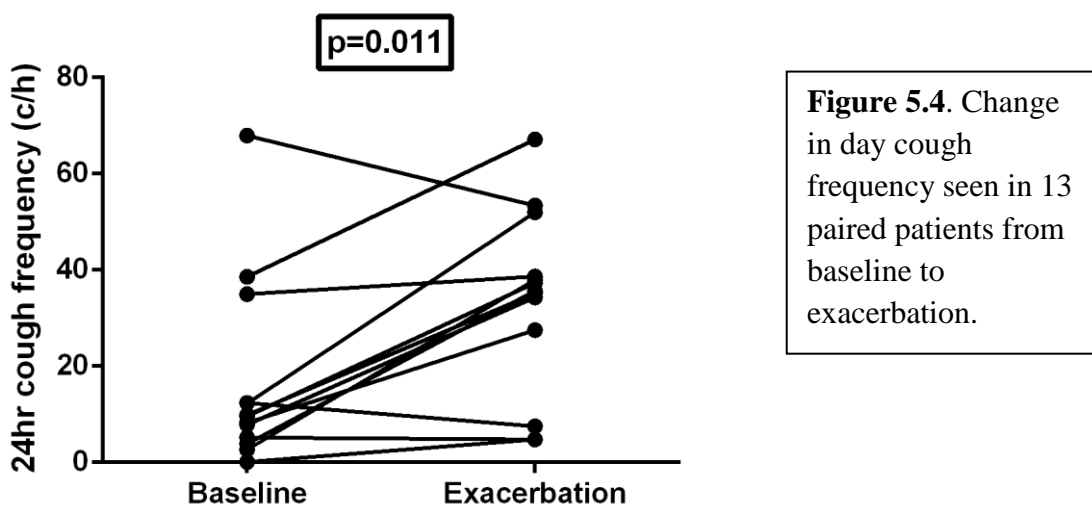
Night exacerbation cough rate 13.2 (8.8-23.1) vs. 0.7 (0-4.2) coughs/h, p= 0.005 (**Figure 5.5**).

Figure 5.5. Change in nocturnal cough frequency seen in 13 paired patients from baseline to exacerbation.
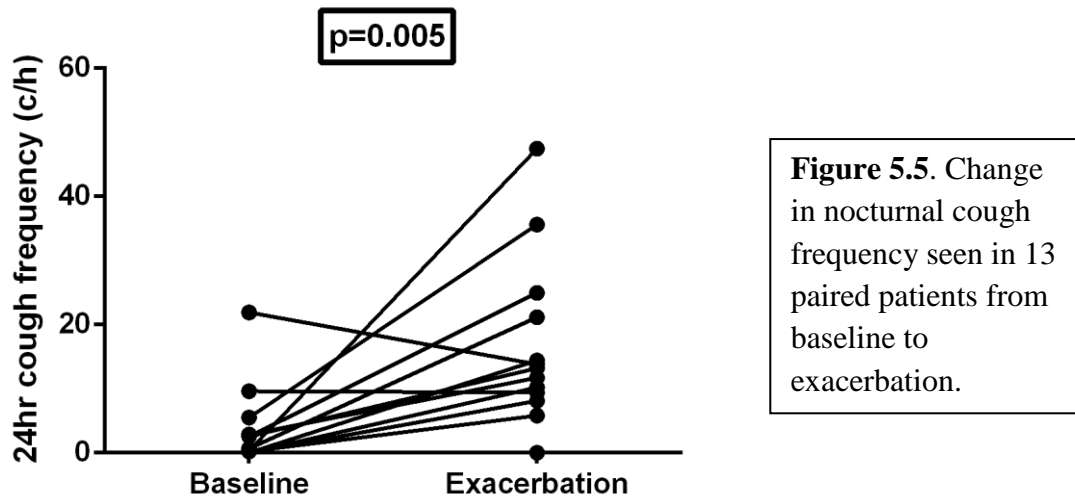
Exacerbation Phenotype

Eosinophilic exacerbations (blood eosinophilic percentage ≥ 2%) did not have higher cough frequency than non-eosinophilic events (n=6, median exacerbation 24-hr cough rate 25.2 (7.6- 34.3) vs. n=9, 30.7 (12.4- 38.0) coughs/h, p= 0.578).

Exacerbation Severity

No significant correlation was seen between 24-hour cough rate at exacerbation and serum inflammatory markers measured concurrently (see **Table 5.2**).

Serum Inflammatory Marker	Spearman r value	P value
CRP	-0.373	0.157
Fibrinogen	-0.207	0.489
Total white cell count	-0.214	0.436
Absolute Eosinophil Count	0.043	0.882
Percentage Eosinophils	0.014	0.964

Table 5.2. Relationship between 24-hour cough rate at exacerbation and serum inflammatory markers measured concurrently.

Furthermore, cough frequency was not significantly related to contemporaneous spirometry, as measured by FEV_1. No significant correlation was seen between the rise in 24 hour cough rate and the fall in FEV_1 from baseline to exacerbation (Spearman r = 0.132, p = 0.665).

Exacerbation Length

It was possible to calculate exacerbation length in 15 of 17 exacerbations. 2 patients did not accurately complete symptom diary cards and so an accurate exacerbation length could not be calculated. Median exacerbation length was 13 days (7-24).

Cough frequency at exacerbation was not related to exacerbation length: no significant correlation was seen between 24 hour cough rate and exacerbation length (Spearman r = -0.057, p = 0.832). Furthermore, no significant correlation was seen between the rise in 24 hour cough rate from baseline to exacerbation and exacerbation length (Spearman r = -0.169, p = 0.442).

Exacerbation Recurrence

Cough monitoring at exacerbation was not useful to predict exacerbation recurrence. Those index exacerbations which were followed by a second recurrent exacerbation with 56 days did not have a significantly higher exacerbation cough rate than those not followed by a recurrent exacerbation (n=6, median cough rate 23.3 (13.5-47.0) vs. n=10, 27.8 (6.0-31.2) coughs/h, p= 0.366, **Figure 5.6**).

Figure 5.6. Median 24 hour cough frequency in index exacerbations which were followed by a recurrent exacerbation (n=6) and those without exacerbation recurrence (n=10). Bars represent Interquartile Range.
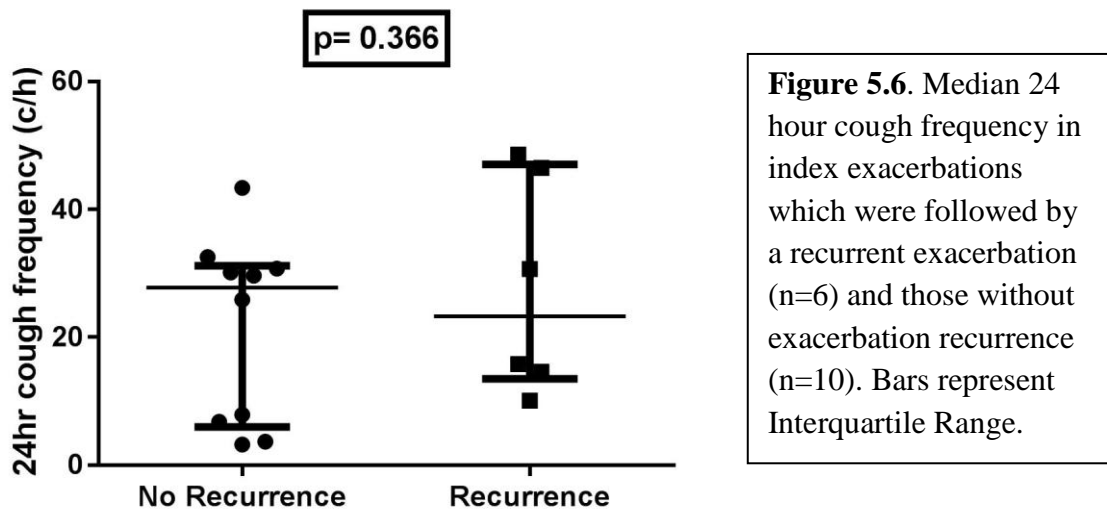
10 patients experienced a further exacerbation within 99 days of the index event at which monitoring was performed. In those 10 patients where time to next exacerbation could be calculated, no significant correlation was seen between the 24 hour cough rate at index exacerbation presentation and time to next exacerbation (Spearman r = -0.176, p = 0.613). Furthermore, no significant correlation was seen between the rise in 24-hour cough rate and time to next exacerbation (Spearman r = 0.342, p = 0.459).

Timecourse of cough frequency during exacerbation recovery

6 patients completed paired recordings at exacerbation presentation and during exacerbation recovery 2 weeks after their index visit. Cough frequency at exacerbation presentation was significantly higher than at 2 weeks (n=6, median cough rate 31.7 (26.7-44.7) vs. 16.5 (8.4-24.8) coughs/h, p= 0.031). Exacerbation cough frequency fell further from 2 weeks to 5 weeks post exacerbation presentation, although due to limited numbers this was not statistically significant (n=3, median cough rate 13.7 (1.5-19.3) coughs/h vs. 3.0 (1.3-10.4) coughs/h, p= 0.250).
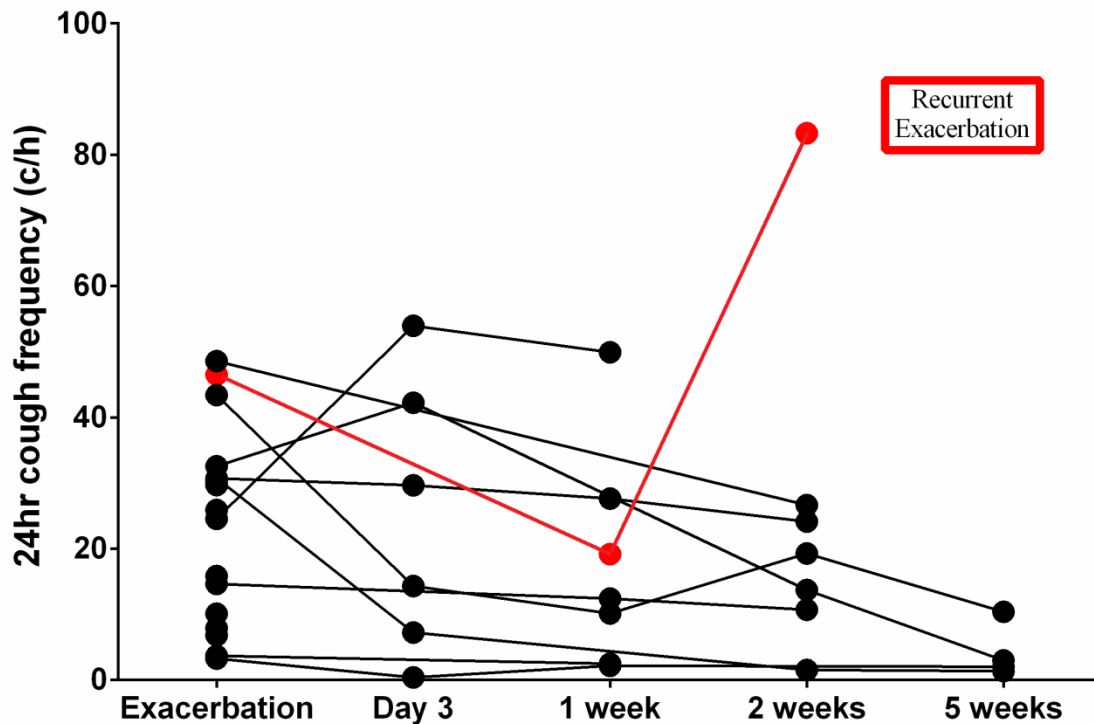
Figure 5.7. Timecourse of cough frequency during exacerbation recovery.

11 of 17 patients performed cough monitoring at least once during the 5 weeks following exacerbation recovery. One patient also performed cough monitoring at a recurrent exacerbation occurring 14 days after presentation for the index exacerbation. In this event the patient experienced an acute rise in cough frequency following symptomatic recovery, which had been achieved one week after exacerbation presentation (**Figure 5.7**).

5.5 Discussion

This novel study is the first to measure objective cough frequency in COPD patients at baseline and at exacerbation onset prior to the initiation of systemic treatment. In the stable state, increasing cough frequency was associated with worsening health related quality of life. Cough frequency increases acutely from baseline levels at exacerbation and falls during subsequent recovery. However, the rise in cough frequency at exacerbation onset did not predict exacerbation severity, as reflected by decline in lung function and systemic inflammatory levels. Furthermore, cough frequency at exacerbation onset did not predict recovery time or the likelihood of recurrence.

Cough is one of the primary symptoms of COPD (1). Chronic cough and mucous hypersecretion has been associated with lung function decline (29) and more frequent COPD exacerbations (30) and is an important area of unmet need in COPD. Previous work has validated objective ambulatory cough recording in COPD and shown that smoking and neutrophilic airway inflammation are key determinants of cough frequency in COPD patients (34).

This study complements this work by reinforcing the importance of cough in COPD patients. In the stable state patients with increasing cough frequency had worse health related quality of life as measured by both the SGRQ and the CAT. Bronchitic patients had higher baseline cough rates than non-bronchitic patients and this is consistent with previous data that reported sputum production is an important determinant of cough frequency (34). The presence of chronic bronchitis is an important phenotype amongst COPD patients (125) and targeting this phenotype using specific therapies improves outcomes in COPD patients (94). Prophylactic azithromycin has recently been shown to improve cough-specific health status in COPD-

patients with chronic productive cough to a clinically relevant degree (126) and our study reaffirms that further specific treatments are also needed to target COPD patients with increased cough frequency to improve health related quality of life.

The presence of chronic bronchitis amongst COPD patients is associated with increased exacerbation frequency compared to non-bronchitic patients (127). Patients with a history of frequent exacerbations have worse quality of life (11), increased risk of hospitalisation (114) and greater mortality (12). Frequent exacerbators also exhibit faster decline in lung function (8) and may have worse functional status, as measured by time outdoors (115). We hypothesised that frequent exacerbators would experience increased cough frequency in the stable state than infrequent exacerbators. However, frequent exacerbators did not have higher baseline cough frequency than infrequent exacerbators and we found no relationship between cough rates and exacerbation frequency in the year prior to assessment. No relationship was seen when all exacerbations (both treated and diary card defined, untreated events) or just treated, health care utilisation exacerbations were included in this analysis.

It is possible that our sample size may be underpowered to detect such a difference, although significant differences in cough frequency have been seen between COPD smokers and non-smokers in groups of similar size (34), reducing this possibility. More likely, it may be unrealistic to expect a single baseline cough recording to identify patients who are more susceptible to exacerbations. Exacerbations are heterogenous and complex events that occur when a susceptible individual is exposed to a sufficient trigger, which is usually an infective pathogen (128). The relationship between bronchitis and increased exacerbation frequency is well established (125, 127) and a significant contributor to this relationship is the presence of bacterial colonisation (129). This study assessed the relationship between baseline cough

rates and exacerbation frequency rather than examining the effects of cough and sputum .The presence of sputum purulence is a robust indicator of increasing bacterial load (39) and therefore appears to be a superior indicator of frequent exacerbations than cough frequency alone.

Comorbidities are common and extremely important in COPD, often contributing to symptoms, exacerbations, hospital admissions and mortality (130). In particular, gastro-oesophageal reflux symptoms occur in up to 60% of COPD patients and a history of heartburn or reflux is associated with increased exacerbation frequency (7). However, in this study, the presence of self-reported comorbidities, including gastro-oesophageal reflux, did not affect baseline cough frequency.

In addition to reinforcing the significance of cough in the baseline state, this study has also highlighted the importance of cough as a feature of acute exacerbations in COPD patients. Cough frequency increased significantly from baseline stable levels at exacerbation. In paired recordings, exacerbation cough frequency increased almost 5 fold from recordings conducted in the stable state. However, cough frequency at exacerbation onset did not relate to the intensity of the event as measured by fall in FEV_1 and by concurrent systemic inflammatory markers. Furthermore, cough frequency at exacerbation did not relate to the length of the exacerbation and there was no difference in cough rates between exacerbations followed by a recurrent exacerbation and those without. Thus, objective cough monitoring at exacerbation was not an effective tool to measure exacerbation severity, predict recovery time or the likelihood of recurrence.

We collected limited data on the use of objective cough monitoring during the recovery phase of exacerbations. These preliminary results suggested that cough frequency reduced during exacerbation recovery and therefore cough monitoring may potentially be a useful objective measure of symptomatic (cough) recovery post-exacerbation. However, few patients performed cough monitoring during exacerbation recovery and further studies are required to assess the utility of cough monitoring during the recovery phase.

In addition to the low numbers of recordings obtained during the recovery phase, a significant limitation of this study is the generalisability of cough monitoring in a clinical context. At present cough monitoring equipment is relatively bulky and expensive and the data generated requires sophisticated analysis at specialist centres. Therefore, this technique is not currently suitable for use in clinical practice. However, objective cough monitoring may be a useful tool in trials of new therapies to reduce cough frequency both in the stable state and at exacerbation, to measure the efficacy of such interventions.

5.6 Conclusion

In conclusion, increasing cough frequency was associated with worsening health related quality of life. Cough frequency increases acutely from baseline levels at exacerbation and falls during subsequent recovery. Objective cough monitoring may be a useful tool to measure the efficacy of novel interventions to reduce cough frequency both in the stable state and at exacerbation. However, the rise in cough frequency at exacerbation onset did not predict exacerbation severity, and further study is required to assess the usefulness of cough monitoring during exacerbation recovery.

6

SYSTEMIC AND AIRWAY BIOMARKERS TO EVALUATE COPD EXACERBATION RECOVERY AND RECURRENCE

The previous chapter has demonstrated that cough is a critical symptom at COPD exacerbation but that objective cough monitoring was not a useful method of predicting exacerbation length. As discussed in the previous chapter, cough and sputum is associated with increased airway inflammation and the following chapter evaluates whether direct measurement of systemic and airway inflammatory biomarkers can predict exacerbation non-recovery and recurrence.

6.1 Introduction

Exacerbations are frequently triggered by infective agents and associated with increased airway and systemic inflammation. Not all exacerbations recover and patients are susceptible to another or recurrent exacerbation after an index event. Previous work has demonstrated that in almost 25% of cases, symptoms do not fully resolve 35days after the onset of an exacerbation (56). Furthermore, in the same study 22% of patients had a recurrent exacerbation within 50 days of the first (index) exacerbation.

Patients who failed to recover within 5 weeks of exacerbation onset had persistently higher levels of serum CRP during the recovery period than patients who made a full symptomatic recovery during that time (56). The inflammatory burden 14 days after exacerbation onset may be a critical time, with those patients with a recurrent exacerbation having a significantly higher CRP level 14 days after the index exacerbation, compared to those who did not have a recurrence (56).

It is unclear why airway and systemic inflammation remain persistently high in some patients treated appropriately at an initial exacerbation. It is possible that there is persistence of the initial infective agent, acquisition of a new organism or bacterial type (41), or an interaction between pathogens present in the airways in the stable state (61) and also when these pathogens act as exacerbation triggers. However it has not previously been possible to identify which COPD patients are likely to have a prolonged recovery period based on their biochemical characteristics at exacerbation onset, or which features of the index exacerbation predict recurrence (56). Identification of patients at risk of abnormal recovery or recurrence at their initial exacerbation presentation would enable those individuals to be closely followed up and enable early intervention to attempt to enhance recovery and prevent recurrence.

6.2 Aim

We hypothesised that elevated systemic and airway biomarkers at exacerbation onset predict abnormal exacerbation recovery and recurrent exacerbations within 8 weeks after an index event. Therefore, patients in the London COPD cohort were intensively sampled at exacerbation, during their recovery and also subsequent recurrent events were captured. Samples were analysed for systemic and airway biomarkers specifically chosen to inform on exacerbation severity and aetiology.

6.3 Methods

Full methodology for this chapter is described in methods section 3.4. This study involved 67 COPD patients enrolled in the London COPD cohort between 1st May 2010 and 1st July 2013. Patients attended at exacerbation, within 7 days of symptom onset, prior to the onset of systemic therapy and thereafter at recovery monitoring visits at Days 3 (±1), 7 (±2), 14 (±2), 35 (±3) after the initial exacerbation visit. The median interval from diary card exacerbation onset to the day of assessment was 4 days (IQR 3-5). Recurrent exacerbations occurring within 56 days after exacerbation onset were assessed and sampled in the same manner as for an index exacerbation. Exacerbations were treated according to the prevailing guidelines and clinical judgment with increased inhaled therapy, antibiotics and/or oral steroids.

At each visit, patients were assessed, had diary cards reviewed, and sputum and blood was collected for analysis. The following biomarkers were selected based upon their biological/mechanistic plausibility to inform on exacerbation recovery and recurrence. For full details regarding rationale of biomarker selection see **Table 9.1** in Appendix 1.

Blood biomarkers: Total white cell count, eosinophils (absolute and percentage), neutrophils, Fibrinogen, CRP, IL-6, IL-8, IP-10, PLGF, TNFα, MMP-1, MMP-8, MMP-9, sRAGE, PARC, α2-macroglobulin, CC16, SAA

Sputum Biomarkers: IFNγ, IL-18, IL-1β, IL-5, IL-6, TNFα, IL-8, MMP-1, MMP-8, MMP-9, MPO, TIMP-1, α2-macroglobulin

Sputum was also collected at exacerbation presentation for PCR detection of *Haemophilus influenzae, Streptococcus pneumoniae*, *Moraxella catarrhalis* and *Human Rhinovirus (HRV).*

Statistical Analysis

Data were analysed using GraphPad PRISM version 6.0 (GraphPad Software Inc., San Diego, CA, USA) and PASW Statistics version 21 (SPSS Inc., Chicago, IL, USA). Normally distributed data were expressed as mean and standard deviation (SD) and non-parametric data as median and interquartile range (IQR). Exacerbations from the same patient were considered as separate events because their trigger (viral or bacterial), inflammatory response and recovery time could differ within the same individual. Differences between groups were analysed by paired t-test, Mann-Whitney U Test, Wilcoxon-matched pairs, or Kruskal-Wallis analysis, depending on the sample population being investigated. Survival analyses were performed using Log-rank (Mantel-Cox) test. A probability of $p < 0.05$ was considered to be statistically significant.

Patient Characteristics

Full baseline clinical characteristics of the 67 patients included in this chapter are reported in

Table 6.1. The patients had moderate-severe disease with a mean FEV_1 % predicted of 49%.

	Baseline Values n=67
	Mean±SD
Age (years)	71.1±17.0
FEV_1 (L)	1.2±0.4
FEV_1 (% predicted)	49.1±18.0
FVC (L)	2.6±0.8
FEV_1 / FVC ratio (%)	47.5±13.0
Smoking pack years	54.5±42.3
SpO_2 (%)	94.3±2.3
BMI (kg/m^2)	26.4±5.5
	Median (IQR)
Total Exacerbation frequency (treated and untreated events)	2.3 (1.3-3.3)
HCU exacerbations in year prior to assessment	2.0 (1.0-3.0)
	N (%)
Male gender	41 (61.2)
Current Smokers	20 (29.9)

Table 6.1. Stable state clinical characteristics of the 67 patients included in the biomarker analysis.

Isolated Exacerbation Analysis

58 patients were sampled at 75 separate isolated exacerbations. All events were physician confirmed and treated with systemic therapy. 62 exacerbations received antibiotics and oral corticosteroids, 11 antibiotics alone, and 2 oral steroids alone. Mean paired FEV_1 was 1.2 (SD 0.4) L at baseline and 1.1 (0.4) L at exacerbation presentation ($p < 0.001$).

Changes in Blood Biomarkers between Baseline and Exacerbation

Concentrations of the blood biomarkers, at baseline and exacerbation presentation, are reported in **Table 6.2** (2 exacerbations did not have a paired baseline). Serum TNFα levels were below the detectable threshold of the assay and are therefore not reported. Significant differences were observed between baseline and exacerbation for 10 blood biomarkers: CRP, Fibrinogen, IL-6, IL-8, IP-10, PLGF, MMP-1, sRAGE, α2-macroglobulin, SAA (**Figure 6.1**).

Blood Biomarker	Units	Baseline Median (IQR)	Exacerbation Median (IQR)	p-value (Wilcoxon)
CRP	mg/L	3.0 (1.0-7.25)	9.0 (3.5-33.5)	< 0.001
Fibrinogen	g/L	3.6 (3.3-4.1)	4.2 (3.5-5.1)	< 0.001
Total wcc	10^9/L	7.3 (6.4-8.8)	7.8 (6.2-10.1)	0.248
Neutrophils	10^9/L	4.6 (3.9-6.1)	5.0 (3.8-6.7)	0.096
Eosinophils	10^9/L	0.20 (0.11-0.35)	0.15 (0.10-0.29)	0.107
IL-6	pg/ml	8.2 (5.25-12.6)	11.6 (7.3-29.0)	< 0.001
IL-8	pg/ml	0.3 (0.0-1.9)	1.4 (0.5-2.6)	0.010
IP-10	pg/ml	337.8 (261.0-490.6)	266.6 (157.1-404.4)	< 0.001
PLGF	pg/ml	2.3 (0.1-4.5)	4.5 (3.4-7.1)	< 0.001
MMP-1	pg/ml	3601 (1662-6866)	10157 (6488-13449)	< 0.001
MMP-8	pg/ml	2028.0 (1004.0-4614.0)	869.8 (131.6-4850.0)	0.188
MMP-9	pg/ml	1.0 (0.7-1.3) x10^6	1.0 (0.7-1.8) x10^6	0.192
sRAGE	pg/ml	127.5 (57.2-218.5)	155.0 (102.3-271.2)	< 0.001
PARC	pg/ml	137615 (108035-184632)	151714 (110145-199870)	0.070
α2-macroglobulin	pg/ml	1.8 (1.2-3.1) x10^{10}	1.0 (0.6-1.5) x10^{10}	< 0.001
CC16	pg/ml	7400 (4363-14306)	6654 (4266-11882)	0.540
SAA	pg/ml	0.3 (0.2-0.9) x10^8	1.0 (0.3-3.6) x10^8	< 0.001

Table 6.2. Differences in Blood Biomarkers between baseline and exacerbation (73 pairs)
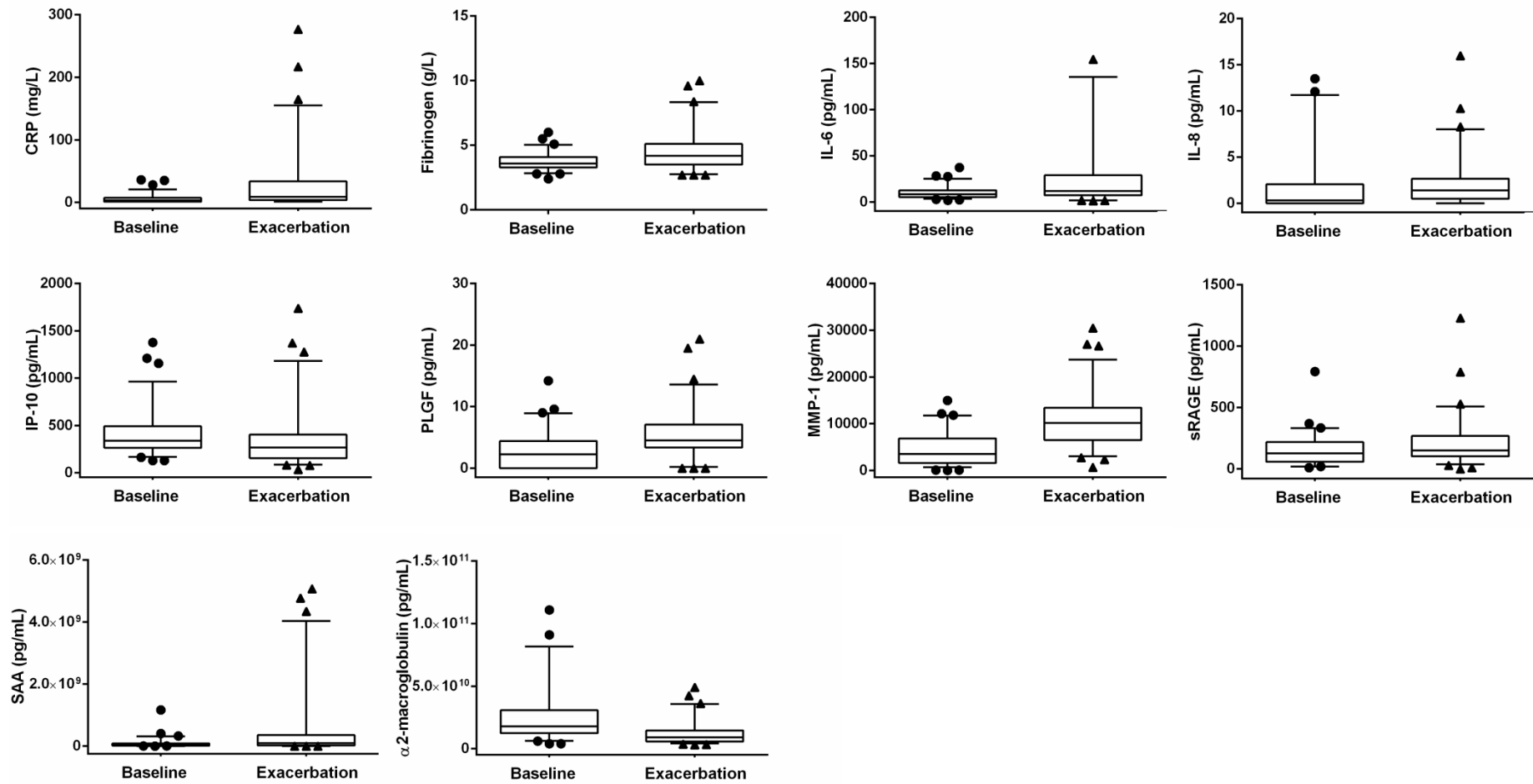
Figure 6.1. Blood biomarkers present at significantly different concentrations between baseline and exacerbation. N=73. Box plots represent median and interquartile range, and whiskers represent 5-95% range with outliers plotted separately.

Median isolated recovery time was 21.5 days (n=70, IQR 12.0-40.25; 5 exacerbations had exacerbation length >99days). Data was analysed to determine the ability of individual biomarkers at exacerbation onset to predict non-recovery (failure of an exacerbation to symptomatically recover) at 35 days after exacerbation onset. This time-point was chosen because previous pivotal studies have analysed non-recovery at 35 days (56). In addition, significant financial penalties can apply to healthcare organizations if patients are readmitted to hospital within 30 days of discharge following an exacerbation of COPD, the median length of stay in hospital prior to discharge being 5 days (131).

Survival analyses were performed on all blood biomarkers which were significantly increased between baseline and exacerbation to determine if elevated biomarkers at exacerbation onset predicted non-recovery at 35 days after exacerbation onset. For the purpose of analysis, elevated biomarkers were defined as those above the median value.

IP-10 and $\alpha_2$macroglobulin fell at exacerbation. For these biomarkers log rank testing examined whether levels below the median value predicted non-recovery, no significant relationship was found.

Median serum IL-6 value at exacerbation presentation in 74 events was 11.75 (7.3-29.0) pg/mL. Log-rank (Mantel-Cox) testing showed that elevated serum IL-6 at exacerbation presentation significantly predicted non-recovery at 35 days (p=0.039, **Figure 6.2**).
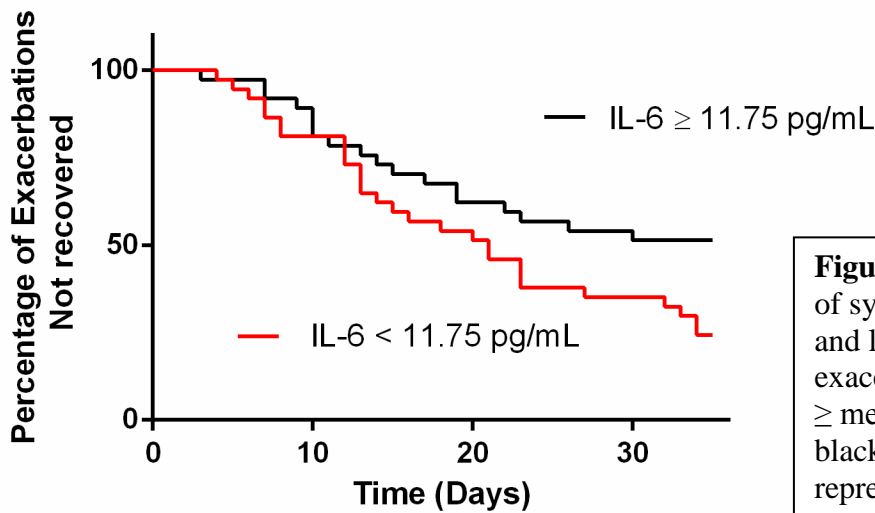
Figure 6.2. Kaplan-Meier plot of symptom resolution by high and low serum IL-6 at exacerbation onset. Serum IL-6 ≥ median is represented by black line and IL-6 < median is represented by red line.

No other blood biomarker showed significant ability to predict exacerbation non-recovery (**Table 6.3**):

Blood Biomarker	p value (Log-rank test)
CRP	0.593
Fibrinogen	0.292
IL-8	0.227
IP-10	0.216
PLGF	0.279
MMP-1	0.525
sRAGE	0.805
α2-macroglobulin	0.600
SAA	0.253

Table 6.3. Results of Log-rank (Mantel-Cox) testing of blood biomarkers at exacerbation onset to predict non-recovery.

Data was also analysed to determine if the change in biomarker levels from baseline to exacerbation onset predicted non-recovery at 35 days after exacerbation onset. Again change in serum IL-6 levels showed predictive ability but there was no such predictive ability in any of the other blood biomarkers (p>0.05 for all), although change in serum IL-8 from baseline to exacerbation approached statistical significance, p=0.065 (**Figure 6.3**). Median change in serum IL-8 value at from baseline to exacerbation presentation in 73 pairs was 0.5 (-0.1-1.8) pg/mL.
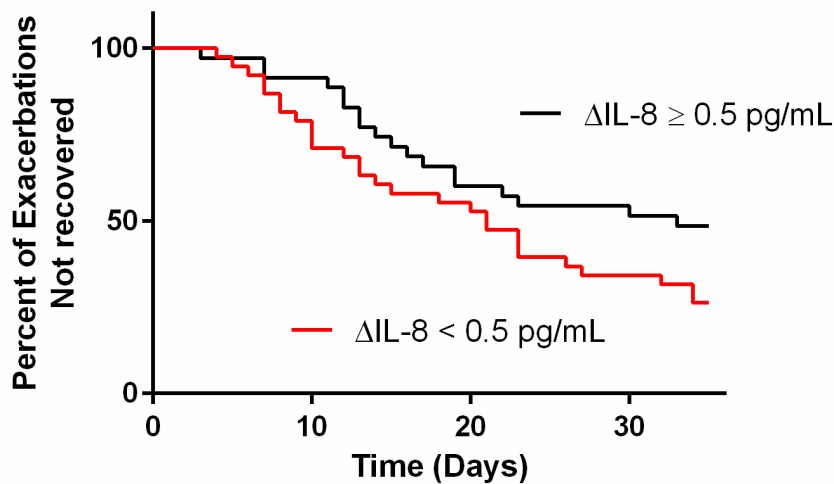
Figure 6.3. Kaplan-Meier plot of symptom resolution by high and low change in serum IL-8 (ΔIL-8) from baseline to exacerbation onset (73 pairs). Change in serum IL-8 ≥ median is represented by black line and change in serum IL-8 < median is represented by red line.

Median change in serum IL-6 value at from baseline to exacerbation presentation in 73 pairs was 4.4 (-0.7-20.5) pg/mL. Log-rank testing showed that elevated change in serum IL-6 from baseline to exacerbation predicted non-recovery at 35 days (p= 0.042, **Figure 6.4**).
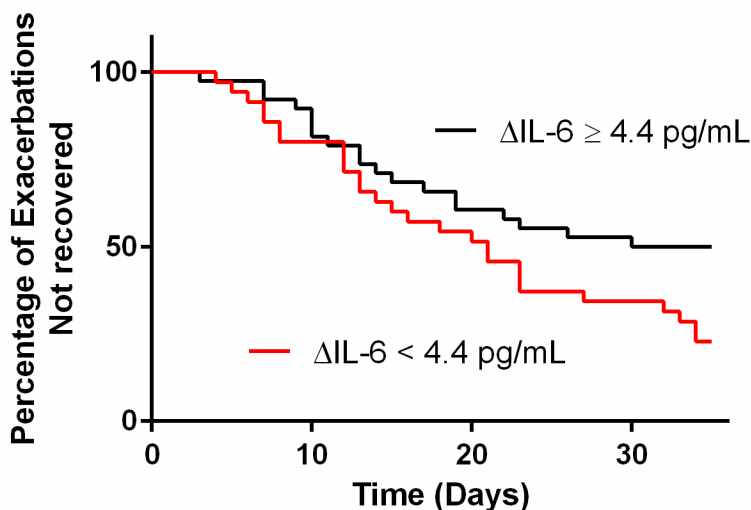
Figure 6.4. Kaplan-Meier plot of symptom resolution by high and low change in serum IL-6 (ΔIL-6) from baseline to exacerbation onset (73 pairs). Change in serum IL-6 ≥ median is represented by black line and change in serum IL-6 < median is represented by red line.

Those exacerbations whose inflammation had returned to baseline values by 2 weeks had faster recovery than exacerbations whose serum inflammatory levels were still elevated. This was reflected in levels of total white cell count, neutrophil count and serum IL-6. Log-rank testing showed that exacerbations with persistently elevated levels of total white cell count and neutrophil count (above paired baseline levels) at 14 days post exacerbation presentation were significantly associated with non-recovery at 35 days compared to exacerbations where inflammatory levels had returned to baseline at 14 days (**Figure 6.5a**: p=0.016, **Figure 6.5b**: p=0.031 respectively). Elevated serum IL-6 at 14 days post exacerbation presentation also approached statistical significance (**Figure 6.5c**: p=0.138).
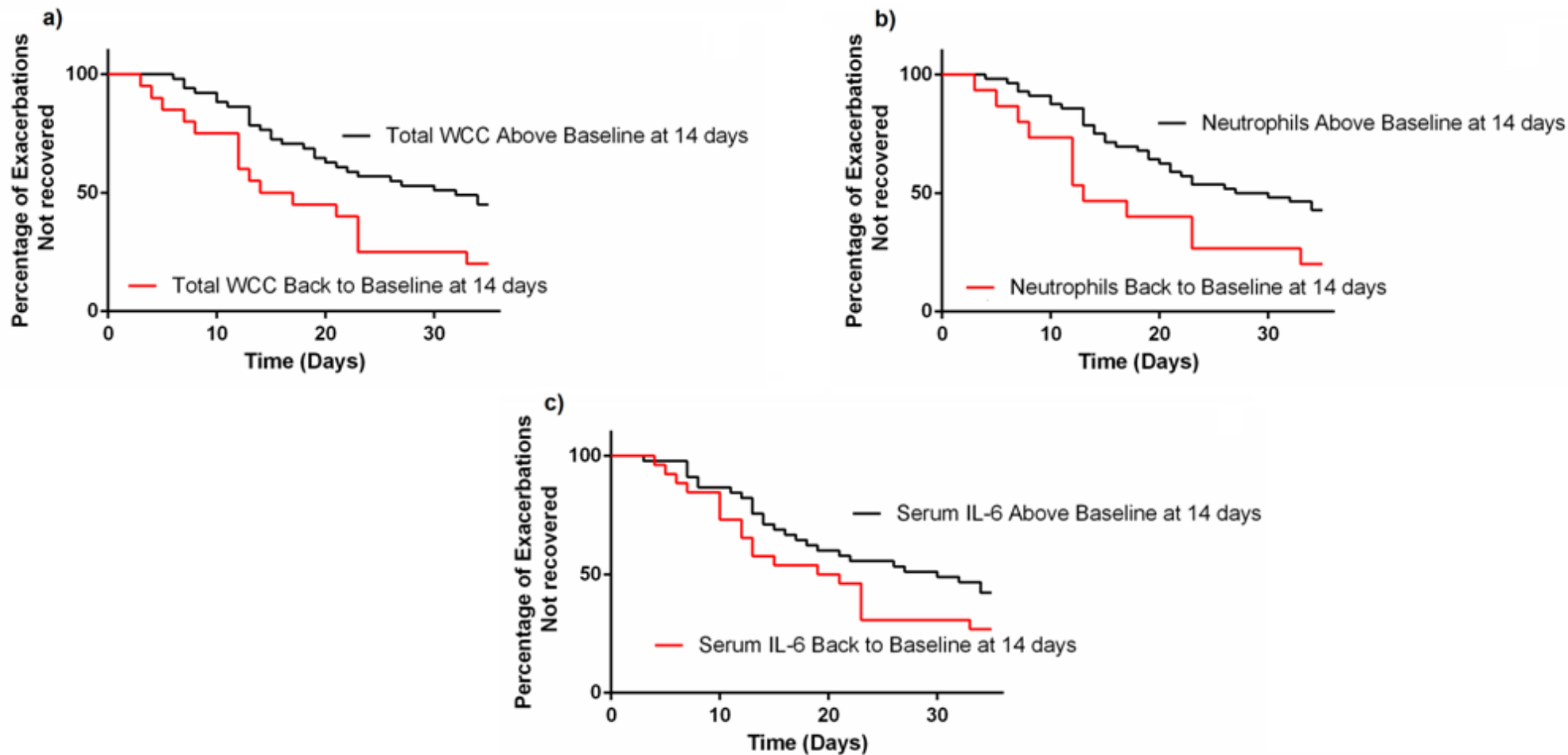
Figure 6.5. Kaplan-Meier plot of symptom resolution determined by biomarker levels at 14 days (71 exacerbations). Black lines represent exacerbations where biomarker levels remained above paired baseline levels at 14 days. Exacerbations where biomarker levels have returned to baseline at 14 days are represented by red line. a) Total white cell count (wcc), b) Absolute neutrophil counts, c) Serum IL-6.

Changes in Sputum Biomarkers between Baseline and Exacerbation

Concentrations of the sputum biomarkers, at baseline and exacerbation presentation, are reported in **Table 6.4**. Not all patients produced a viable sputum sample at baseline and exacerbation so results were available for 69 pairs. The majority of sputum IFNγ and IL-5 levels were below detectable threshold of assay and are therefore not reported.

Sputum Biomarker	Units	Baseline Median (IQR)	Exacerbation Median (IQR)	p-value (Wilcoxon)
IL-18	pg/ml	474.9 (218.2-1077.0)	762.0 (439.6-1770.0)	0.043
IL-1β	pg/ml	377.8 (112.6-2553.0)	2038.0 (502.8-16790.0)	< 0.001
IL-6	pg/ml	2783.0 (1434.0-6168.0)	6165.0 (1360.0-13438.0)	0.002
TNFα	pg/ml	56.1 (0.0-186.8)	195.3 (19.4-1617.0)	< 0.001
IL-8	pg/ml	75732.0 (34469.0-178487.0)	182732.0 (66883.0-473399.0)	< 0.001
MMP-1	pg/ml	9599.0 (4979.0-24088.0)	48328.0 (24396.0-73377.0)	< 0.001
MMP-8	pg/ml	$2.4 (1.2-10.0) \times 10^{6}$	$12.2 (2.2-47.6) \times 10^{6}$	< 0.001
MMP-9	pg/ml	$0.8 (0.6-3.7) \times 10^{7}$	$5.3 (0.8-21.1) \times 10^{7}$	< 0.001
MPO	pg/ml	$1.8 (1.1-5.1) \times 10^{6}$	$4.3 (1.7-8.6) \times 10^{6}$	0.001
TIMP-1	pg/ml	$1.2 (0.6-2.3) \times 10^{6}$	$3.9 (2.6-5.3) \times 10^{6}$	< 0.001
α2-macroglobulin	pg/ml	$4.3 (2.2-16.4) \times 10^{7}$	$6.4 (2.4-26.8) \times 10^{7}$	0.149

Table 6.4. Concentrations of the sputum biomarkers, at baseline and exacerbation presentation (69 pairs)

Significant differences were observed between baseline and exacerbation for 11 sputum biomarkers: TIMP-1, IL-18, IL-1β, IL-6, TNFα, IL-8, MMP-1, MMP-8, MMP-9, MPO and are illustrated in **Figure 6.6**.
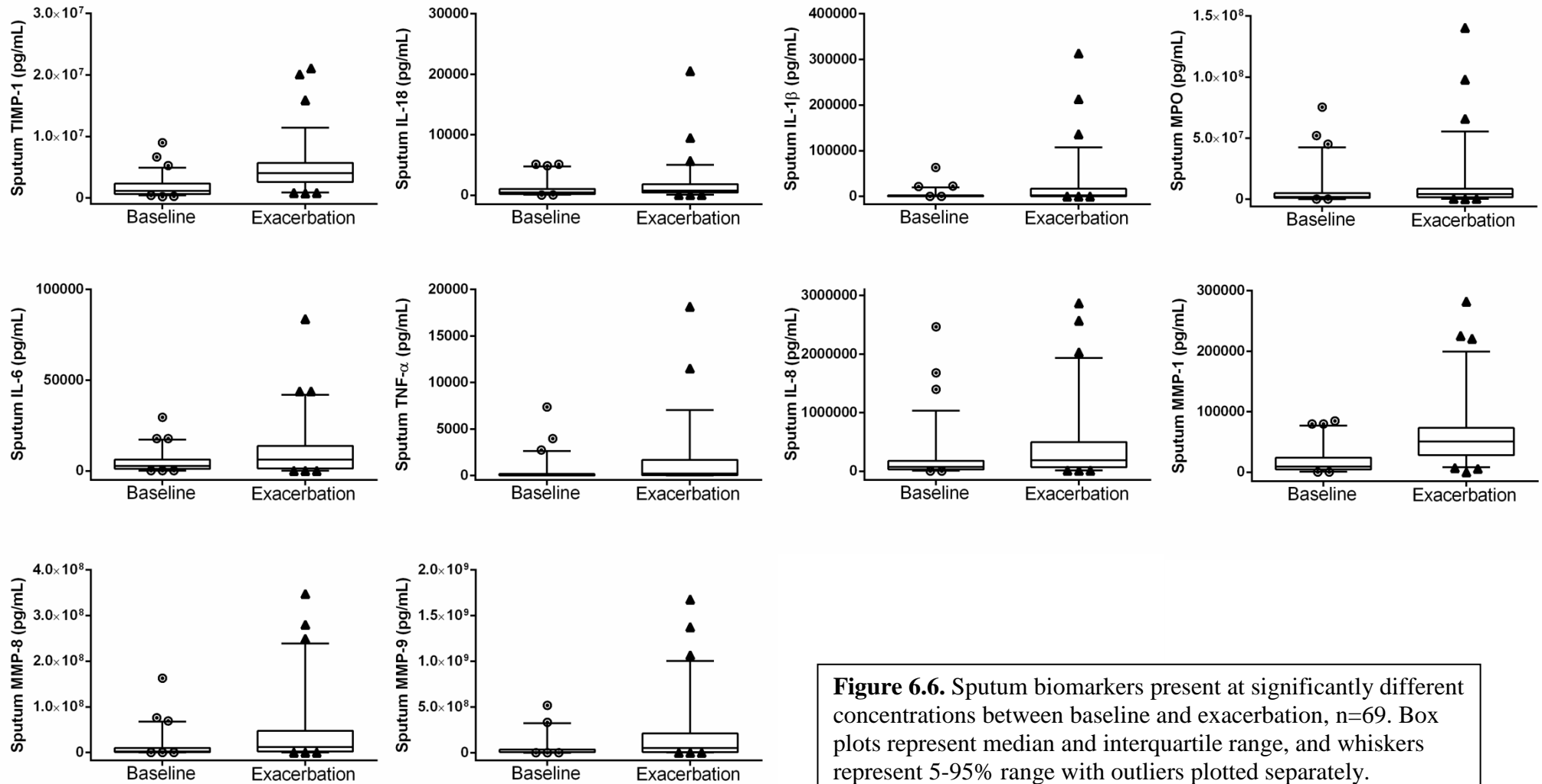
Figure 6.6. Sputum biomarkers present at significantly different concentrations between baseline and exacerbation, n=69. Box plots represent median and interquartile range, and whiskers represent 5-95% range with outliers plotted separately.

Use of Sputum Biomarkers in the prediction of Exacerbation Recovery

Survival analyses were performed on all sputum biomarkers which were significantly different between baseline and exacerbation to determine if elevated sputum biomarkers (defined as above the median value) at exacerbation onset predicted non-recovery at 35 days after exacerbation onset. No sputum biomarker showed significant ability to predict exacerbation non-recovery (all p>0.05, **Table 6.5**), although sputum IL-18 approached statistical significance. Median sputum IL-18 value at exacerbation presentation in 73 events was 806.0 (450.0-1855.0) pg/mL. Log-rank (Mantel-Cox) p=0.066, **Figure 6.7**).

Table 6.5. Results of Log-rank (Mantel-Cox) testing of elevated sputum biomarkers at exacerbation onset to predict non-recovery.

Sputum Biomarker	p value (Log-rank test)
IL-1β	0.892
IL-6	0.676
TNFα	0.473
IL-8	0.653
MMP-1	0.626
MMP-8	0.604
MMP-9	0.911
MPO	0.429
TIMP-1	0.201

Figure 6.7. Kaplan-Meier plot of symptom resolution by high and low sputum IL-18 at exacerbation onset. Sputum IL-18 ≥ median is represented by black line and IL-18 < median is represented by red line.
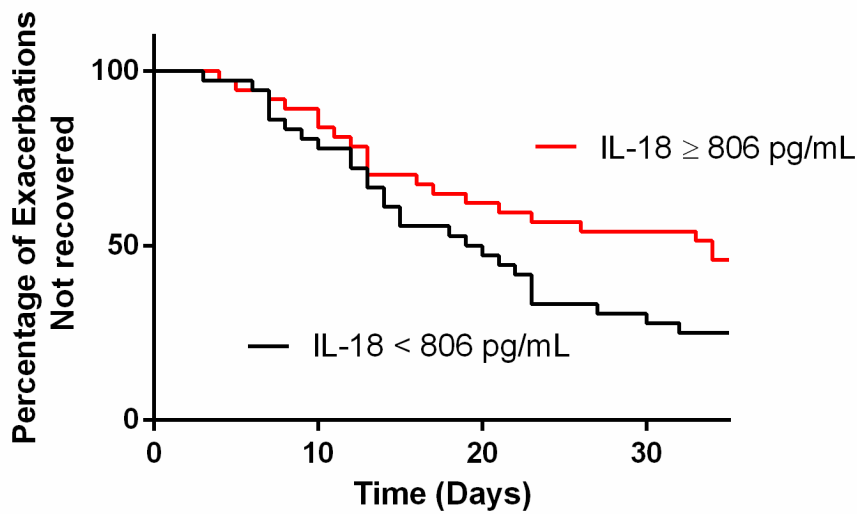
Sputum data was also analysed to determine if the change in biomarker levels from baseline to exacerbation onset predicted non-recovery at 35 days after exacerbation onset. Again no biomarker showed significant predictive ability, although change in sputum IL-18 from baseline to exacerbation was the closest to statistical significance, p=0.111 (**Figure 6.8**). Median change in sputum IL-18 value at from baseline to exacerbation presentation in 69 pairs was 285.8 (-242.8-680.0) pg/mL.
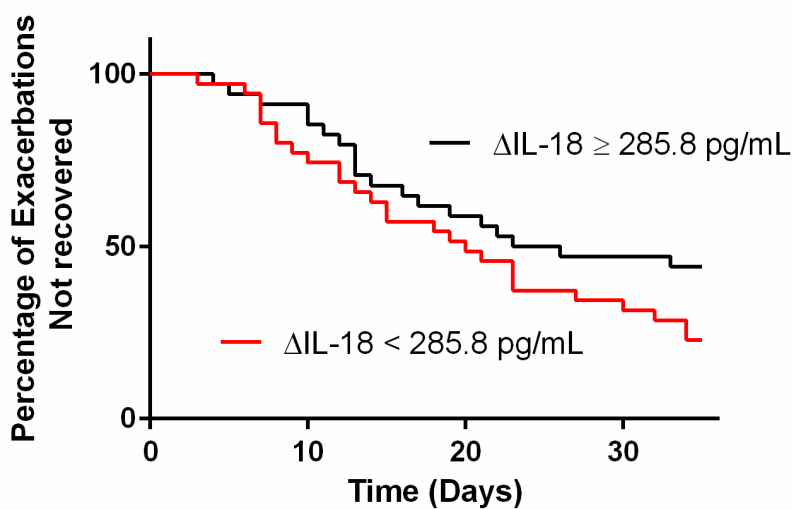
Figure 6.8. Kaplan-Meier plot of symptom resolution by high and low change in sputum IL-18 (ΔIL-18) from baseline to exacerbation onset (69 pairs). Change in sputum IL-18 ≥ median is represented by black line and change in serum IL-18 < median is represented by red line.

Effect of exacerbation aetiology on recovery
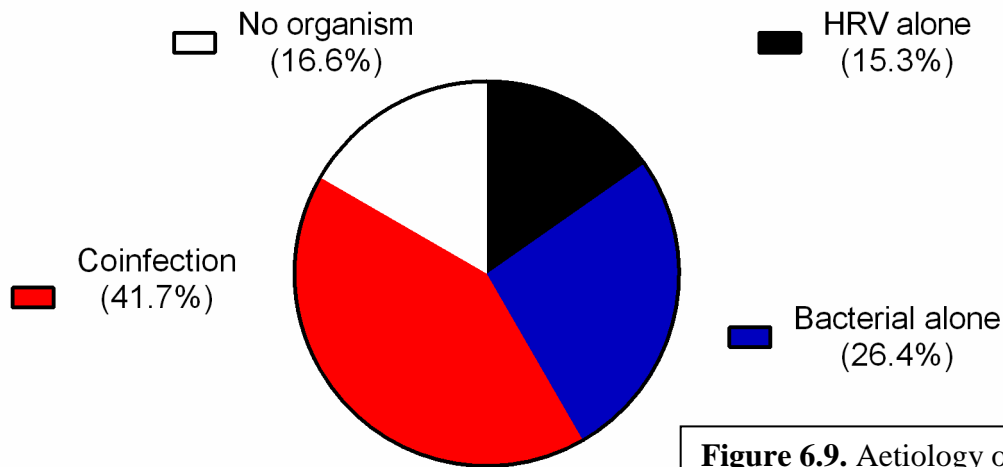
Figure 6.9. Aetiology of 66 isolated exacerbations.

The aetiology of exacerbations did not significantly affect the recovery time of isolated exacerbations. 4 exacerbations did not have sufficient sputum for both bacterial and HRV PCR testing. 5 exacerbations had exacerbation length >99days. For the remaining 66 exacerbations, there was no significant difference in exacerbation length between exacerbations depending on infection status at exacerbation presentation (Kruskal-Wallis test, p=0.2045, **Figure 6.9**, **Table 6.6**).

Table 6.6. Exacerbation Length depending upon aetiology at exacerbation onset

Aetiology	Bacterial Positive Alone	HRV Positive Alone	Coinfection	No organism detected
N	19	9	28	10
Median Exacerbation Length (IQR)	23.0 (15.0-37.0)	23.0 (12.5-46.0)	14.0 (8.3-33.5)	48.5 (9.5-60.8)

Furthermore, there was no significant difference in median exacerbation length between bacterial positive and bacterial negative exacerbations (n=47, 18.0 (11.0-37.0) days vs n=19, 23.0 (12.0-50.0) days, p=0.091) and no significant difference in exacerbation length between viral positive and viral negative exacerbations (n=37, 16.0 (10.5-36.0) vs n=29, 23.0 (14.0-41.0) days, p=0.185). Additionally, for bacterial positive exacerbations, there was no significant relationship between exacerbation length and bacterial load at exacerbation onset (r= -0.137, p= 0.271) and for HRV positive exacerbations, no significant relationship was seen between exacerbation length and viral load at exacerbation onset (r= -0.099, p= 0.431).

Index and Recurrent Exacerbation Characteristics

15 patients were included in this substudy who experienced 17 index exacerbations each followed by a recurrent exacerbation occurring within 8 weeks of the onset of the index exacerbation. Data was examined to determine if the inflammatory profile of index exacerbations which were followed by a recurrent exacerbation were different from isolated exacerbations not followed by recurrent events.

Exacerbations with an eosinophilic profile at exacerbation onset appeared more likely to be followed by a recurrent event than non-eosinophilic exacerbations. Median percentage blood eosinophils was significantly higher at exacerbation onset in index exacerbations which were followed by recurrent events, than in isolated exacerbations (n=17, 3.86 (1.67- 7.18) vs. n=75, 2.24 (1.24-3.73), p= 0.031, **Figure 6.10**). Median absolute blood eosinophil count in index exacerbations was also greater than in isolated exacerbations, although this did not quite reach the threshold for statistical significance (n=17, 0.25 (0.15-0.41) $\times 10^9$ vs. n= 75, 0.15 (0.10-0.29) $\times 10^9$, p=0.053).
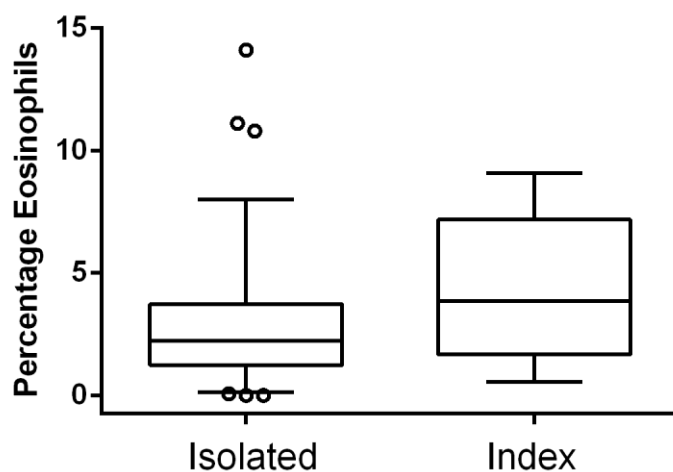
Figure 6.10. Percentage eosinophils present at significantly higher level at index (those followed by recurrent event) compared to isolated exacerbations (no-recurrence). Box plots represent median and interquartile range, and whiskers represent 5-95% range with outliers plotted separately.

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Furthermore, index exacerbations displayed significantly higher sputum IL-18 and sputum MMP-1 levels than isolated exacerbations. Median sputum IL-18 in index exacerbations was significantly higher than at exacerbation onset of isolated exacerbations (n=16, 2196.0 (759.0-3934.0) vs. n=73, 806.0 (450.0-1855.0), p=0.019, **Figure 6.11**). Median sputum MMP-1 at exacerbation onset in index exacerbations was also significantly higher than at exacerbation onset of isolated exacerbations (n=16, 80264.0 (50503.0-111057.0) vs. n=73, 51094.0 (28464.0-73377.0), p=0.016, **Figure 6.12**).
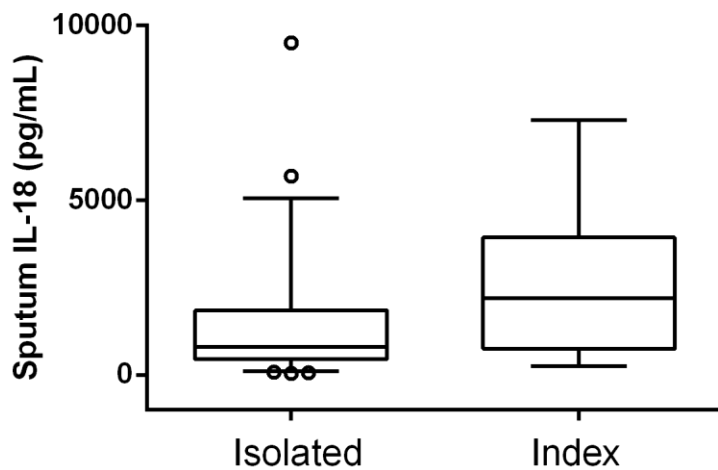
Figure 6.11. Sputum IL-18 present at significantly higher level at index (those followed by recurrent event) compared to isolated exacerbations (no-recurrence). Box plots represent median and interquartile range, and whiskers represent 5-95% range with outliers plotted separately.
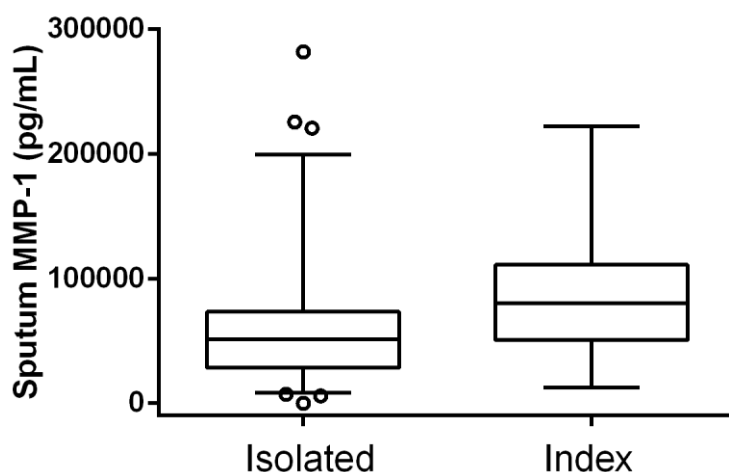
Figure 6.12. Sputum MMP-1 present at significantly higher level at index (those followed by recurrent event) compared to isolated exacerbations (no-recurrence). Box plots represent median and interquartile range, and whiskers represent 5-95% range with outliers plotted separately.

Median concentrations of all other blood and sputum biomarker levels, at isolated and index exacerbation presentation, are reported in **Table 6.7**.

Blood Biomarker	Units	Isolated Exacerbation Median (IQR) N=74	Index Exacerbation Median (IQR) N=17	p-value
CRP	mg/L	9.0 (3.5-33.5)	10.0 (1.3-26.8)	0.279
Fibrinogen	g/L	4.2 (3.5-5.1)	4.3 (3.2-4.9)	0.675
Total wcc	10^9/L	7.8 (6.2-10.1)	6.1 (5.1-9.8)	0.199
Neutrophils	10^9/L	5.0 (3.8-6.7)	4.0 (3.2-6.7)	0.219
IL-6	pg/ml	11.8 (7.3-29.0)	9.9 (6.5-48.0)	0.886
IL-8	pg/ml	1.4 (0.5-2.7)	1.7 (1.0-3.9)	0.298
IP-10	pg/ml	265.5 (153.6-401.4)	299.6 (200.1-632.1)	0.476
PLGF	pg/ml	4.5 (3.4-7.1)	5.7 (4.2-6.6)	0.139
MMP-1	pg/ml	10184 (6511-13428)	10694 (7554-18501)	0.351
MMP-8	pg/ml	875.5 (133.4-4800.0)	291.2 (44.0-1044.0)	0.112
MMP-9	pg/ml	1.0 (0.7-1.8) $\times 10^6$	0.8 (0.5-2.0) $\times 10^6$	0.619
sRAGE	pg/ml	150.5 (101.8-268.7)	116.2 (45.8-218.9)	0.161
PARC	pg/ml	150878.0 (109323.0-199851.0)	194932.0 (131060.0-247725.0)	0.067
α2-macroglobulin	pg/ml	9.2 (5.8-14.6) $\times 10^9$	7.0 (5.9-9.9) $\times 10^9$	0.201
CC16	pg/ml	6559.0 (4159.0-11582.0)	5185.0 (4257.0-10267.0)	0.472
SAA	pg/ml	9.5 (2.2-36.0) $\times 10^7$	5.9 (1.2-13.0) $\times 10^7$	0.115

Sputum Biomarker	Units	Isolated Exacerbation Median (IQR) N=73	Index Exacerbation Median (IQR) N=16	p-value
IL-1β	pg/ml	2038.0 (553.8-16790.0)	3588.0 (871.2-7198.0)	0.938
IL-6	pg/ml	6310.0 (1444.0-13855.0)	12029.0 (5099.0-16299.0)	0.075
TNFα	pg/ml	195.3 (21.2-1680.0)	466.7 (66.0-1210.0)	0.777
IL-8	pg/ml	190586.0 (71923.0-498167.0)	163001.0 (112521.0-411692.0)	0.878
MMP-8	pg/ml	1.2 (0.2-4.8) $\times 10^7$	1.1 (0.3-4.9) $\times 10^7$	0.989
MMP-9	pg/ml	5.3 (0.8-21.1) $\times 10^7$	4.6 (1.5-16.8) $\times 10^7$	0.803
MPO	pg/ml	4.3 (1.8-8.6) $\times 10^6$	5.5 (2.4-9.9) $\times 10^6$	0.310
TIMP-1	pg/ml	4.0 (2.6-5.7) $\times 10^6$	4.7 (3.5-6.8) $\times 10^6$	0.232
α2-macroglobulin	pg/ml	6.4 (2.4-26.8) $\times 10^7$	16.2 (4.3-32.8) $\times 10^7$	0.113

Table 6.7. Differences in Biomarker levels between isolated and index exacerbations

Timecourse of inflammation during exacerbation recovery

The timecourse of airway and systemic inflammatory biomarkers during isolated exacerbations is shown in **Table 6.8** and **Table 6.9** respectively

(p value <0.05 are marked with asterix*).

Sputum Biomarker	Baseline	Exacerbation Presentation	p-value Baseline -onset	Day 3	p-value Baseline -day 3	Day 7	p-value Baseline -day 7	Day 14	p-value Baseline -day 14	Day 35	p-value Baseline -day 35
IL-18 (pg/mL)	474.9 (218.2-1077.0)	762.0 (439.6-1770.0)	0.043*	537.0 (204.3-957.1)	0.958	422.0 (251.8-796.0)	0.643	459.6 (245.3-1301.0)	0.603	523.0 (264.6-847.0)	0.797
IL-1β (pg/mL)	377.8 (112.6-2553.0)	2038.0 (502.8-16790.0)	< 0.001*	570.1 (214.0-3142.0)	0.219	678.4 (237.5-2461.0)	0.141	789.5 (305.3-6844.0)	0.032*	746.0 (241.5-2545.0)	0.184
IL-6 (pg/mL)	2783.0 (1434.0-6168.0)	6165.0 (1360.0-13438.0)	0.002*	4286.0 (1726.0-11911.0)	0.268	4785.0 (2089.0-8756.0)	0.102	5463.0 (900.0-13620.0)	0.042*	5531.0 (1318.0-11291.0)	0.179
TNFα (pg/mL)	56.1 (0.0-186.8)	195.3 (19.4-1617.0)	< 0.001*	40.0 (10.8-173.2)	0.561	58.5 (11.9-338.0)	0.270	67.5 (14.0-599.8)	0.063	85.5 (14.5-277.5)	0.059
IL-8 (pg/mL)	75732.0 (34469.0-178487.0)	182732.0 (66883.0-473399.0)	< 0.001*	128065.0 (54374.0-240769.0)	0.130	113128.0 (77880.0-225181.0)	0.019*	185632.0 (66917.0-371185.0)	0.002*	95165.0 (49752.0-232452.0)	0.134
MMP-1 (pg/mL)	9599.0 (4979.0-24088.0)	48328.0 (24396.0-73377.0)	< 0.001*	45212.0 (13674.0-74801.0)	< 0.001*	42151.0 (23699.0-64718.0)	< 0.001*	36771.0 (20820.0-81747.0)	< 0.001*	36360.0 (20411.0-73033.0)	< 0.001*
MMP-8 (pg/mL)	2.4×10^6 (1.2-10.0)	12.2×10^6 (2.2-47.6)	< 0.001*	5.5×10^6 (1.5-21.3)	0.051	3.4×10^6 (1.9-15.5)	0.078	$5.2 (1.8-28.8) \times 10^6$	0.013*	2.2×10^6 (1.0-8.4)	0.900
MMP-9 (pg/mL)	0.8×10^7 (0.6-3.7)	5.3×10^7 (0.8-21.1)	< 0.001*	2.1×10^7 (0.6-9.7)	0.031*	2.2×10^7 (0.8-6.7)	0.005*	$2.9 (1.0-13.1) \times 10^7$	0.002*	1.0×10^7 (0.5-3.6)	0.668
MPO (pg/mL)	1.8×10^6 (1.1-5.1)	4.3×10^6 (1.7-8.6)	0.001*	3.2×10^6 (1.3-5.2)	0.273	2.8×10^6 (1.8-4.7)	0.093	$2.7 (1.4-4.7) \times 10^6$	0.193	2.2×10^6 (1.2-3.7)	0.948
TIMP-1 (pg/mL)	1.2×10^6 (0.6-2.3)	3.9×10^6 (2.6-5.3)	< 0.001*	5.1×10^6 (2.7-7.4)	< 0.001*	5.0×10^6 (3.6-6.6)	< 0.001*	$4.7 (2.9-7.2) \times 10^6$	< 0.001*	4.5×10^6 (2.8-6.9)	< 0.001*
α2-macroglobulin (pg/mL)	4.3×10^7 (2.2-16.4)	6.4×10^7 (2.4-26.8)	0.149	2.9×10^7 (1.1-8.8)	0.025*	2.5×10^7 (0.9-6.7)	< 0.001*	$4.5 (1.3-8.5) \times 10^7$	0.057	3.1×10^7 (1.4-7.4)	0.043*

Table 6.8. Time course of airway inflammatory markers during isolated COPD exacerbations

Serum Biomarker	Baseline	Exacerbation Presentation	p-value Baseline -onset	Day 3	p-value Baseline -day 3	Day 7	p-value Baseline -day 7	Day 14	p-value Baseline -day 14	Day 35	p-value Baseline -day 35
CRP (mg/L)	3.0 (1.0-7.25)	9.0 (3.5-33.5)	< 0.001*	4.0 (2.0-10.0)	0.153	3.0 (1.0-7.0)	0.555	6.0 (3.0-15.0)	<0.001*	3.0 (2.0-7.0)	0.937
Fibrinogen (g/L)	3.6 (3.3-4.1)	4.2 (3.5-5.1)	< 0.001*	3.5 (3.0-4.2)	0.501	3.1 (2.8-3.5)	<0.001*	4.2 (3.5-4.8)	<0.001*	3.7 (3.4-4.2)	0.366
Total wcc (10^9/L)	7.3 (6.4-8.8)	7.8 (6.2-10.1)	0.248	10.2 (8.4-11.7)	< 0.001*	10.9 (8.3-13.6)	< 0.001*	8.9 (7.0-11.5)	<0.001*	7.8 (6.5-9.7)	0.501
Neutrophils (10^9/L)	4.6 (3.9-6.1)	5.0 (3.8-6.7)	0.096	8.0 (6.2-9.0)	< 0.001*	7.1 (5.4-9.0)	< 0.001*	6.1 (4.8-8.5)	<0.001*	5.0 (3.8-6.3)	0.379
Eosinophils (10^9/L)	0.20 (0.11-0.35)	0.15 (0.10-0.29)	0.107	0.05 (0.01-0.10)	< 0.001*	0.13 (0.05-0.27)	< 0.001*	0.17 (0.11-0.27)	0.316	0.17 (0.11-0.33)	0.570
IL-6 (pg/mL)	8.2 (5.25-12.6)	11.6 (7.3-29.0)	< 0.001*	5.4 (3.5-10.8)	< 0.001*	8.3 (5.1-13.8)	0.753	11.4 (6.2-17.5)	0.084	9.0 (5.4-11.6)	0.528
IL-8 (pg/mL)	0.3 (0.0-1.9)	1.4 (0.5-2.6)	0.010*	1.3 (0.5-1.9)	0.014*	1.4 (0.7-2.4)	< 0.001*	1.0 (0.4-2.1)	< 0.001*	1.0 (0.4-2.5)	0.006*
IP-10 (pg/mL)	337.8 (261.0-490.6)	266.6 (157.1-404.4)	< 0.001*	168.4 (96.5-277.3)	< 0.001*	144.7 (91.7-248.6)	< 0.001*	246.0 (139.4-398.5)	< 0.001*	237.4 (140.5-335.2)	< 0.001*
PLGF (pg/mL)	2.3 (0.1-4.5)	4.5 (3.4-7.1)	< 0.001*	4.6 (3.4-7.7)	< 0.001*	5.7 (3.8-7.6)	< 0.001*	4.8 (3.5-6.7)	< 0.001*	4.5 (3.5-6.2)	< 0.001*
MMP-1 (pg/mL)	3601 (1662-6866)	10157 (6488-13449)	< 0.001*	10349 (6924-16330)	< 0.001*	11114 (6869-15711)	< 0.001*	9074 (6037-12794)	< 0.001*	9910 (5904-13480)	< 0.001*
MMP-8 (pg/mL)	2028.0 (1004.0-4614.0)	869.8 (131.6-4850.0)	0.188	1418.0 (357.2-5456.0)	0.943	1334.0 (347.3-4495.0)	0.390	873.5 (203.3-3540.0)	0.170	458.4 (134.6-2622)	0.002*
MMP-9 (pg/mL)	1.0 x10^6 (0.7-1.3)	1.0 x10^6 (0.7-1.8)	0.192	1.3 x10^6 (0.8-2.0)	0.014*	1.5 x10^6 (0.9-1.9)	< 0.001*	1.2 x10^6 (0.8-1.8)	0.094	0.9 x10^6 (0.6-1.4)	0.471
sRAGE (pg/mL)	127.5 (57.2-218.5)	155.0 (102.3-271.2)	< 0.001*	109.7 (69.5-194.6)	0.702	125.3 (71.9-203.6)	0.438	152.1 (97.7-281.1)	0.023*	155.3 (94.2-308.4)	0.008*
PARC (pg/mL)	137615 (108035-184632)	151714 (110145-199870)	0.070	129866 (88540-176886)	0.207	109305 (80643-153359	0.003*	143002 (96664-186609)	0.957	127572 (101181-186100)	0.528
α2-macroglobulin (pg/mL)	1.8 x10^{10} (1.2-3.1)	1.0 x10^{10} (0.6-1.5)	< 0.001*	0.9 x10^{10} (0.5-1.6)	< 0.001*	0.8 x10^{10} (0.5-1.5)	< 0.001*	0.8 x10^{10} (0.5-1.4)	< 0.001*	0.9 x10^{10} (0.6-1.4)	< 0.001*
CC16 (pg/mL)	7400 (4363-14306)	6654 (4266-11882)	0.540	6811 (3615-10252)	0.249	5493 (3563-10504)	0.153	5677 (3817-10354)	0.226	5760 (3759-10945)	0.187
SAA (pg/mL)	0.3 x10^8 (0.2-0.9)	1.0 x10^8 (0.3-3.6)	< 0.001*	0.9 x10^8 (0.4-2.0)	< 0.001*	0.6 x10^8 (0.3-1.1)	< 0.001*	0.5 x10^8 (0.2-1.2)	0.017*	0.3 x10^8 (0.1-0.6)	0.779

Table 6.9. Time course of serum inflammatory markers during isolated COPD exacerbations

Timecourse of inflammation during exacerbation recovery

Systemic and airway biomarkers followed distinct patterns during exacerbation recovery and can be categorised as follows:

1) **Acute rise and rapid fall**

2) **Acute rise and slow fall**

3) **Delayed rise and slow fall**

4) **Persistent rise**

5) **Fall**

Figure 6.13. Acute Rise and Rapid Fall of Biomarkers during Exacerbation Recovery. (a) Timecourse of serum CRP, arrow demonstrates rapid rise at exacerbation onset. (b) Time course of plasma Fibrinogen, arrow demonstrates fall in biomarker levels below baseline. (c) Timecourse of serum PARC, arrow demonstrates rise in biomarker level at 2 weeks post exacerbation onset. Points represent median and interquartile range.

1) Acute rise and rapid fall:

Serum Biomarkers:

CRP, Fibrinogen, Serum IL-6, PARC, sRAGE.

Sputum Biomarkers:

Sputum IL-1β, Sputum IL-6, Sputum IL-8, Sputum IL-18, Sputum a2M, Sputum MMP-8, Sputum TNFα, Sputum MPO(3),

The above biomarkers displayed a rapid rise at exacerbation onset followed by a rapid fall (by day 7) to return to baseline levels (**Figure 6.13a**) often at 3 days post exacerbation presentation. Within this group inflammation often fell below baseline levels at 1 week post presentation (**Figure 6.13b**), probably reflecting the effect of exacerbation treatment.

Biomarkers within this group also displayed a rebound rise at 2 weeks post exacerbation onset (**Figure 6.13c**).

Figure 6.14. Acute Rise and Slow Fall of Biomarkers during Exacerbation Recovery. Time course is shown for (a) SAA, (b) Sputum MMP-1 and (c) Serum IL-8. Points represent median and interquartile range.

2) Acute rise and slow fall:

Serum Biomarkers:

SAA, Serum IL-8.

Sputum Biomarkers:

Sputum MMP-1. Sputum MMP-9

The above biomarkers displayed an acute rise at exacerbation onset followed by a slow descent back towards baseline levels. SAA and sputum MMP-9 levels had returned to baseline levels 5 weeks post exacerbation onset (**Figure 6.14a**), whilst serum IL-8 and sputum MMP-1 levels remained persistently higher than baseline levels at day 35 after exacerbation onset (**Figure 6.14b** and **Figure 6.14c**).

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a)

3) Delayed rise and slow fall:

Biomarkers:

Total white blood cells, neutrophils, Serum MMP-9.
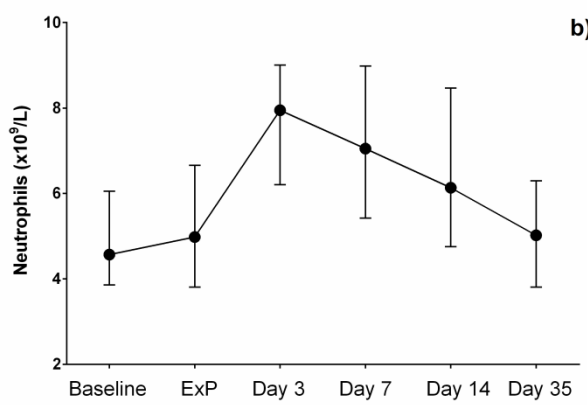
b)

There was a delayed rise in total white cell counts (**Figure 6.15a**). No significant difference was seen between total white cell counts (wcc) at baseline and exacerbation presentation, however at day 3 post exacerbation presentation wcc levels were significantly greater than baseline. This rise persisted for 2 weeks post exacerbation presentation, although levels did start to fall from week 1 to week 2.
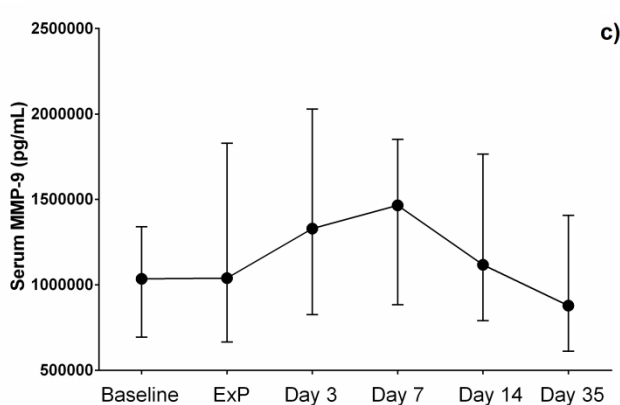
c)

Furthermore neutrophil counts followed a similar pattern (**Figure 6.15b**), along with serum MMP-9 (**Figure 6.15c**) which displayed a

Figure 6.15. Slow Rise and Slow Fall of Biomarkers during Exacerbation Recovery. The timecourse is shown for (a) Blood total white cell counts, (b) Blood neutrophils and (c) Serum MMP-9. Points represent median and interquartile range.

slow rise following exacerbation onset, reaching a peak level at 1 week post exacerbation onset before falling to baseline levels at 14 days.
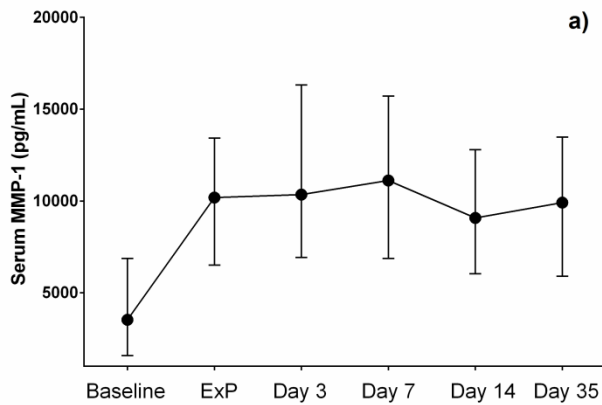
4) Persistent Rise:

Serum Biomarkers:

Serum MMP-1, PLGF.

Sputum Biomarkers:

Sputum TIMP-1.
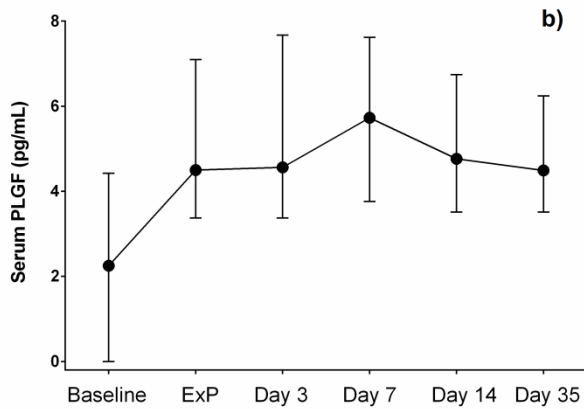
The above biomarkers rose acutely at exacerbation onset and remained persistently elevated throughout the recovery period. Biomarker levels at day 35 remained significantly higher than baseline levels.
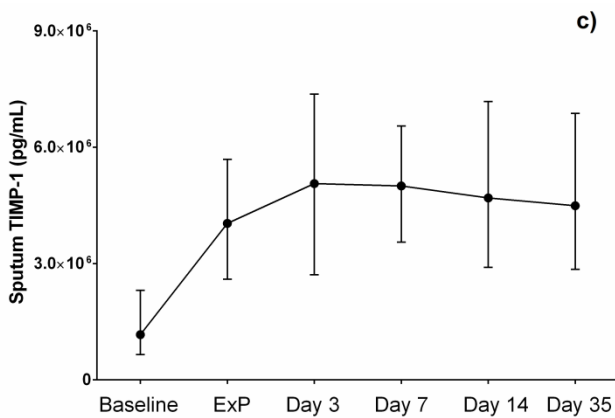
Figure 6.16. Persistent Rise of Biomarkers during Exacerbation Recovery. Timecourse is shown for (a) Serum MMP-1, (b) Serum PLGF and (c) Sputum TIMP-1. Points represent median and interquartile range.
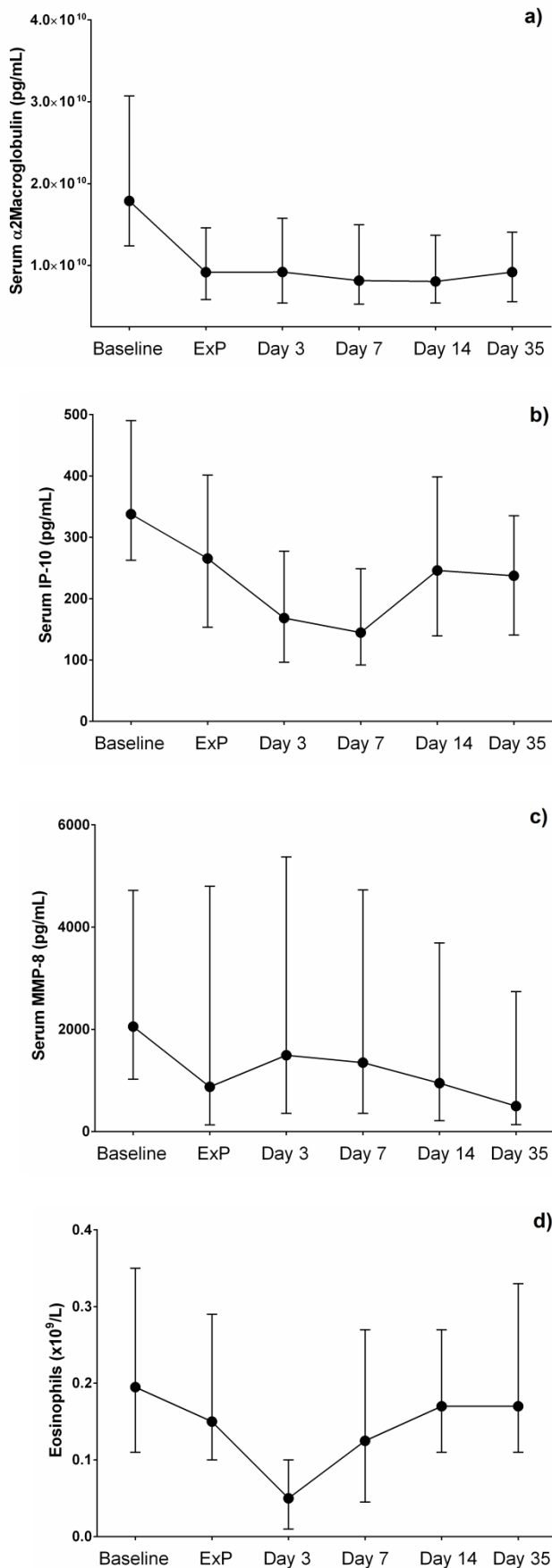
Figure 6.17. Fall of Biomarkers during Exacerbation Recovery. Timecourse is shown for (a) Serum α2Macroglobulin, (b) Serum IP-10, (c) Serum MMP-8 and (d) Blood Eosinophils. Points represent median and interquartile range.

5) Fall:

Biomarkers:

Serum α2Macroglobulin, Serum MMP-8, Serum IP-10, Blood Eosinophils.

Serum α2Macroglobulin (**Figure 6.17a**) and Serum IP-10 levels (**Figure 6.17b**) fell acutely at exacerbation and remained significantly below baseline levels throughout recovery. Serum MMP-8 (**Figure 6.17c**) levels were also reduced following exacerbation but levels were only significantly below baseline at day 35 post exacerbation onset.

Eosinophil levels fell significantly following exacerbation presentation, being significantly reduced compared to baseline at day 3 and 1 week before returning to baseline at 2 weeks (**Figure 6.17d**). These changes may be the result of treatment with oral corticosteroids which were administered at exacerbation presentation in the majority of exacerbations (64 (85%) of 75 events).

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6.5 Discussion

This study examined the detailed inflammatory profile of exacerbations of COPD in a well characterised cohort and for the first time demonstrated that increased systemic inflammation, as measured by absolute levels and change in IL-6, at exacerbation onset predicts clinical non-recovery at 35 days. Airway inflammation also increased at exacerbation, although no sputum biomarker showed significant ability to predict exacerbation non-recovery. Systemic and airway biomarkers followed distinct patterns during exacerbation recovery and slower resolution of systemic markers of neutrophilic inflammation was associated with prolonged clinical recovery. Uniquely, this study also investigated the inflammatory profile of exacerbations which were followed by recurrent events. Exacerbations with an eosinophilic profile at exacerbation onset appeared more likely to be followed by a recurrent event than non-eosinophilic exacerbations.

Exacerbation non-recovery and recurrence are critical clinical adverse outcomes and are a major cause of morbidity, mortality and financial burden to health care systems. Exacerbation non-recovery in this study was defined as failure of an exacerbation to symptomatically recover 35 days after exacerbation onset. 35 days is a key time-point, since significant financial penalties can apply to healthcare organizations if patients are readmitted to hospital within 30 days of discharge following an exacerbation of COPD, the median length of stay in hospital prior to discharge being 5 days (131). Furthermore, previous pivotal studies have analysed non-recovery at 35 days (56).

In almost 25% of cases symptoms do not fully resolve 35 days after the onset of an exacerbation (56). Previous biomarker analysis has revealed that serum, plasma and sputum IL-6 levels increase from stable values at exacerbation (37, 38, 52, 56, 111) and serum IL-6

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levels fall during recovery (56, 112). Prior research has also shown that patients who have not symptomatically recovered at day 35 after exacerbation onset have a persistently higher serum CRP concentration during recovery (56). However, preceding work has failed to identify any single biomarker that can be measured at exacerbation onset which enables identification of patients at risk of prolonged recovery.

In this study, significant increases were observed between baseline and exacerbation for the following blood biomarkers: CRP, Fibrinogen, IL-6, IL-8, PLGF, MMP-1, sRAGE, and SAA. This is consistent with previous studies which have shown elevated systemic biomarkers at exacerbation (52, 56, 111, 112, 132, 133). However, this work advances the field because survival analyses showed that elevated serum IL-6 at exacerbation presentation predicted non-recovery at 35 days. This finding has important clinical implications since it may now be possible to stratify patients at high risk of non-recovery based on a single blood biomarker taken at exacerbation presentation.

In addition to early identification of exacerbations at risk of non-recovery, this study confirms that serum biomarkers such as serum IL-6 may be used to monitor the trajectory of recovery. Biomarkers showed distinct patterns during exacerbation recovery and serum IL-6 was amongst those biomarkers characterised by an acute rise and rapid fall during the exacerbation recovery timecourse. In addition to serum IL-6, this group of biomarkers contained serum CRP, Fibrinogen, serum PARC, sRAGE, sputum IL-1beta, sputum IL-6, sputum IL-8, sputum IL-18, sputum a2M, sputum MMP-8, sputum TNFα, and sputum MPO. A number of these biomarkers including IL-8, IL-18 (134) and TNFα are important neutrophil chemoattractants, and MPO is a strong oxidant stored in primary granules of neutrophils with potent antibacterial (135). Furthermore, IL-6 itself is a key regulator of

neutrophil trafficking during the inflammatory response and acts by orchestrating chemokine production and leukocyte apoptosis (136).

This study further reinforces the importance of neutrophilic inflammation at COPD exacerbations by demonstrating that exacerbations with persistently elevated total white cell (leukocyte) counts, neutrophil counts and serum IL-6 levels above paired baseline at 14 days were more likely to have failed to symptomatically recover at 35 days compared to exacerbations where inflammatory levels had returned to baseline (although in the case of serum IL-6 this did not meet statistical significance).

Such biomarker data may explain previous symptomatic studies which have shown that COPD exacerbations exhibit two distinct patterns-sudden and gradual onset (137). Patients who experience sudden onset exacerbations have earlier and higher magnitude peak symptoms, along with a shorter median recovery time back to baseline. Conversely, gradual onset exacerbations appear more likely to be associated with a longer duration of recovery. From the data shown in this chapter it can be inferred that patients with rapid symptom onset and resolution have a fast onset and rapid resolution of their neutrophilic inflammatory profile. Thus, the persistence of excess neutrophilic inflammation at day 14 despite standard therapy (systemic antibiotics and/or corticosteroids) may be driving the increased symptomatic burden seen in patients with abnormal, prolonged recovery. Therefore, excessive neutrophilic inflammation may provide a novel target to improve exacerbation recovery. The use of the anti-neutrophilic agent Roflumilast in a novel acute intervention study is further discussed in **Chapter 7**.

In addition to systemic inflammatory markers, this work examined airway inflammation during exacerbation recovery. Sputum inflammatory markers also increased at exacerbation: IL-18, IL-1β, IL-6, TNFα, IL-8, MMP-1, MMP-8, MMP-9, MPO, TIMP-1. However no sputum biomarker showed significant ability to predict exacerbation non-recovery. Sputum IL-18 levels however did show important differences between exacerbations which were followed by a recurrent event and isolated exacerbations not followed by a separate exacerbation within 8 weeks.

Recurrent exacerbations are an important clinical problem for COPD patients. In outpatient exacerbations, approximately 27% of first exacerbations are followed by a second discrete exacerbation over the subsequent 8 weeks (15). In the UK national COPD audit, despite current therapies and attempts to prevent such events, 34% of hospitalised exacerbations were readmitted in the subsequent 3 months (138). Previous studies have shown that elevated serum CRP during the recovery period of a first exacerbation is related to a shorter time to second event (56) and it has been theorised that failure to suppress inflammation during recovery may predispose to exacerbation recurrence (15). This is supported by clinical trial data showing an increased risk of exacerbation following steroid withdrawal (139). Corticosteroids are predominantly effective against eosinophilic inflammation and form the mainstay of treatment for asthma. However, up to 30% of COPD exacerbations are characterised by an eosinophilic phenotype (52) and COPD patients with high baseline blood eosinophil levels appear to experience greater reduction in exacerbation frequency with steroid-containing inhaler therapy than individuals with lower eosinophil levels (54).

In this study, a recurrent exacerbation was defined as an exacerbation that had an onset within 8 weeks of a preceding exacerbation as per prior research (15). Exacerbations with an eosinophilic profile at exacerbation onset were more likely to be followed by a recurrent event than non-eosinophilic exacerbations. Blood eosinophils were higher at exacerbation onset in index exacerbations which were followed by recurrent events, than in isolated exacerbations, although the numbers of patients included in this sub-analysis were small. Furthermore, index exacerbations displayed significantly higher sputum IL-18 and sputum MMP-1 levels than isolated exacerbations, cytokines which have been implicated in the aetiology and severity of bronchial asthma (140-143).

These findings have potentially important implications for the treatment of exacerbations. In this study the majority of exacerbations received 7 days of corticosteroid therapy in the form oral prednisolone but it may be that exacerbations with an eosinophilic/asthmatic phenotype require a longer course of corticosteroid therapy. Trial data has shown that peripheral blood eosinophil counts can be used as a biomarker to direct corticosteroid therapy during COPD exacerbations (55), and although a recent Cochrane review has shown no difference in rates of treatment failure, relapse or time to next exacerbation between short and prolonged courses of corticosteroids (144), this review was largely dominated by a recent trial (145) which did not stratify patients based upon eosinophilia at exacerbation. Therefore future studies should prospectively evaluate the efficacy of corticosteroids to reduce rates of recurrence following eosinophilic COPD exacerbations.

Whilst this study showed important relationships between inflammation, recovery length and recurrence, it also investigated the relationship between exacerbation aetiology and symptom

recovery. No significant difference in exacerbation length was seen between exacerbations depending on infection status at exacerbation presentation. This is inconsistent with previous work which has shown that exacerbations with both cold symptoms (a marker of viral infection) and a bacterial pathogen, have higher symptom burden than those with a bacterial pathogen alone (48). Furthermore, in studies of hospitalised exacerbations, patients coinfected with virus and bacteria had a significantly longer length of hospital stay (97, 146). Although none of the above studies specifically examined the relationship between exacerbation aetiology and total exacerbation length as in this chapter.

A significant limitation of this work, in particular the study of recurrent exacerbations, is the relatively small number of exacerbations that have been studied. However, this work was conducted in a well characterised cohort where patients are specifically trained to recognise and report the symptoms of an exacerbation. Patients subsequently presented early after the onset of symptoms and prior to starting additional systemic therapy for an exacerbation thus this study is likely to provide an accurate insight into the early inflammatory patterns at exacerbation and during recovery. Patients do present at variable time from symptom onset, however this is unlikely to have significantly influenced results since the median time from diary card exacerbation onset to presentation was just 4 days and patients were only included if they attended and were sampled within 7 days of symptom onset. A further potential limitation is that the statistical analysis did not include a correction for the multiple biomarkers that were assessed at each timepoint. However, using a full Bonferroni correction would be highly conservative for a proof of mechanism analysis of changes in biomarker concentrations at exacerbation, potentially missing real differences. To overcome these concerns regarding adequate power and potential type 1 errors, the findings of this chapter should be replicated in larger studies as a priority.

Additionally, the results of this work should also be examined in more severe hospitalised exacerbations. The population in this research includes patients seen commonly in both primary care and hospital clinics with moderate and severe COPD who have a history of exacerbations and so the results are also likely to be applicable to exacerbations that warrant inpatient therapy, since frequent exacerbators are at higher risk of hospitalisation for exacerbations (7).

6.6 Conclusion

In conclusion, this work provides further evidence that neutrophilic inflammation is a key therapeutic target for exacerbations of COPD. Reducing neutrophilic inflammation may improve recovery time. The use of the anti-neutrophilic agent Roflumilast in a novel acute intervention study is further discussed in the next section of this thesis (**Chapter 7**). Furthermore, eosinophilic exacerbations appeared more likely to be followed by a recurrent event than non-eosinophilic exacerbations. Future studies should prospectively evaluate the efficacy of corticosteroids to reduce rates of recurrence following eosinophilic events.

7

TREATMENT WITH ROFLUMILAST AT EXACERBATION (TREAT)

Chapter 6 showed that persistently elevated neutrophilic inflammation was associated with longer clinical recovery and that exacerbations with an eosinophilic profile at exacerbation onset appeared more likely to be followed by a recurrent event. Roflumilast reduces neutrophilic and eosinophilic inflammation and the following chapter describes the TREAT (**T**reatment with **R**oflumilast at **ExA**cerba**T**ion) study: a novel, proof-of-mechanism trial to investigate if roflumilast can reduce exacerbation severity and improve recovery when administered as an acute exacerbation therapy.

7.1 Introduction

COPD exacerbations are critical events that lead to acute hospital admission (14), poor health status (11), increased risk of cardiovascular events (10), and mortality (12). However, treatment options for acute exacerbations remain limited and current standard therapy of moderate to severe exacerbations (not requiring ventilatory support) consists of increased bronchodilators, oral corticosteroids and antibiotics (49).

However, evidence for the efficacy of antibiotics is inconsistent and limited (64), and antibiotic resistance is become increasingly prevalent in both hospitals and the community. The merits of corticosteroids in the treatment of COPD exacerbations are also debatable. Multiple studies have found significant short-term benefits of corticosteroids including improving lung function in the first three to five days of treatment (68-70) and oxygenation in the first 72 hours in comparison to placebo (70, 71). Corticosteroids have also been shown to reduce hospitalisation length (68, 69), and the likelihood of treatment failure (72). However, recent research suggests that the benefit of corticosteroid therapy at exacerbation may be limited to patients with an eosinophilic inflammatory profile, and that in fact use of corticosteroids in non-eosinophilic exacerbations may impair recovery (55).

Patients suffer from repeated exacerbations (7, 11) and may receive repeated courses of corticosteroids, exposing patients to significant side effects including cataracts, osteoporosis and suppression of the immune system (77). To quickly respond to the symptoms of a COPD exacerbation, healthcare systems encourage widespread self-management of exacerbations which may lead to uncontrolled and unnecessary corticosteroid use with all its associated complications. Crucially, 27% of patients fail treatment with combined antibiotic and corticosteroid therapy by 30 days, and treatment failure rates rise to 37% by 90 days (69, 79).

Thus, new therapies for management of acute exacerbations are urgently needed, in particular specifically targeting the increased inflammation seen at exacerbation.

Exacerbations are inflammatory events, and persistently elevated systemic inflammation is associated with prolonged recovery and recurrence (56). Reduction of inflammation may reduce the intensity and length of exacerbations. Roflumilast is a phosphodiesterase 4 (PDE4) inhibitor and a non-steroid, anti-inflammatory agent, designed to target both the systemic and pulmonary inflammation associated with COPD. The mechanism of action is the inhibition of PDE4, the major cyclic adenosine monophosphate (cAMP)-metabolising enzyme in structural and inflammatory cells important in the pathogenesis of COPD. Inhibition of PDE4 increases intracellular cAMP and typically leads to an anti-inflammatory effect. Roflumilast and its major active metabolite, roflumilast N-oxide, demonstrate potent anti-inflammatory effects (91, 92).

COPD exacerbations are associated with an increased neutrophilic inflammation (38) which is relatively insensitive to corticosteroids. In contrast to corticosteroids, roflumilast demonstrates broad anti-inflammatory activities in-vitro and in animal studies, including the modulation of mediator release from neutrophils, and has been shown to reduce neutrophilic airway inflammation in COPD patients (92). The efficacy and safety of roflumilast in stable COPD has been investigated in numerous clinical trials in patients with moderate to very severe COPD. In all trials roflumilast improved lung function and reduced exacerbations when taken daily for 6-12 months (93-95). In these long-term maintenance therapy trials, roflumilast was generally safe and well tolerated. In particular, there is no indication that roflumilast use is associated with an increase of pneumonia as observed for inhaled corticosteroids (94). Diarrhoea is a common side effect and some patients experience

significant weight loss, although this is reversible upon withdrawal. Additionally, roflumilast significantly reduces endotoxin-induced influx of neutrophils, eosinophils, and total cells into the bronchoalveolar compartment in healthy volunteers (96). Thus, we hypothesised that roflumilast will be beneficial in the treatment for COPD exacerbations.

7.2 Aim

Therefore we conducted a randomised, placebo controlled trial to test the hypothesis that Roflumilast reduces neutrophilic inflammation during exacerbations of COPD when added to the usual care of these patients. **Chapter 6** has demonstrated that faster resolution of neutrophilic inflammation is associated with shorter clinical recovery. Thus this study serves as a proof-of-mechanism trial to examine whether roflumilast can improve clinical recovery following COPD exacerbations. In addition, the potential benefit of roflumilast to reduce the length and severity of exacerbations has been assessed using patient diaries and questionnaires (CAT and EXACT).

7.3 Methods

Full methodology for this chapter is described in methods section 3.5. The TREAT (**T**reatment with **R**oflumilast at **ExA**cerba**T**ion) study was a randomised, double-blind, parallel-group, single centre, phase II trial. COPD patients were recruited from the London COPD patient cohort at exacerbation between 14th February 2012 and 21st February 2014. Patients were consented, recruited and randomised at exacerbation presentation prior to commencement of systemic therapy, within 7 days of exacerbation onset as judged by diary cards. Patients received Roflumilast or placebo for 28 days on top of standard exacerbation therapy with 7 days oral amoxicillin (or clarithromycin in event of penicillin allergy) and 10 days oral prednisolone. Patients were followed up for 8 weeks following recruitment (**Figure 3.10**).

Figure 3.10. Clinical trial overview.
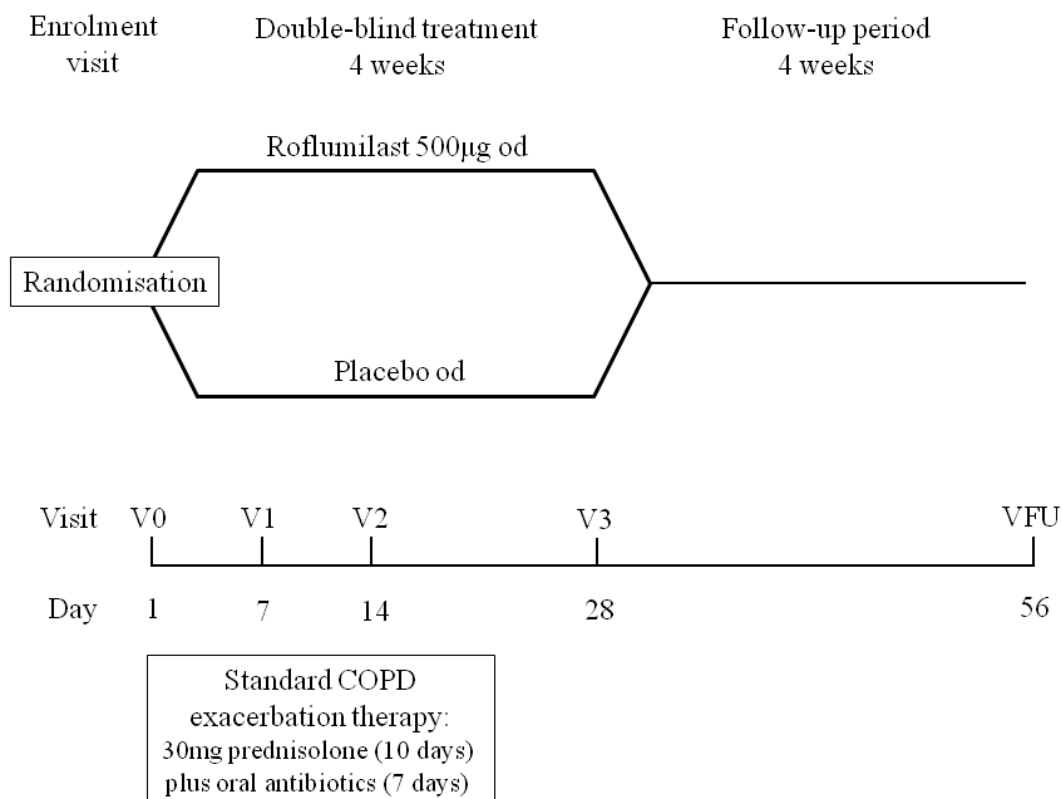
To measure the efficacy of Roflumilast in separate exacerbations within the same patient, repeated exacerbations from the same patient were included if the second event occurred at least 3 months after end of the initial 8 week exacerbation follow-up period. Therefore 2 groups were available for analysis:

1. **Initial Approach**-included just one exacerbation per patient.

   In the initial approach 38 patients were randomised to the Roflumilast group and 43 patients into the placebo group.

2. **Extended Approach**- included repeated exacerbations from the same patient.

   In the extended approach 48 exacerbations were randomised to the Roflumilast group and 47 exacerbations into the placebo group.

The primary endpoint was change in sputum neutrophil counts from Visit V0 to V2, 14 days after randomisation. Secondary endpoints included assessment of change from visit V0 to each post randomisation visit for sputum markers, blood biomarkers, spirometric assessments, PROs and diary card outcomes.

Statistical Analysis

All reported efficacy analyses were predefined. Quantitative endpoints are expressed using descriptive statistics (mean ± SD). Categorical data are expressed descriptively by absolute and relative frequencies (n and %).

The primary endpoint was analysed with an analysis of covariance model. The model contained the primary endpoint as an independent variable and neutrophil count at enrolment and treatment as independent variables. The comparison was performed at a 2-sided significance level α=0.05 and was based on the full analysis set. Descriptive and inferential statistics were used to analyse secondary endpoints. All analyses of secondary endpoints were exploratory.

Sample size calculation was based on the primary endpoint, change in sputum neutrophil counts from enrolment to Day 14 post exacerbation. With the assumption of an effect size of 40%, with the neutrophil count at Day 14 post exacerbation with placebo being 20% higher than baseline (previously reported as 1.48×10^6 (147)) and the neutrophil count on roflumilast 20% lower than baseline, the change in neutrophil count between enrolment and Day 14 was expected to be 0.414×10^6 cells/g with placebo and 1.006×10^6 cells/g with roflumilast. It was also assumed that the standard deviation of the change with these paired samples would be the same as the IQR of the neutrophil count at baseline (1.18×10^6 cells/g (147)). Based on these assumptions, 63 patients in each group (total 126) were required to detect such a difference with 80% power at the 5% significance level. To allow for a 10% drop-out, we aimed to recruit 140 patients.

The scientific oversight of the study was provided by a steering committee responsible for providing scientific advice about the study design, execution, interpretation, and publication of results. As sponsor of this study, Takeda (Takeda Development Centre Europe Ltd, London, UK) was responsible for study oversight and overall project management. Accovion GmbH (Frankfurt, Germany) managed the administration, coordination, and monitoring of the study, including data management and statistical analysis. Statistical analysis was performed by Accovion with statistical support from Takeda in accordance with statistical analysis plan agreed with myself, Professor Wedzicha and Dr Gavin Donaldson.

7.4 Results

Patient Characteristics

Full baseline clinical characteristics of the patients included in this paper are reported in **Table 7.1**. The initial approach included 38 patients who were randomised to the Roflumilast group and 43 patients into the placebo group, with one exacerbation included per patient. The extended approach included repeated exacerbations from the same patient.

Table 7.1. Baseline clinical characteristics.

	Initial Approach		Extended Approach	
	Roflumilast n=38	Placebo n=43	Roflumilast n=48	Placebo n=47
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Age (years)	72.9±8.6	70.5±9.3	72.0±8.9	70.3±9.1
FEV_1 (L)	1.3±0.5	1.4±0.6	1.3±0.5	1.4±0.6
FEV_1 (% predicted)	48.6±14.1	50.1±18.0	49.8±14.1	49.2±17.8
FVC (L)	2.7±0.8	2.9±1.0	2.7±0.9	2.9±1.0
FEV_1 / FVC ratio (%)	46.7±11.7	48.2±13.3	48.5±11.9	47.4±13.0
Smoking pack years	53.1±40.7	56.0±27.1	53.5±38.0	55.0±26.4
BMI (kg/m^2)	26.8±6.4	27.1±5.8	27.6±6.8	26.8±5.8
HCU exacerbations in year prior to enrolment	1.4±1.2	1.3±1.3	1.4±1.2	1.3±1.3
	N (%)	N (%)	N (%)	N (%)
Male gender	22 (57.9)	28 (65.1)	26 (54.2)	31 (66.0)
Current Smokers	8 (21.1)	11 (25.6)	10 (79.2)	13 (27.7)
Chronic Bronchitics	24 (63.2)	26 (60.5)	31 (64.6)	29 (61.7)
Frequent Exacerbators (≥2 HCU exacerbations in year prior to enrolment)	10 (26.3)	15 (34.9)	14 (29.2)	16 (34.0)

Table 7.1. Stable state clinical characteristics of patients included in the initial approach with one patient per exacerbation, and the extended approach with repeated exacerbations included.

Figure 7.1: CONSORT diagram for flow of patients through the study.

Primary Outcome: Change in sputum neutrophil count from Exacerbation Presentation (V0) to 14 days post exacerbation (V2)

No significant difference in sputum neutrophil count from V0 to V2 (cells/g sputum) was seen between the Roflumilast group and the placebo group, in either the initial or the extended approach (**Figure 7.2**). By the initial approach, LS mean changes from Day 1 to Day 14 were -18.705 (95% CI -23.228, -14.183) cells/g sputum and -20.109 (95% CI -24.095, -16.123) cells/g sputum for roflumilast and placebo, respectively. The between-treatment difference did not reach statistical significance (1.404 [95% CI -4.731, 7.538] cells/g sputum; p=0.6491). By the extended approach, these values were -19.569 (95% CI -23.367, -15.771) and -19.157 (95% CI -22.854, -15.459) for roflumilast and placebo respectively. The between-treatment difference was -0.412 (-95% CI 5.766, 4.943; p=0.8786).
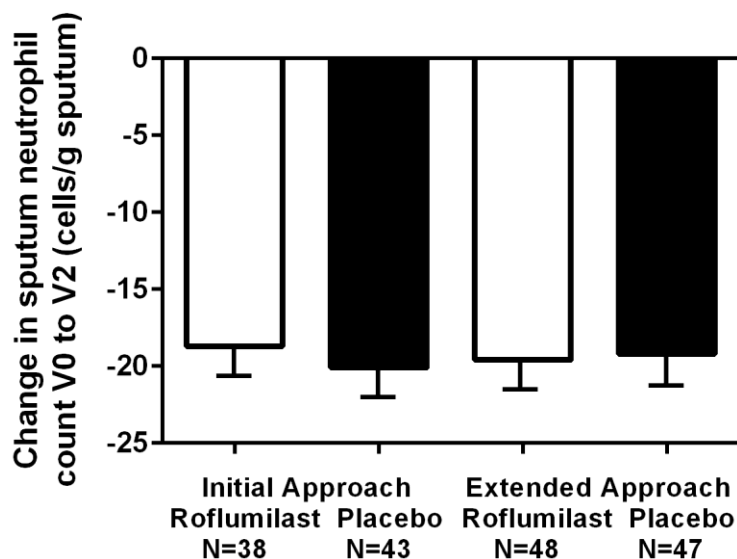
Figure 7.2. Primary Outcome: Change in sputum neutrophil count from V0 to V2 (cells/g sputum), initial and extended approach. Roflumilast treated events shown as white filled bars with black border, placebo treated events shown as solid black bars. Lines represent standard errors.

Secondary Outcomes: Airway Inflammation

Roflumilast use led to significant reductions in airway inflammation as shown by change from V0 to V3 (exacerbation presentation, Day 1 to day 28 post exacerbation onset) in % sputum neutrophils (p=0.0486 initial approach; p=0.0208 extended approach), and myeloperoxidase concentration (p=0.018 initial approach; p=0.0149 extended approach). Roflumilast use also led to enhanced reductions in airway inflammation from V0 to V1 that approached statistical significance for IL-6 and MPO (IL-6 p=0.0546 initial approach, p=0.1312 extended approach; MPO p=0.0884 initial approach, p=0.0881 extended approach). Full results for all sputum biomarkers are shown in **Table 7.2A and 7.2B**).

Secondary Outcomes: Systemic Inflammation

No significant differences were seen in serum inflammatory markers between patients treated with Roflumilast or placebo in the initial approach. However, in the extended approach, patients treated with Roflumilast displayed a small rise in serum IL-6 and serum CRP from V0-V2 (Day 1-14), leading to a statistically significant change compared to placebo (change from V0-V2, Roflumilast vs placebo, p=0.0337 for IL-6, and p=0.0318 for CRP). Whilst statistically significant, the absolute rise in serum IL-6 and serum CRP from V0-V2 were numerically small at 1.607pg/mL and 5.720mg/L respectively, and thus are unlikely to be clinically significant. Full results for all blood biomarkers are shown in **Table 7.3A and 7.3B**).

Table 7.2A: LS mean change in sputum inflammatory markers (initial approach)

	Change from V0 to V1 (Day 1–7)			Change from V0 to V2 (Day 1–14)			Change from V0 to V3 (Day 1–28)			Change from V0 to VFU (Day 1–56)		
	ROF	PBO	ROF vs PBO	ROF	PBO	ROF vs PBO	ROF	PBO	ROF vs PBO	ROF	PBO	ROF vs PBO
Sputum markers												
Sputum neutrophil count (10^6 cells/g sputum)	-20.445 (-26.104, -14.786)	-24.024 (-29.156, -18.893)	3.579 (-4.072, 11.231), p=0.3544	-18.705 (-23.228, -14.183)*	-20.109 (-24.095, -16.123)*	1.404 (-4.731, 7.538) p=0.6491*	-25.704 (-32.602, -18.807)	-19.188 (-25.013, -13.364)	-6.516 (-15.563, 2.532) p=0.1555	-18.400 (-24.901, -11.899)	-22.817 (-28.293, -17.341)	4.417 (-4.093, 12.927) p=0.3045
Total cells (10^6 /g)	-25.559 (-32.585, -18.532)	-27.563 (-34.200, -20.927)	2.005 (-7.677, 11.686) p=0.6813	-21.813 (-27.201, -16.425)	-25.111 (-30.098, -20.125)	3.299 (-4.085, 10.682) p=0.3765	-29.200 (-37.207, -21.193)	-22.218 (-28.910, -15.525)	-6.982 (-17.449, 3.484) p=0.1880	-24.477 (-29.867, -19.087)	-26.474 (-31.135, -21.814)	1.997 (-5.164, 9.159) p=0.5803
Neutrophils (%)	-11.325 (-18.969, -3.680)	-14.484 (-21.575, -7.394)	3.160 (-7.277, 13.597) p=0.5483	-16.770 (-24.705, -8.836)	-20.346 (-27.418, -13.274)	3.576 (-7.089, 14.240) p=0.5062	-27.760 (-38.267, -17.252)	-13.837 (-22.797, -4.876)	-13.923 (-27.760, -0.086) p=0.0486	-19.663 (-30.742, -8.584)	-17.047 (-25.472, -8.622)	-2.615 (-16.563, 11.332) p=0.7098
Macrophages (%)	11.393 (3.749, 19.036)	15.430 (8.339, 22.520)	-4.037 (-14.467, 6.393) p=0.4431	16.062 (8.234, 23.890)	20.774 (13.789, 27.758)	-4.712 (-15.225, 5.802) p=0.3748	27.535 (17.009, 38.060)	13.382 (4.411, 22.352)	14.153 (0.304, 28.002) p=0.0453	16.593 (5.559, 27.627)	15.877 (7.567, 24.187)	0.716 (-13.117, 14.548) p=0.9182
Eosinophils (%)	-0.259 (-0.747, 0.228)	-0.100 (-0.548, 0.347)	-0.159 (-0.825, 0.507) p=0.6362	-0.110 (-0.759, 0.539)	-0.188 (-0.762, 0.386)	0.078 (-0.791, 0.947) p=0.8584	0.043 (-2.366, 2.451)	1.597 (-0.434, 3.628)	-1.555 (-4.706, 1.597) p=0.3289	2.187 (-2.351, 6.725)	1.086 (-2.432, 4.605)	1.101 (-4.642, 6.843) p=0.7036
Lymphocytes (%)	0.229 (-0.474, 0.932)	0.182 (-0.468, 0.831)	0.047 (-0.911, 1.005) p=0.9224	0.430 (-0.077, 0.936)	0.295 (-0.154, 0.743)	0.135 (-0.542, 0.813) p=0.6923	0.039 (-0.326, 0.404)	-0.325 (-0.633, -0.016)	0.363 (-0.116, 0.843) p=0.1355	-0.095 (-0.467, 0.278)	-0.051 (-0.324, 0.222)	-0.043 (-0.505, 0.419) p=0.8529
IL-6 (pg/mL)	-1317.400 (-1603.823, -1030.976)	-934.624 (-1200.065, -669.182)	-382.776 (-773.373, 7.821) p=0.0546	-822.715 (-1263.721, -381.708)	-695.955 (-1106.819, -288.091)	-126.760 (-729.574, 476.055) p=0.6764	-1232.929 (-1656.668, -809.109)	-955.365 (-1303.530, -607.200)	-277.564 (-826.228, 271.101) p=0.3167	-1042.097 (-1491.651, -592.542)	-996.485 (-1388.476, -604.494)	-45.612 (-642.166, 550.943) p=0.8793
IL-8 (pg/mL)	-174718.396 (-265342.682, -84094.111)	-227274.816 (-310605.023, -143944.610)	52556.420 (-70496.843, 175609.682) p=0.3975	-149198.139 (-243622.453, -54773.826)	-199515.687 (-287119.434, -111911.940)	50317.548 (-78505.910, 179141.005) p=0.4389	-255317.294 (-396618.822, -114015.765)	-160587.365 (-276044.886, -45129.844)	-94729.929 (-277217.297, 87757.440) p=0.3043	-204731.356 (-305164.180, -104298.533)	-223918.341 (-309676.552, -138160.130)	19186.984 (-112898.540, 151272.508) p=0.7731
Neutrophil elastase (µg/mL)	-222995.020 (-252658.522, -193331.517)	-224606.396 (-251844.437, -197368.355)	1611.376 (-38762.691, 41985.444) p=0.9368	-149211.318 (-227963.551, -70459.086)	-125385.276 (-198372.069, -52398.483)	-23826.042 (-131241.297, 83589.212) p=0.6598	-200905.068 (-251581.394, -150228.742)	-149865.936 (-190811.843, -108920.029)	-51039.132 (-116304.330, 14226.066) p=0.1234	-141985.838 (-198332.110, -85639.566)	-169389.587 (-216748.120, -122031.054)	27403.749 (-46270.048, 101077.546) p=0.4609
Myeloperoxidase (ng/mL)	-9253.786 (-13233.048, -5274.525)	-4521.397 (-8235.233, -807.561)	-4732.389 (-10193.012, 728.233) p=0.0884	-8072.784 (-12346.581, -3798.987)	-6318.878 (-10309.822, -2327.935)	-1753.906 (-7623.747, 4115.936) p=0.5534	-13744.824 (-18678.606, -8811.041)	-5944.184 (-10012.135, -1876.233)	-7800.640 (-14241.610, -1359.670) p=0.0183	-10248.612 (-15385.680, -5111.544)	-9478.460 (-13930.276, -5026.644)	-770.151 (-7607.330, 6067.027) p=0.8230

Data are LS mean (95% CI) in the intention-to-treat population. LS means are from Mixed Model Repeated Measurement (MMRM) for all secondary endpoints. *LS means are from ANCOVA for the primary endpoint. ROF=roflumilast. PBO=placebo.

Table 7.2B: LS mean change in sputum inflammatory markers (extended approach)

	Change from V0 to V1 (Day 1–7)			Change from V0 to V2 (Day 1–14)			Change from V0 to V3 (Day 1–28)			Change from V0 to VFU (Day 1–56)		
	ROF	PBO	ROF vs PBO	ROF	PBO	ROF vs PBO	ROF	PBO	ROF vs PBO	ROF	PBO	ROF vs PBO
Sputum markers												
Sputum neutrophil count (10^6 cells/g sputum)	-21.265 (-26.389, -16.140)	-22.326 (-27.380, -17.271)	1.061 (-6.141, 8.263) p=0.7704	-19.569 (-23.367, -15.771)*	-19.157 (-22.854, -15.459)*	-0.412 (-5.766, 4.943) p= 0.8786*	-24.112 (-30.203, -18.020)	-17.969 (-23.559, -12.378)	-6.143 (-14.421, 2.135) p=0.1438	-12.788 (-21.283, -4.292)	-21.892 (-28.821, -14.963)	9.104 (-1.865, 20.073) p=0.1026
Total cells (10^6 /g)	-26.634 (-32.653 - 20.615)	-26.335 (-32.541, -20.129)	-0.299 (-8.962, 8.364) p=0.9455	-23.512 (-28.376, -18.648)	-25.332 (-30.239, -20.424)	1.820 (-5.130, 8.769) p=0.6042	-29.023 (-35.672, -22.373)	-22.920 (-28.774, -17.066)	-6.102 (-14.984, 2.779) p=0.1757	-19.551 (-27.339, -11.764)	-26.855 (-33.879, -19.831)	7.304 (-3.198, 17.806) p=0.1705
Neutrophils (%)	-14.637 (-22.088, -7.185)	-16.060 (-23.441, -8.678)	1.423 (-9.081, 11.927) p=0.7883	-20.690 (-28.160, -13.220)	-21.052 (-28.364, -13.740)	0.362 (-10.124, 10.848) p=0.9455	-29.848 (-39.233, -20.462)	-14.664 (-23.353, -5.974)	-15.184 (-28.004, -2.363) p=0.0208	-21.327 (-31.283, -11.370)	-17.328 (-25.652, -9.003)	-3.999 (-17.009, 9.012) p=0.5428
Macrophages (%)	14.679 (7.245, 22.113)	17.228 (9.864, 24.592)	-2.549 (-13.023, 7.925) p=0.6297	20.331 (12.885, 27.777)	21.599 (14.309, 28.889)	-1.268 (-11.713, 9.177) p=0.8099	29.510 (20.132, 38.888)	14.463 (5.781, 23.144)	15.048 (2.245, 27.850) p=0.0218	19.075 (9.129, 29.021)	16.322 (8.060, 24.584)	2.753 (-10.201, 15.706) p=0.6737
Eosinophils (%)	-0.341 (-0.749, 0.068)	-0.141 (-0.542, 0.260)	-0.200 (-0.776, 0.376) p=0.4925	-0.174 (-0.713, 0.364)	-0.214 (-0.738, 0.309)	0.040 (-0.713, 0.792) p=0.9167	0.031 (-2.045, 2.107)	1.518 (-0.374, 3.411)	-1.487 (-4.297, 1.323) p=0.2957	1.714 (-2.200, 5.627)	0.913 (-2.378, 4.203)	0.801 (-4.312, 5.914) p=0.7562
Lymphocytes (%)	0.109 (-0.502, 0.720)	-0.015 (-0.618, 0.588)	0.124 (-0.735, 0.983) p=0.7747	0.309 (-0.188, 0.805)	0.195 (-0.289, 0.679)	0.113 (-0.580, 0.807) p=0.7463	-0.118 (-0.462, 0.225)	-0.431 (-0.744, -0.118)	0.313 (-0.153, 0.778) p=0.1849	-0.203 (-0.530, 0.123)	-0.208 (-0.470, 0.055)	0.004 (-0.414, 0.422) p=0.9843
IL-6 (pg/mL)	-1207.678 (-1472.709, -942.646)	-923.325 (-1182.859, -663.791)	-284.353 (-655.351, 86.645) p=0.1312	-725.454 (-1114.998, -335.910)	-705.849 (-1089.366, -322.331)	-19.605 (-566.291, 527.082) p=0.9433	-1115.741 (-1514.125, -717.357)	-877.871 (-1221.742, -533.999)	-237.870 (-764.005, 288.265) p=0.3712	-860.191 (-1270.349, -450.032)	-962.342 (-1339.748, -584.935)	102.151 (-455.166, 659.468) p=0.7164
IL-8 (pg/mL)	-193222.856 (-271948.714, -114496.997)	-215296.477 (-291710.432, -138882.522)	22073.621 (-87606.768, 131754.011) p=0.6901	-172069.810 (-254267.450, -89872.170)	-195751.307 (-276176.478, -115326.135)	23681.497 (-91333.372, 138696.366) p=0.6833	-264788.038 (-388936.322, -140639.754)	-160441.028 (-267734.979, -53147.077)	-104347.010 (-268439.773, 59745.753) p=0.2096	-168204.970 (-271118.435, -65291.506)	-205822.814 (-298231.512, -113414.117)	37617.844 (-100676.823, 175912.511) p=0.5900
Neutrophil elastase (µg/mL)	-202235.344 (-228069.915, -176400.772)	-206912.465 (-231851.593, -181973.337)	4677.121 (-31294.505, 40648.747) p=0.7966	-143831.510 (-211519.846, -76143.175)	-110144.325 (-176177.838, -44110.811)	-33687.186 (-128273.181, 60898.809) p=0.4808	-183452.324 (-228308.127, -138596.522)	-130613.019 (-168898.812, -92327.225)	-52839.306 (-111867.516, 6188.905) p=0.0787	-118129.436 (-168625.560, -67633.313)	-156544.533 (-201380.082, -111708.984)	38415.096 (-29147.155, 105977.348) p=0.2614
Myeloperoxidase (ng/mL)	-8606.357 (-12080.048, -5132.666)	-4380.803 (-7786.800, -974.807)	-4225.553 (-9094.548, 643.441) p=0.0881	-8380.855 (-12102.755, -4658.956)	-5929.775 (-9595.757, -2263.794)	-2451.080 (-7683.078, 2780.919) p=0.3543	-12692.279 (-17106.977, -8277.581)	-5351.001 (-9196.240, -1505.763)	-7341.277 (-13217.127, -1465.428) p=0.0149	-9424.227 (-13949.488, -4898.966)	-8527.766 (-12660.203, -4395.329)	-896.461 (-7042.991, 5250.069) p=0.7725

Data are LS mean (95% CI) in the intention-to-treat population. LS means are from MMRM for all secondary endpoints. *LS means are from ANCOVA for the primary endpoint. ROF=roflumilast. PBO=placebo.

Table 7.3A: LS mean change in blood biomarkers (initial approach)

	Change from V0 to V1 (Day 1–7)			Change from V0 to V2 (Day 1–14)			Change from V0 to V3 (Day 1–28)			Change from V0 to VFU (Day 1–56)		
	ROF	PBO	ROF vs PBO	ROF	PBO	ROF vs PBO	ROF	PBO	ROF vs PBO	ROF	PBO	ROF vs PBO
Blood biomarkers												
IL-6 (pg/mL)	-12.469 (-14.026, -10.911)	-11.904 (-13.382, -10.427)	-0.564 (-2.716, 1.588) p=0.6032	-1.973 (-5.792, 1.847)	-5.728 (-9.171, -2.285)	3.755 (-1.390, 8.900) p=0.1502	-8.108 (-12.807, -3.409)	-7.243 (-11.219, -3.267)	-0.865 (-7.022, 5.293) p=0.7806	-11.665 (-13.914, -9.417)	-9.431 (-11.296, -7.565)	-2.235 (-5.161, 0.692) p=0.1325
IL-1β (pg/mL)	1.274 (-0.084, 2.631)	0.378 (-0.910, 1.665)	0.896 (-0.975, 2.767) p=0.3434	0.217 (-0.499, 0.932)	0.495 (-0.150, 1.139)	-0.278 (-1.241, 0.685) p=0.5674	0.657 (-0.201, 1.516)	0.541 (-0.188, 1.270)	0.116 (-1.010, 1.243) p=0.8376	-0.003 (-0.657, 0.652)	0.464 (-0.109, 1.037)	-0.467 (-1.336, 0.403) p=0.2885
CRP (mg/L)	-15.579 (-16.546, -14.613)	-16.518 (-17.437, -15.600)	0.939 (-0.398, 2.275) p=0.1660	4.836 (-3.623, 13.296)	-4.369 (-11.958, 3.220)	9.205 (-2.160, 20.571) p=0.1109	-9.179 (-17.362, -0.996)	-8.018 (-15.051, -0.985)	-1.161 (-11.952, 9.629) p=0.8309	-14.889 (-17.665, -12.112)	-13.057 (-15.312, -10.802)	-1.832 (-5.412, 1.748) p=0.3115
Fibrinogen (µmol/L)	-3.916 (-4.487, -3.345)	-3.976 (-4.496, -3.457)	0.060 (-0.713, 0.833) p=0.8771	0.404 (-0.841, 1.648)	-0.184 (-1.321, 0.953)	0.588 (-1.099, 2.274) p=0.4894	0.210 (-1.137, 1.557)	0.014 (-1.116, 1.144)	0.196 (-1.562, 1.955) p=0.8247	-2.127 (-3.373, -0.882)	-1.144 (-2.171, -0.117)	-0.983 (-2.597, 0.631) p=0.2287

Table 7.3B. LS mean change in blood biomarkers (extended approach)

	Change from V0 to V1 (Day 1–7)			Change from V0 to V2 (Day 1–14)			Change from V0 to V3 (Day 1–28)			Change from V0 to VFU (Day 1–56)		
	ROF	PBO	ROF vs PBO	ROF	PBO	ROF vs PBO	ROF	PBO	ROF vs PBO	ROF	PBO	ROF vs PBO
Blood biomarkers												
IL-6 (pg/mL)	-12.314 (-13.609, -11.019)	-11.802 (-13.124, -10.480)	-0.512 (-2.363, 1.340) p=0.5844	1.607 (-3.444, 6.658)	-6.069 (-11.015, -1.124)	7.676 (0.607, 14.746) p=0.0337	-7.587 (-11.734, -3.441)	-7.619 (-11.269, -3.970)	0.032 (-5.493, 5.557) p=0.9909	-10.811 (-12.925, -8.697)	-9.604 (-11.428, -7.780)	-1.207 (-4.000, 1.587) p=0.3932
IL-1β (pg/mL)	0.350 (-0.823, 1.522)	-1.002 (-2.198, 0.193)	1.352 (-0.322, 3.027) p=0.1122	-0.394 (-1.099, 0.311)	-0.841 (-1.533, -0.148)	0.447 (-0.542, 1.435) p=0.3721	-0.139 (-0.960, 0.682)	-0.757 (-1.489, -0.025)	0.618 (-0.483, 1.718) p=0.2676	-0.799 (-1.459, -0.138)	-0.806 (-1.426, -0.187)	0.008 (-0.898, 0.914) p=0.9864
CRP (mg/L)	-16.166 (-16.980, -15.352)	-16.901 (-17.729, -16.072)	0.734 (-0.434, 1.903) p=0.2152	5.720 (-1.429, 12.870)	-5.257 (-12.245, 1.731)	10.977 (0.979, 20.975) p=0.0318	-8.250 (-15.801, -0.700)	-9.254 (-15.958, -2.550)	1.004 (-9.095, 11.102) p=0.8440	-15.152 (-17.573, -12.730)	-13.793 (-15.847, -11.738)	-1.359 (-4.538, 1.819) p=0.3980
Fibrinogen (µmol/L)	-3.971 (-4.460, -3.481)	-3.839 (-4.324, -3.353)	-0.132 (-0.822, 0.558) p=0.7046	0.051 (-0.984, 1.087)	-0.148 (-1.180, 0.885)	0.199 (-1.263, 1.661) p=0.7873	0.147 (-1.044, 1.337)	-0.091 (-1.133, 0.952)	0.237 (-1.345, 1.820) p=0.7664	-2.155 (-3.213, -1.096)	-1.162 (-2.079, -0.244)	-0.993 (-2.395, 0.408) p=0.1625

Data are LS mean (95% CI) in the intention-to-treat population. LS means are from Mixed Model Repeated Measurement (MMRM) for all secondary endpoints. *LS means are from ANCOVA for the primary endpoint. ROF=roflumilast. PBO=placebo.

Secondary Outcomes: Lung Function

Roflumilast use led to improvements in lung function and full results are shown in **Table 7.4A and 7.4B**. FEV_1 was improved in the Roflumilast treated group compared to placebo at V1 (1week) and V2 (2 weeks) by approximately 50ml, although these improvements did not meet the threshold for statistical significance. However, at V3 (4 weeks post exacerbation) FEV_1 was greater in the Roflumilast arm by 94ml in the initial approach (p=0.0546) and 87ml in the extended approach which was statistically significant (p=0.0434, **Figure 7.3**).
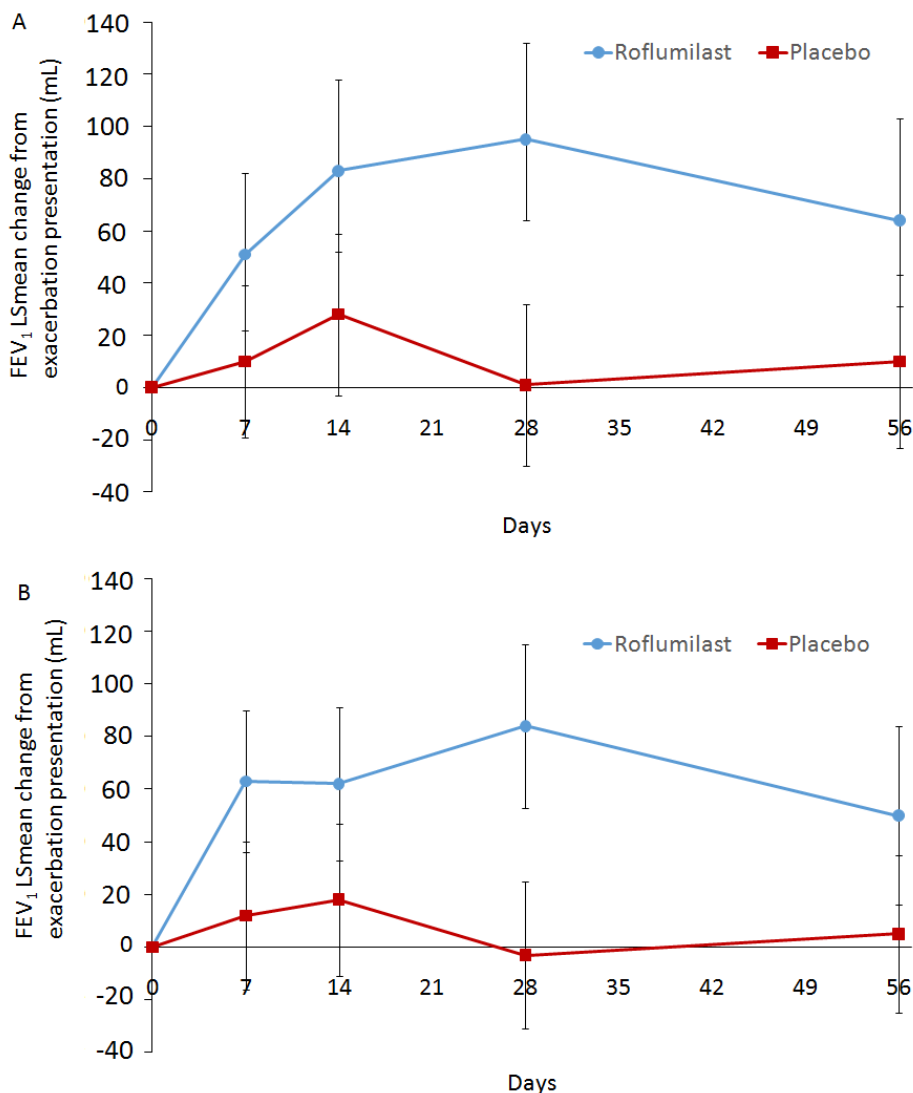
Figure 7.3: Change in FEV_1 from Baseline. A) Initial approach. B) Extended approach. Data are LSmeans ± SE in the intention-to-treat population.

Furthermore, FEV_1% predicted was significantly improved from V0 to V3 (Day 1–28) compared with placebo (p=0.0372 initial approach; p=0.0192 extended approach). The FEV_1/FVC ratio improved statistically significantly more from V0 (exacerbation presentation) to each post-randomisation visit in the roflumilast group compared with the placebo group (p=0.0421, 0.088, 0.0275 and 0.0106 from Day 1 to 7, 14, 28 and 56 respectively initial approach; p=0.0353, 0.081, 0.0158 and 0.0046 from Day 1 to 7, 14, 28 and 56 respectively extended approach). No significant difference was seen in FVC between patients who received Roflumilast or placebo.

Table 7.4A. LS mean change in spirometry outcomes (initial approach)

	Change from Day 1–7			Change from Day 1–14			Change from Day 1–28			Change from Day 1–56		
	ROF	PBO	ROF vs PBO	ROF	PBO	ROF vs PBO	ROF	PBO	ROF vs PBO	ROF	PBO	ROF vs PBO
FEV$_1$ (L)	0.051 (-0.010, 0.112)	0.010 (-0.048, 0.067)	0.041 (-0.043, 0.125) p=0.3328	0.083 (0.013, 0.152)	0.028 (-0.034, 0.090)	0.055 (-0.039, 0.148) p=0.2463	0.095 (0.022, 0.168)	0.001 (-0.062, 0.063)	0.094 (-0.002, 0.191) p=0.0546	0.064 (-0.013, 0.141)	0.010 (-0.055, 0.075)	0.053 (-0.047, 0.154) p=0.2947
FEV$_1$ (% predicted)	2.108 (-0.135, 4.351)	-0.111 (-2.205, 1.983)	2.219 (-0.851, 5.288) p=0.1541	3.370 (0.978, 5.761)	0.759 (-1.404, 2.922)	2.610 (-0.614, 5.835) p=0.1111	3.203 (0.697, 5.709)	-0.314 (-2.461, 1.833)	3.517 (0.215, 6.820) p=0.0372	1.988 (-0.605, 4.581)	-0.294 (-2.460, 1.872)	2.282 (-1.100, 5.664) p=0.1830
FVC (L)	0.002 (-0.117, 0.122)	0.057 (-0.054, 0.168)	-0.055 (-0.218, 0.109) p=0.5077	0.039 (-0.084, 0.162)	0.166 (0.054, 0.278)	-0.127 (-0.294, 0.040) p=0.1341	0.039 (-0.104, 0.181)	0.075 (-0.048, 0.198)	-0.036 (-0.225, 0.153) p=0.7037	0.044 (-0.100, 0.188)	0.086 (-0.037, 0.209)	-0.042 (-0.232, 0.148) p=0.6613
FEV$_1$/FVC (%)	1.575 (-0.283, 3.433)	-1.064 (-2.796, 0.668)	2.639 (0.097, 5.181) p=0.0421	1.874 (-0.142, 3.889)	-1.796 (-3.615, 0.023)	3.670 (0.953, 6.386) p=0.0088	2.350 (0.085, 4.615)	-1.029 (-2.984, 0.927)	3.379 (0.385, 6.373) p=0.0275	1.636 (-0.258, 3.531)	-1.669 (-3.317, -0.022)	3.306 (0.793, 5.819) p=0.0106

Table 7.4B. LS mean change in spirometry outcomes (extended approach)

	Change from Day 1–7			Change from Day 1–14			Change from Day 1–28			Change from Day 1–56		
	ROF	PBO	ROF vs PBO	ROF	PBO	ROF vs PBO	ROF	PBO	ROF vs PBO	ROF	PBO	ROF vs PBO
FEV$_1$ (L)	0.063 (0.008, 0.117)	0.012 (-0.042, 0.067)	0.050 (-0.027, 0.127) p=0.2007	0.062 (0.004, 0.121)	0.018 (-0.039, 0.075)	0.044 (-0.038, 0.125) p=0.2913	0.084 (0.021, 0.146)	-0.003 (-0.060, 0.053)	0.087 (0.003, 0.171) p=0.0434	0.050 (-0.018, 0.118)	0.005 (-0.055, 0.065)	0.045 (-0.046, 0.136) p=0.3290
FEV$_1$ (% predicted)	2.533 (0.572, 4.495)	-0.093 (-2.068, 1.882)	2.626 (-0.157, 5.410) p=0.0641	2.717 (0.682, 4.752)	0.401 (-1.585, 2.388)	2.316 (-0.528, 5.159) p=0.1092	2.971 (0.802, 5.141)	-0.532 (-2.486, 1.423)	3.503 (0.583, 6.423) p=0.0192	1.653 (-0.694, 4.001)	-0.487 (-2.544, 1.571)	2.140 (-0.981, 5.261) p=0.1766
FVC (L)	0.048 (-0.056, 0.152)	0.062 (-0.043, 0.166)	-0.014 (-0.161, 0.134) p=0.8558	0.046 (-0.061, 0.153)	0.146 (0.041, 0.250)	-0.100 (-0.250, 0.050) p=0.1898	0.053 (-0.071, 0.177)	0.067 (-0.046, 0.179)	-0.014 (-0.182, 0.154) p=0.8697	0.034 (-0.095, 0.162)	0.084 (-0.031, 0.199)	-0.050 (-0.224, 0.123) p=0.5645
FEV$_1$/FVC (%)	1.282 (-0.298, 2.861)	-1.128 (-2.714, 0.459)	2.409 (0.170, 4.648) p=0.0353	1.394 (-0.294 3.083)	-1.830 (-3.487 -0.174)	3.225 (0.858, 5.591) p=0.0081	2.045 (0.110, 3.979)	-1.198 (-2.962, 0.566)	3.243 (0.624, 5.862) p=0.0158	1.393 (-0.250, 3.036)	-1.881 (-3.401, -0.361)	3.274 (1.035, 5.513) p=0.0046

Data are LS mean (95% CI) in the intention-to-treat population. LS means are from MMRM. ROF=roflumilast. PBO=placebo. FEV$_1$=forced expiratory volume in 1s. FVC=forced vital capacity.

Secondary Outcomes: Patient Reported Outcomes (CAT & EXACT scores)

Reduction in CAT score from exacerbation onset to convalescence (Week 1 to Day 56) was greater in patients treated with Roflumilast compared to placebo and narrowly missed statistical significance (initial approach p=0.0504, extended approach p=0.0753, (**Figures 7.4A and 7.4B, and Tables 7.5A and 7.5B**). However, by both the initial and extended approach, changes from Day 56 in weekly average EXACT-PRO scores did not differ statistically significantly between the roflumilast and the placebo groups at any time point (**Figures 7.4A and 7.4B, and Tables 7.5A and 7.5B**) and no difference was seen in AUC of changes from V0 to V3 or VFU in daily EXACT scores.

Figure 7.4A: Weekly change in patient reported outcomes (initial approach)
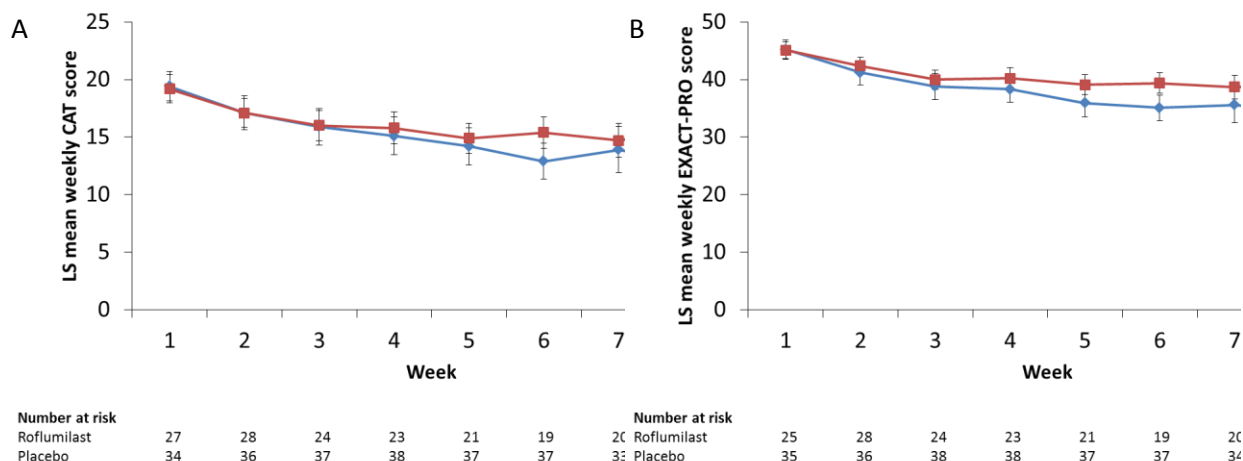
(A) CAT score and (B) EXACT-PRO score. Data are LS means ± SE in the intention to treat population. LS means are from MMRM. CAT=Chronic Obstructive Pulmonary Disease Assessment Test. EXACT-PRO=EXAcerbations of Chronic pulmonary disease Test-Patient-Reported Outcome. Blue line=roflumilast. Redline=placebo

Figure 7.4B: Weekly change in patient reported outcomes (extended approach)
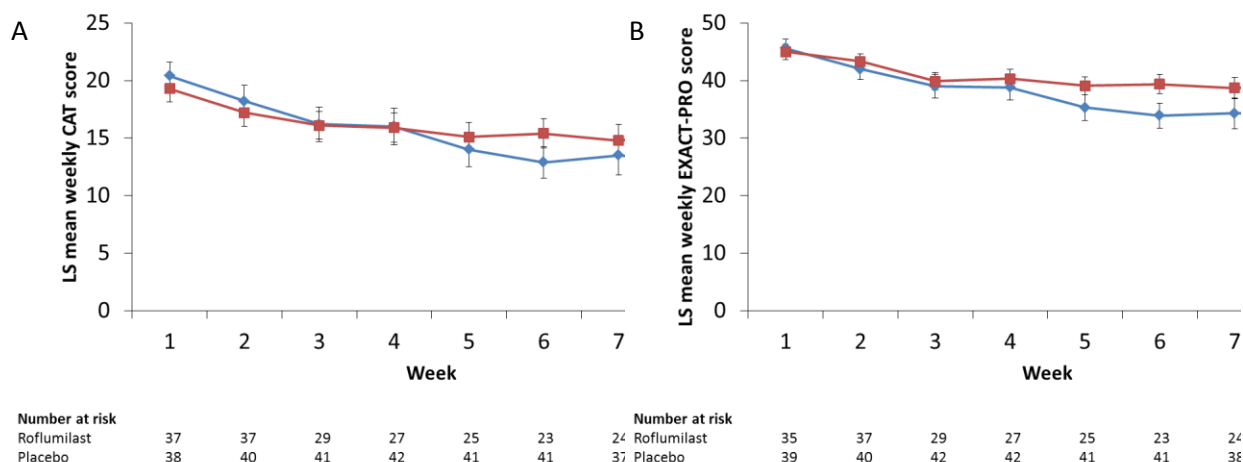
(A) CAT score and (B) EXACT-PRO score. Data are LS means ± SE in the intention to treat population. LS means are from MMRM. CAT=Chronic Obstructive Pulmonary Disease Assessment Test. EXACT-PRO=EXAcerbations of Chronic pulmonary disease Test-Patient-Reported Outcome. Blue line=roflumilast. Redline=placebo

Table 7.5A: Change from stable state (Day 56) in weekly average CAT and EXACT-PRO scores (initial approach)

		Weekly average CAT	Weekly average EXACT-PRO
Day 56 to week 1	**ROF**	6.448 (3.411, 9.485)	6.182 (-0.429, 12.792)
	PBO	2.695 (0.481, 4.910)	4.753 (-0.067, 9.573)
	ROF vs PBO	3.752 (-0.007, 7.511) p=0.0504	1.429 (-6.753, 9.610) p=0.7260
Day 56 to week 2	**ROF**	3.778 (1.273, 6.283)	4.423 (-0.706, 9.552)
	PBO	0.804 (-1.037, 2.645)	1.558 (-2.208, 5.325)
	ROF vs PBO	2.974 (-0.135, 6.083) p=0.0603	2.865 (-3.499, 9.229) p=0.3683
Day 56 to week 3	**ROF**	2.002 (0.153, 3.851)	2.080 (-2.045, 6.205)
	PBO	0.278 (-1.075, 1.631)	0.317 (-2.702, 3.335)
	ROF vs PBO	1.724 (-0.568, 4.015) p=0.1363	1.763 (-3.350, 6.875) p=0.4899
Day 56 to week 4	**ROF**	0.559 (-0.935, 2.053)	0.002 (-4.046, 4.051)
	PBO	0.277 (-0.806, 1.360)	0.657 (-2.292, 3.606)
	ROF vs PBO	0.282 (-1.563, 2.127) p=0.7589	-0.655 (-5.664, 4.354) p=0.7930
Day 56 to week 5	**ROF**	-0.412 (-1.652, 0.828)	-1.058 (-4.590, 2.475)
	PBO	-0.392 (-1.297, 0.512)	-0.307 (-2.887, 2.272)
	ROF vs PBO	-0.019 (-1.555, 1.516) p=0.9797	-0.750 (-5.125, 3.625) p=0.7307
Day 56 to week 6	**ROF**	-0.924 (-1.754, -0.093)	-1.688 (-3.740, 0.365)
	PBO	-0.062 (-0.663, 0.539)	-0.002 (-1.493, 1.489)
	ROF vs PBO	-0.862 (-1.887, 0.164) p=0.0972	-1.686 (-4.224, 0.852) p=0.1871
Day 56 to week 7	**ROF**	-0.228 (-0.920, 0.464)	-0.773 (-2.183, 0.638)
	PBO	0.048 (-0.463, 0.559)	0.002 (-1.038, 1.041)
	ROF vs PBO	-0.276 (-1.136, 0.585) p=0.5206	-0.774 (-2.529, 0.981) p=0.3779
Day 56 to week 8	**ROF**	0.002 (-0.118, 0.122)	-0.190 (-0.713, 0.334)
	PBO	-0.048 (-0.136, 0.041)	-0.154 (-0.539, 0.232)
	ROF vs PBO	0.050 (-0.102, 0.201) p=0.5127	-0.036 (-0.694, 0.622) p=0.9121

Data are LS means (95% CI) in the intention to treat population. LS means are from MMRM. CAT=Chronic Obstructive Pulmonary Disease Assessment Test. EXACT-PRO=EXAcerbations of Chronic pulmonary disease Test-Patient-Reported Outcome. ROF=roflumilast. PBO=placebo.

Table 7.5B: Change from stable state (Day 56) in weekly average CAT and EXACT-PRO scores (extended approach)

		Weekly average CAT	Weekly average EXACT-PRO
Day 56 to week 1	**ROF**	6.232 (3.364, 9.099)	7.163 (1.408, 12.919)
	PBO	3.009 (0.890, 5.128)	4.697 (0.517, 8.878)
	ROF vs PBO	3.223 (-0.343, 6.788) p=0.0753	2.466 (-4.648, 9.580) p=0.4888
Day 56 to week 2	**ROF**	3.558 (1.186, 5.930)	4.839 (0.292, 9.386)
	PBO	1.074 (-0.690, 2.837)	1.433 (-1.892, 4.757)
	ROF vs PBO	2.484 (-0.472, 5.440) p=0.0974	3.406 (-2.227, 9.040) p=0.2298
Day 56 to week 3	**ROF**	2.044 (0.338, 3.750)	2.988 (-0.776, 6.753)
	PBO	0.458 (-0.805, 1.722)	0.179 (-2.564, 2.922)
	ROF vs PBO	1.586 (-0.538, 3.709) p=0.1396	2.809 (-1.850, 7.468) p=0.2310
Day 56 to week 4	**ROF**	0.545 (-0.928, 2.019)	0.909 (-2.838, 4.655)
	PBO	0.429 (-0.646, 1.505)	0.743 (-1.962, 3.448)
	ROF vs PBO	0.116 (-1.708, 1.940) p=0.8986	0.165 (-4.456, 4.787) p=0.9429
Day 56 to week 5	**ROF**	-0.347 (-1.599, 0.904)	-0.657 (-3.786, 2.472)
	PBO	-0.170 (-1.095, 0.755)	-0.318 (-2.591, 1.954)
	ROF vs PBO	-0.177 (-1.734, 1.379) p=0.8195	-0.338 (-4.207, 3.530) p=0.8610
Day 56 to week 6	**ROF**	-0.668 (-1.478, 0.142)	-0.889 (-2.855, 1.076)
	PBO	-0.043 (-0.636, 0.551)	-0.140 (-1.562, 1.282)
	ROF vs PBO	-0.625 (-1.630, 0.379) p=0.2163	-0.749 (-3.177 1.679) p=0.5376
Day 56 to week 7	**ROF**	-0.121 (-0.746, 0.504)	-0.311 (-1.628, 1.005)
	PBO	0.014 (-0.453, 0.481)	-0.075 (-1.039, 0.889)
	ROF vs PBO	-0.135 (-0.915, 0.645) p=0.7294	-0.237 (-1.872, 1.399) p=0.7722
Day 56 to week 8	**ROF**	-0.010 (-0.133, 0.112)	-0.240 (-0.731, 0.251)
	PBO	-0.031 (-0.122, 0.060)	-0.115 (-0.474, 0.244)
	ROF vs PBO	0.021 (-0.134, 0.176) p=0.7895	-0.125 (-0.742, 0.492) p=0.6855

Data are LS means (95% CI) in the intention to treat population. LS means are from MMRM.
CAT=Chronic Obstructive Pulmonary Disease Assessment Test. EXACT-PRO=EXAcerbations of Chronic pulmonary disease Test-Patient-Reported Outcome. ROF=roflumilast. PBO=placebo.

Secondary Outcomes: Exacerbation Length

By the initial approach, Kaplan Meier estimates for exacerbation length in the intention to treat population, were 12.0 (95% CI 8.0, 18.0) days for roflumilast and 14.0 (95% CI 10.0, 17.0) days for placebo. The between treatment difference did not reach statistical significance, with a log rank 2-sided p value of 0.4733. Median exacerbation length in the intention to treat population was 12 days for roflumilast and 10 days for placebo. By the extended approach, Kaplan Meier estimates for exacerbation length in the intention to treat population, were 13.0 (95% CI 9.0, 19.0) days for roflumilast and 14.0 (95% CI 11.0, 17.0) days for placebo. The between treatment difference did not reach statistical significance, with a log rank 2-sided p value of 0.4427. Median exacerbation length in the intention to treat population was 12 days for roflumilast and 11 days for placebo.

Adverse Events

By the initial approach, adverse events were reported by 37 (97.4%) of 38 patients who received roflumilast and by 30 (69.8%) of 43 patients who received placebo; serious adverse events were reported by 4 (10.5%) patients and 1 (2.3%) patient in the roflumilast and placebo groups, respectively (**Table 7.6**). The most frequently reported adverse events were diarrhoea, chronic obstructive pulmonary disease exacerbations, and insomnia (**Table 7.7**). Adverse events led to withdrawal from the study in 10 (26.3%) patients in the roflumilast group compared with 2 (4.7%) patients in the placebo group (**Table 7.8**). By the extended approach, adverse events were reported by 47 (97.9%) of 48 patients who received roflumilast and by 33 (70.2%) of 47 patients who received placebo; serious adverse events were reported by 4 (8.3%) patients and 1 (2.1%) patient in the roflumilast and placebo groups, respectively. The most frequently reported adverse events were diarrhoea, chronic

obstructive pulmonary disease exacerbations, and insomnia. Adverse events led to withdrawal from the study in 15 (31.3%) patients in the roflumilast group compared with 2 (4.3%) patients in the placebo group. No deaths occurred over the entire course of the study.

Table 7.6: Total Adverse Events

	Initial Approach		Extended Approach	
	Roflumilast (N=38)	**Placebo (N=43)**	**Roflumilast (N=48)**	**Placebo (N=47)**
All AEs	37 (97.4%)	30 (69.8%)	47 (97.9%)	33 (70.2%)
Non-serious AEs	36 (94.7%)	30 (69.8%)	46 (95.8%)	33 (70.2%)
Serious AEs	4 (10.5%)	1 (2.3%)	4 (8.3%)	1 (2.1%)
• **Deaths**	0	0	0	0
• **Other SAEs**	4 (10.5%)	1 (2.3%)	4 (8.3%)	1 (2.1%)
AEs with suggested relationship to IMP	33 (86.8%)	16 (37.2%)	42 (87.5%)	17 (36.2%)
AEs with suggested relationship to trial procedures	0	1 (2.3%)	0	1 (2.1%)
AEs leading to withdrawal	10 (26.3%)	2 (4.7%)	15 (31.3%)	2 (4.3%)
AEs not recovered at trial termination	0	0	0	0
AEs with changes in trial treatment or concomitant medication	32 (84.2%)	18 (41.9%)	40 (83.3%)	20 (42.6%)

Table 7.7: Frequent Adverse Events (≥5% of patients)

	Initial Approach		Extended Approach	
	Roflumilast (N=38)	Placebo (N=43)	Roflumilast (N=48)	Placebo (N=47)
Diarrhoea	25 (65.8%)	10 (23.3%)	32 (66.7%)	11 (23.4%)
COPD	17 (44.7%)	14 (32.6%)	19 (39.6%)	16 (34.0%)
Insomnia	12 (31.6%)	2 (4.7%)	14 (29.2%)	2 (4.3%)
Nausea	6 (15.8%)	3 (7.0%)	9 (18.8%)	3 (6.4%)
Dizziness	6 (15.8%)	1 (2.3%)	6 (12.5%)	1 (2.1%)
Decreased appetite	4 (10.5%)	2 (4.7%)	6 (12.5%)	2 (4.3%)
Headache	3 (7.9%)	2 (4.7%)	4 (8.3%)	2 (4.3%)
Abdominal pain	2 (5.3%)	1 (2.3%)		
Constipation	2 (5.3%)	1 (2.3%)		
Gastrooesophageal reflux	2 (5.3%)	1 (2.3%)	3 (6.3%)	1 (2.1%)
Cellulitis	2 (5.3%)	0		
Flatulence	2 (5.3%)	0		
Muscle spasms	2 (5.3%)	0		
Oral candidiasis	2 (5.3%)	0		
Pneumonia	2 (5.3%)	0		
Vomiting			3 (6.3%)	1 (2.1%)

Table 7.8: Trial Discontinuation Rates

	Initial Approach		Extended Approach	
	Roflumilast (N=38)	Placebo (N=43)	Roflumilast (N=48)	Placebo (N=47)
Total number of early discontinued patients	11 (28.9%)	3 (7.0%)	16 (33.3%)	3 (6.4%)
Death	0	0	0	0
Adverse event	10 (26.3%)	2 (4.7%)	15 (31.3%)	2 (4.3%)
Withdrawal by patient	1 (2.6%)	1 (2.3%)	1 (2.1%)	1 (2.1%)
Total number of trial completers	27 (71.1%)	40 (93.0%)	32 (66.7%)	44 (93.6%)

Weight Loss

By the initial approach, patients in the roflumilast group lost 1.96kg (SD 1.631) in body weight during the course of double-blind treatment; in the placebo group, patients gained 0.11kg (SD 0.886) over the same timeframe. By the end of follow up, body weight partially recovered to enrolment values in patients assigned to roflumilast (-1.29kg [SD 1.277]). By the extended approach, patients in the roflumilast group lost 1.89kg (SD 1.704) in body weight during the course of double-blind treatment; in the placebo group, patients gained 0.14kg (SD 0.865) over the same timeframe. By the end of follow up, body weight partially recovered to enrolment values in patients assigned to roflumilast (-1.21kg [SD 1.275]).

7.5 Discussion

This is the first randomised placebo-controlled trial to evaluate the use of the PDE4 inhibitor, Roflumilast during COPD exacerbations. Roflumilast did not accelerate reduction of sputum neutrophils from exacerbation onset to 2 weeks post exacerbation when given on top of standard therapy, but did lead to significantly enhanced recovery of other airway inflammatory markers. Patients treated with Roflumilast also experienced significantly improved lung function compared to patients receiving steroids and antibiotics alone. However, Roflumilast use was associated with higher rates of adverse events, and further study is required to determine the optimal timing and duration of Roflumilast therapy at exacerbation.

As shown in **Chapter 6**, inflammation rises acutely at exacerbation onset and falls during subsequent recovery. Persistently elevated inflammation during exacerbations is associated with clinical non-recovery and recurrence (56). Roflumilast reduces neutrophilic airway inflammation in the stable state (92) and reduces endotoxin-induced influx of neutrophils, eosinophils, and total cells into the bronchoalveolar compartment in healthy volunteers (96). Thus, this study examined whether Roflumilast would enhance inflammation reduction at acute exacerbations of COPD.

No significant difference was detected in the primary outcome: change of sputum neutrophils from exacerbation onset to 2 weeks thereafter between the groups treated with Roflumilast or placebo. However at 4 weeks, patients treated with Roflumilast experienced a statistically significant greater reduction in percentage sputum neutrophils and a larger reduction in absolute numbers of sputum neutrophils (although the enhanced reduction in absolute neutrophil count was not statistically significant). Furthermore, Roflumilast use led to

significant improvements in sputum myeloperoxidase after 4 weeks of treatment, and numerical superiority in sputum IL-8, sputum IL-6, and sputum neutrophil elastase levels at this time-point.

Exacerbations are typically triggered by infective pathogens and it is likely that a significant proportion of the rise in neutrophilic inflammation seen at exacerbation onset is a direct consequence of increased bacterial and/or viral load (40, 47, 49). Antibiotics improve exacerbation recovery (63), potentially by reducing bacterial load and the resultant neutrophilic inflammation. Therefore, it appears that during the early phase of acute exacerbations, the ability of Roflumilast to reduce airway inflammation is superseded by the efficacy of standard antibiotic treatment and the main benefits from Roflumilast in reducing airway inflammation are seen between 2 and 4 weeks post exacerbation onset.

Importantly, Roflumilast use led to significant clinical benefits in exacerbation recovery, in particular in lung function. Patients treated with Roflumilast in the initial approach had over 40ml improvement at 1 week, over 50ml improvement at 2 weeks, and almost 100ml additional improvement in FEV_1 at 4 weeks compared to patients treated with antibiotics and steroids alone which was statistically significant in the extended approach. The benefits of Roflumilast also persisted 4 weeks after treatment discontinued, although were not statistically significant. Previous trials at acute exacerbation have shown that patients receiving systemic corticosteroid therapy experience approximately 50-100ml improvement in FEV_1 compared to placebo (68, 69). Although there is no clear established minimal clinically important difference (MCID) in FEV_1 at acute exacerbation, an improvement of 100ml compared to placebo has been proposed as the MCID for pharmacological trials

conducted in the stable state (148), so an additional improvement of the magnitude observed in this study on top of 10 days of oral prednisolone is highly beneficial.

Patients treated with Roflumilast also displayed greater reduction in CAT scores from the start of the exacerbation to follow-up 8 weeks later, although these benefits narrowly missed statistical significance. However, these findings were not replicated in the EXACT analyses, and the PRO results may have been compromised by relatively low numbers of patients completing these tools throughout recovery so should be interpreted cautiously.

Patients treated with Roflumilast did not have significantly shorter exacerbation length than those patients who received antibiotics and corticosteroids alone, perhaps since this study was not powered to detect a change in exacerbation duration. Long-term erythromycin use has been shown to reduce exacerbation duration (149), however few acute exacerbation studies have previously examined exacerbation length as an endpoint, instead focusing on treatment failure or clinical cure rate as an outcome measure. Antibiotics have been shown to increase clinical cure rate and prolong time to next exacerbation in mild to moderate exacerbations (63), in addition to reducing mortality and length of inpatient stay in severe exacerbations (65). Oral corticosteroids have also been shown to reduce rates of treatment failure and length of inpatient stay by 1-2 days (68, 69) compared to placebo. Observational data has shown that patients who were initiated on Roflumilast during hospitalised exacerbations of COPD had short lengths of inpatient stay (150). In this study exacerbation length was reduced by 1-2 days when assessed using Kaplan Meier estimates in patients treated with Roflumilast but importantly this was in addition to the beneficial effects of oral corticosteroids and antibiotics, so the ability of Roflumilast to reduce exacerbation length when used at acute exacerbation should be explored in future, larger studies.

A recent trial of the tumour necrosis factor α (TNFα) antagonist Etanercept failed to show any benefit at acute exacerbations of COPD compared to prednisolone, although subgroup analyses suggested that patients without peripheral blood eosinophilia did equally well with etanercept or steroids (83). This study is the first trial of a novel acute exacerbation agent to show additional benefits on top of standard therapy with antibiotics and corticosteroids. However, adverse events (in particular diarrhoea, insomnia and weight loss) and withdrawal rates were higher in the Roflumilast arm of the study.

These adverse events are well recognised in long-term studies using Roflumilast as maintenance therapy to prevent COPD exacerbations, although side effects and withdrawal rates in this study exceeded those seen in previous industry sponsored trials (94, 95), where 10-15% of patients withdrew due to adverse events, as opposed to approximately 30% in this trial. Diarrhoea and insomnia are common side effects of antibiotics and corticosteroids respectively, as well as Roflumilast alone. The high adverse event rates observed in this study suggest that these side effects are potentiated when Roflumilast is given concurrently with antibiotics and corticosteroids during acute exacerbations. Despite this, withdrawal rates in this study remain lower than reported in some recent "real-world" reports (151, 152) where over half of patients discontinued therapy due to adverse events.

Therefore further trials are now urgently required to confirm and accurately define the optimum use of Roflumilast as an acute exacerbation treatment. The nadir of gastrointestinal side effects in trials of Roflumilast as maintenance therapy occurs early after initiation of treatment and then adverse events stabilise over time (93, 94). Studies are already underway attempting to improve adherence to Roflumilast (NCT02018432, NCT02165826), by

reducing the initial dosage from 500μg to 250μg per day, or administering full dose treatment on alternate days (NCT01849341) although this may reduce efficacy in the acute setting. Standard exacerbation therapy with oral corticosteroids is associated with serious adverse health effects when patients who take frequent courses of therapy (77), therefore future studies might also seek to establish whether Roflumilast can effectively replace oral corticosteroids at exacerbation. Since corticosteroids appear to be most beneficial in eosinophilic exacerbations (55), future studies may also examine whether exacerbation therapy should vary according to patient phenotype, with corticosteroids being reserved for eosinophilic events and Roflumilast used in non-eosinophilic exacerbations. In this way, Roflumilast may be used as part of a personalised exacerbation treatment strategy.

This study was designed as a proof-of-mechanism trial and meant to support a potential second larger study to investigate the ability of Roflumilast to improve acute exacerbation recovery and reduce recurrence rates. As such, our sample size was not large, and in fact our study did not reach the original power calculation because recruitment was curtailed early by the scientific oversight committee. This early stoppage was due to the sputum cell counts exceeding those used in the power calculation and were consistent with previously seen levels of severe COPD exacerbations (97). The high neutrophil counts may result from the inclusion criteria of this study which enrolled only bronchitic exacerbations which are known to have higher airway inflammation than non-bronchitic exacerbations (50). Thus the oversight committee decided that fewer events would be required to show a significant difference in the primary outcome. This could potentially have impacted the ability of the study to meet the primary endpoint.

However, analysis of sputum data from the ECLIPSE study suggested that to detect a difference of 10 percentage points in mean sputum neutrophil % between two groups with 80% power would require 34 subjects per treatment arm based on a two-sample t-test and alpha level 0.05, which was exceeded by recruitment of this study (153). Furthermore, no improvement was seen in reduction of airway inflammation between the 2 treatment groups at 14 days on any of the airway inflammatory markers measured in this study. This suggests a type 2 error is unlikely and as discussed previously, in this first very early phase of acute exacerbations, the ability of Roflumilast to reduce airway inflammation appears to be superseded by the efficacy of standard therapy treatment. Nevertheless, a priority is now a larger clinical trial to confirm the important clinical benefits that have been indicated in this work and to greater phenotype which patients and exacerbations might benefit from the addition of Roflumilast. Future studies should also include patients undergoing severe exacerbations that require hospitalisation to determine whether Roflumilast reduces length of inpatient stay and readmission rates.

7.6 Conclusion

In conclusion, Roflumilast is a promising novel addition to current exacerbation therapy. Although the primary outcome of this trial was negative, patients treated with Roflumilast on top of standard therapy had improved lung function and reduced airway inflammation compared to patients treated with corticosteroids alone. However, patients treated with Roflumilast also experienced more adverse events. Further studies are required to confirm and accurately define the use of Roflumilast as an acute exacerbation treatment. In particular, Roflumilast may be useful as part of a personalised strategy that treats patients according to exacerbation phenotype.

8

CONCLUSIONS AND SUGGESTIONS FOR FUTURE STUDIES

This chapter summarises the main findings of this thesis, with reference to the stated Hypothesis and Aims of **Chapter 2**, and concludes with suggestions for future studies based on the present results.

8.1 Conclusions

The key findings of this thesis may be summarised thus:

- Baseline CAT scores are significantly elevated in stable COPD patients with a history of frequent exacerbations.

- At exacerbation, CAT scores were significantly elevated from paired baseline values.

- CAT scores reflect exacerbation severity as measured by exacerbation length, increased systemic inflammation and reduction in lung function.

- EXACT is as an effective tool to assess exacerbation severity. EXACT scores increase at COPD exacerbation, the magnitude of which reflects the severity of the event in terms of treatment, systemic inflammation, airflow limitation and symptom recovery time.

- However, the EXACT is not a reliable tool to detect exacerbations. Approximately 50% of both diary card-defined and HCU exacerbations crossed the EXACT event threshold but only approximately one third fulfilled the criteria for an EXACT event. Baseline disease severity appears to play an important role in symptom reporting and physician prescribing thresholds at exacerbation.

- In the stable state, increasing cough frequency was associated with worsening health related quality of life.

- Cough frequency increases acutely from baseline levels at exacerbation and falls during subsequent recovery. However, the rise in cough frequency at exacerbation onset did not predict exacerbation severity, and cough frequency at exacerbation onset did not predict recovery time or the likelihood of recurrence.

- Increased systemic inflammation, as measured by absolute levels and change in IL-6, at exacerbation onset predicts clinical non-recovery at 35 days.

- Airway inflammation also increased at exacerbation, although no sputum biomarker showed significant ability to predict exacerbation non-recovery.

- Slower resolution of systemic markers of neutrophilic inflammation was associated with longer clinical recovery.

- Exacerbations with an eosinophilic profile at exacerbation onset appeared more likely to be followed by a recurrent event than non-eosinophilic exacerbations.

- Roflumilast did not accelerate reduction of sputum neutrophils from exacerbation onset to 2 weeks post exacerbation when given on top of standard therapy. Although Roflumilast use led to enhanced reduction of other airway inflammatory markers and improved lung function recovery.

- Roflumilast use was associated with high rates of adverse events and study withdrawal.

Therefore, with regard to the specific Aims of the thesis, described in Chapter 2:

1. ***Can patient reported outcomes, specifically the CAT and EXACT questionnaires, be used to monitor COPD exacerbation severity and recovery?***

   Yes, both the CAT and EXACT questionnaires are effective instruments to monitor COPD exacerbation severity and recovery. Due to its simplicity, ease of use and availability the CAT is more suitable for clinical practice, where as the more detailed EXACT may be more suitable for pharmaceutical studies of novel acute exacerbation therapies.

2. ***What is the relationship between exacerbations diagnosed using validated London COPD cohort diary cards and physician review, and symptom-defined events captured by the EXACT patient reported outcome?***

   There was only a modest relationship found between exacerbations identified using the London Cohort diary card, physician review and the EXACT. Approximately 50% of both diary card-defined and HCU exacerbations crossed the EXACT event threshold but only approximately one third fulfilled the criteria for an EXACT event. At exacerbation, patients exhibited smaller rises in EXACT score as baseline disease severity increased, suggesting that patients with more severe stable disease are more likely to report and receive additional systemic therapy at exacerbations associated with smaller increases in symptom intensity than patients with milder baseline disease. This result confirms that people seek care and are treated for exacerbations for a variety of reasons, including varied tolerance to symptomatic change, and means that the EXACT cannot reliably be used to detect HCU exacerbations.

3. *Is objective cough monitoring an accurate means of determining the severity of an exacerbation and can this method identify patients at risk of prolonged recovery and recurrence?*

No, the rise in cough frequency at exacerbation onset did not predict exacerbation severity, and cough frequency at exacerbation onset was not capable of predicting recovery time or the likelihood of recurrence.

4. *Can airway and systemic inflammatory biomarkers measured at onset or during the recovery phase of a COPD exacerbation predict prolonged recovery and recurrence?*

Increased systemic inflammation, as measured by absolute levels and change in IL-6, at exacerbation onset predicts clinical non-recovery at 35 days. Exacerbations with an eosinophilic (blood) profile at exacerbation onset appeared more likely to be followed by a recurrent event than non-eosinophilic exacerbations. No airway inflammatory biomarker showed the ability to predict exacerbation non-recovery.

5. *Does Roflumilast reduce neutrophilic inflammation during exacerbations of COPD when added to the usual care of these patients?*

Roflumilast did not accelerate reduction of sputum neutrophils from exacerbation onset to 2 weeks post exacerbation when given on top of standard therapy, although it did enhance reduction of other inflammatory markers.

6. Can Roflumilast reduce exacerbation severity and improve clinical recovery following COPD exacerbations?

Yes. Roflumilast led to statistically and clinically meaningful improvements in lung function on top of standard exacerbation therapy. However, patients treated with Roflumilast experienced significant adverse events that raise concerns regarding the use of this therapy in the acute setting.

8.2 Suggestions for Future Work

8.2.1 Patient Reported Outcomes

A number of other groups have replicated the findings published in the CAT paper in both other outpatient groups and hospitalised patients and confirmed that this instrument can be a useful tool to evaluate exacerbation severity and recovery (154, 155). Furthermore, results of a study of hospitalised patients suggested that patients with higher CAT score increases during the initial days of hospitalisation and with higher area under the curve (AUC) CAT scores for the first 5 days of exacerbation may be more likely to require hospitalisation for a recurrent exacerbation (155). However, the total number of patients in this study was small at 45 with only 6 patients undergoing recurrent exacerbations within 6 months of their initial hospitalisation for an exacerbation.

Future studies should focus on using the CAT in clinical capacities in both the outpatient and inpatient settings. Outpatient studies which use CAT directed treatment algorithms may be useful to stratify patients whose persistently elevated scores indicate the need for close follow-up and perhaps increased treatment post-exacerbation to prevent deterioration or prolonged abnormal recovery. Inpatient studies have shown that exacerbation CAT scores relate to length of stay (156) and so future work should examine using the CAT to direct discharge planning with falling CAT scores indicative of patients suitable for discharge.

Since the EXACT paper was published in the European Respiratory Journal, the results of the ATTAIN study have been released (157). This study examined the ability of aclidinium to reduce exacerbation frequency and included EXACT events as an outcome measure. In

agreement with the work in this thesis, the authors reported low concordance between HCU exacerbations and EXACT events. Future studies should also utilise the EXACT in clinical trials involving novel acute intervention therapies. The data in **Chapter 4** supports the use of the EXACT as an effective tool to measure exacerbation severity and assess recovery, and could provide valuable alternative outcome measures for such studies. Historically many trials of acute exacerbation treatment have used clinical (or treatment) failure, defined as exacerbation symptoms that have not improved or have worsened such that additional or alternate systemic antimicrobial and/or systemic corticosteroid therapy is required, as a key outcome to determine efficacy of acute exacerbation therapies (158). However, this outcome is limited by difficulties discriminating between relapsed exacerbations, where the index event has failed to resolve, and recurrent exacerbations which are separate events that occur shortly after resolution of the initial insult (15). Furthermore, the decision to retreat a patient is subjective and affected by a patient's baseline functioning. As shown in this chapter, it appears that patients with more severe stable disease are more likely to report and receive additional systemic therapy at exacerbations associated with smaller increases in symptom intensity than patients with milder baseline disease. It is likely that more severe patients are more sensitive to change in symptoms and therefore more likely to report earlier and clinicians will naturally be cautious and have a lower threshold to administer repeated treatment courses in such individuals.

EXACT scores are less affected by such biases and can provide an objective measurement of symptomatic change at exacerbation. EXACT scores also accurately track both symptomatic and lung function recovery and thus may discriminate between persistent exacerbation symptoms which may require additional treatment and separate recurrent events. Furthermore, since the EXACT provides a numerical score on a scale from 0-100 it has the

potential to discriminate subtle relative efficacies of new therapies in absolute numerical values, by comparing the EXACT scores of patients taking a novel treatment versus those on placebo one week after administration of treatment, as well as in proportionate means. For example, the relative proportion of patients whose EXACT scores have returned to baseline (or fallen by a predetermined magnitude) by 7 days (the median recovery time in a study evaluating recovery in over 500 exacerbations (13)) could be a valuable novel outcome measure for acute intervention studies. The potential benefit of Roflumilast to reduce the length and severity of exacerbations has been assessed using the CAT and EXACT PROs in **Chapter 7**.

8.2.2 Cough Monitoring

The work presented in Chapter 5 reinforced the importance of cough in COPD patients, and reaffirmed that further specific treatments are also needed to target COPD patients with increased cough frequency to improve health related quality of life. It also confirmed that objective cough monitoring can be successfully used in both the acute setting and at baseline, and thus may be a useful tool in trials of new therapies to reduce cough frequency in COPD patients both in the stable state and at exacerbation, to measure the efficacy of such novel interventions.

Recent trial data has shown that the oral P2X3 antagonist, AF-219, significantly reduces cough frequency in patients with refractory chronic cough (159). Future studies could consider the use of such agents to reduce cough frequency in COPD patients troubled by persistent cough. An important aspect of future studies will be patient selection. The work presented in Chapter 5 suggests that those patients who experienced the largest rise in cough frequency at exacerbation had lower baseline cough frequency, and patients with higher

baseline cough frequency did not always experience a significant rise in cough frequency at exacerbation (**Figure 5.4, Figure 5.5**). This has important implications for the selection of patients to be considered for anti-tussive therapies; patients with high baseline cough frequency may benefit most from long-term therapy to reduce cough frequency but may not gain from such treatments at exacerbation, which might be preferentially offered to patients with low baseline cough frequency.

8.2.3 Biomarker Measurement and Personalised Exacerbation Treatment

Chapter 6 demonstrated that elevated serum IL-6 at exacerbation onset predicts clinical non-recovery at 35 days. This finding has potentially important clinical implications and should be prospectively repeated in a large separate study. If confirmed in this manner then future follow-up of clinical patients could be stratified according to this biomarker level, with those patients with higher IL-6 levels at exacerbation receiving a routine follow-up during recovery (potentially at day 14) so that they could be clinically reviewed and considered for additional treatment to accelerate recovery.

Chapter 6 also found that patients with persistently elevated total white blood cell counts and neutrophil levels (in addition to those with elevated IL-6) during recovery were more likely to have failed to symptomatically recover at 35 days post exacerbation onset. These levels can be easily measured in clinical practice. Studies are currently underway to determine if retreatment at day 14 with antibiotics enhances exacerbation recovery (NCT02300220), and future work should consider targeting patients with elevated levels of these clinically available biomarkers for additional treatment.

Conventional exacerbation treatment also includes systemic corticosteroids. Corticosteroids are particularly effective in combating eosinophilic inflammation. Chapter 6 for the first time showed that blood eosinophils were higher at exacerbation onset in index exacerbations which were followed by recurrent events, than in isolated exacerbations. This result was shown in relatively small numbers and also needs confirmation in future prospective large scale studies, but if confirmed could be used to guide therapy. Patients with eosinophilic inflammation at exacerbation could be considered for prolonged courses of oral corticosteroids in an attempt to reduce the likelihood of recurrence.

The TREAT study showed that Roflumilast could have clinical benefits when added to conventional therapy at exacerbation, particularly in enhancing lung function recovery. However, use of this drug was also associated with significant adverse effects and high withdrawal rates. Future studies could attempt to identify those patients who might receive the maximum benefit from this drug so that it can be used as part of a personalised exacerbation treatment strategy. Future studies might also attempt to mitigate the adverse effects seen with Rofumilast use. Further analysis of large trials of Roflumilast as maintenance therapy could attempt to identify patient characteristics associated with experience of side effects. Future studies could also attempt to identify biomarkers that might reveal patients at increased risk of adverse events. Studies are already underway attempting to improve adherence to Roflumilast (NCT02018432, NCT02165826), by reducing the initial dosage from 500µg to 250µg per day, or administering full dose treatment on alternate days (NCT01849341) although this may reduce efficacy in the acute setting. Additional approaches could also include the addition of anti-emetics and/or anti-diarrhoeal treatment to be taken concurrently with short term exacerbation Roflumilast therapy to improve tolerance.

If it were possible to identify prospectively which patients are more susceptible to the adverse effects that would enable further targeting of patient selection and so enhance personalisation of acute exacerbation therapy with Roflumilast.

9

APPENDICES

APPENDIX 1: Pre-existing Recovery Biomarker data.

9.1 Appendix 1

The following biomarkers were selected for analysis in **<u>Chapter 6</u>** based upon their biological/mechanistic plausibility to inform on exacerbation recovery and recurrence:

- *Exacerbation Aetiology:* Viral markers (IP-10, IFN-γ); Bacterial (IL-1β); Eosinophilic (Blood eosinophils-absolute/%, IL-5)

- *Neutrophilic Markers:* IL-8, MPO, TNFα, Blood Neutrophils

- *Response to clinical status/therapy:* IL-6, SAA, PARC, CRP, Fibrinogen, Total white cell count

- *Oxidative Stress:* sRAGE, CC-16

- *Pathogenesis/Activity:* PLGF, MMP-1, MMP-8, MMP-9, TIMP-1, α2-macroglobulin, IL-18

Past studies examining these biomarkers are summarised in **<u>Table 9.1</u>**.

Table 9.1. Past studies examining biomarkers measured in Chapter 6.

BIOMARKER	MEDIUM	EVIDENCE
α2-macroglobulin	Serum + Sputum	• α_2-macroglobulin forms part of the acute phase response in the coagulation cascade with anti-inflammatory activity through inhibition of oxidative stress. It plays a key role in humoral defence and levels are increased in the sputum in COPD patients. • Serum levels have also been shown to distinguish between asthma and COPD (160).
Clara cell secretory protein (CC16)	Serum	• CC-16 is secreted by the non-ciliated Clara cells 8 which are found predominantly in the respiratory bronchioles, and by non-ciliated columnar cells of the large and small airways. • Serum levels of CC-16 largely reflect protein produced by the lower respiratory tract. • CC-16 acts as an immunosuppressant and provides protection against oxidative stress and carcinogenesis. • Serum levels rise following acute exposure to smoke, chlorine and lipopolysaccharide and can be suppressed by inhaled salmeterol/fluticasone (161). • Serum CC-16 levels are reduced in individuals with COPD and there is a weak correlation with disease severity in former smokers (162). • When stratifying COPD patients into GOLD groups and by current smoking, lower serum CC-16 levels are seen in current smoking COPD patients relative to former smokers in GOLD2 and GOLD3 but not in GOLD4. • In former-smoking COPD patients, a significant inverse correlation is observed between CC-16 and COPD severity. • CC-16 can distinguish between patients with or without reversibility in former smoking COPD patients (162). • Reduced levels of CC-16 are associated with accelerated decline in FEV_1 (163)

C-reactive protein (CRP)	Serum	- CRP is an acute phase protein, mainly induced by interleukin 6 (IL-6) and is a component of the innate immune response - Individuals with a CRP level >3 mg/L compared with those with a value of ≤3 mg/L in the stable state have increased risk of COPD hospitalization and COPD death (164). - CRP increases at exacerbation (111, 132) and higher CRP levels at exacerbation are associated with bacterial aetiology (40). - Patients who do not symptomatically recovery within 5 weeks of exacerbation onset have persistently higher levels of serum CRP during the recovery period than those who recover (56). - Patients who did not recover fully from the symptoms of an exacerbation had a failure to normalize CRP at 1 and 2 weeks post-exacerbation and even had a CRP rise from baseline if they hadn't clinically recovered (56). - A high CRP at day 14 following an index exacerbation predicts exacerbation recurrence (56) and an elevated CRP at discharge from hospitalised exacerbations predicts readmission (165).
Fibrinogen	Plasma	- Fibrinogen is primarily synthesized in the liver, is involved in clotting formation and systemic levels are elevated in an IL-6-stimulated acute phase response. - Plasma fibrinogen is elevated in patients with stable COPD (112). - Bacterial colonisation of the sputum of COPD patients is associated with higher fibrinogen levels (166) and chronic respiratory syncytial virus (RSV) infection is also associated with increased plasma fibrinogen in the stable state (42). - Stable-state plasma fibrinogen levels also increase more quickly over time in frequent exacerbators compared with infrequent exacerbators and elevated plasma fibrinogen is also associated with a faster decline in lung function (167). - High fibrinogen is associated with an increased risk of hospitalised COPD exacerbations and an increased risk of death (168). - Plasma fibrinogen levels increase at exacerbation (112) and reduce significantly during recovery

		(59, 169).
		• The rise in fibrinogen seen at exacerbation responds to corticosteroids, since treatment of exacerbations with intravenous steroid treatment leads to significantly larger reductions in fibrinogen than in patients treated with non-steroid drugs alone (170).
		• Elevated fibrinogen levels seen at exacerbation may relate to infective aetiology, since exacerbations characterised by purulent sputum, increased cough and colds have greater rises in fibrinogen (112).
		• Viral exacerbations in particular are associated with higher plasma fibrinogen concentrations than non-viral exacerbations (42).
Full blood count (total white cell count, neutrophils, eosinophils)	Blood	• Total white cell count and neutrophils are increased at exacerbation compared to stable COPD patients (97, 171), although eosinophils are not always elevated (52).
		• The presence of high total white cell count (172) and blood neutrophilia is associated with increased risk of hospitalization following attendance at the emergency department with COPD exacerbation (172, 173).
		• Eosinopaenia may be associated with increased mortality and length of stay during hospitalized exacerbations (174).
		• Eosinophils are potentially useful to guide corticosteroid therapy at exacerbation (55).
Interferon-γ (IFN-γ)	Sputum	• IFN-γ is a cytokine that is critical for innate and adaptive immunity against viral, some bacterial and protozoal infections. IFN-γ is an important activator of macrophages and inducer of Class I major histocompatibility complex (MHC) molecule expression. In particular IFN- γ directly inhibits viral replication.
		• IFN-γ is produced predominantly by natural killer (NK) and natural killer T (NKT) cells as part of the innate immune response, and by Th1 CD4 and CD8 cytotoxic T lymphocyte (CTL) effector T cells once antigen-specific immunity develops (175).
		• IFN-γ levels are elevated in patients with COPD exacerbation of viral aetiology (176).

Interferon-γ-inducible protein 10 (IP-10)	Serum	• IP-10 is a chemokine secreted by bronchial epithelial cells, monocytes, lymphocytes, and neutrophils in response to interferon-γ and TNF-α (177, 178) • IP-10 is elevated by Human Rhinovirus (HRV) infection (179) • HRV infects and replicates in bronchial epithelial cells. This active replication of HRV triggers production of IP-10 (179) • Serum IP-10 is higher in patients with COPD than controls • HRV-16 induces expression of IP-10 and levels of IP-10 correlate with symptom severity, viral titre, and numbers of lymphocytes in airway secretions of healthy volunteers (179). • In COPD patients, serum IP-10 levels increase significantly from baseline to exacerbation in human rhinovirus (HRV)-positive exacerbations (180), although no change in IP-10 was observed between baseline and exacerbation in HRV-negative exacerbations. • At exacerbation, IP-10 correlates with sputum viral load and In receiver operating characteristics analysis, the combination of IP-10 and coryzal symptoms gave an area under the curve of 0.82 for predicting the presence of HRV at exacerbation (180).
Interleukin-1β (IL-1β)	Sputum	• Interleukin-1 (IL-1) is a superfamily of cytokines. IL-1β is produced by activated macrophages as a proprotein, which is proteolytically processed to its active form by caspase 1. This cytokine is an important mediator of the inflammatory response. • IL-1β induces the transcription of pro-inflammatory cytokines, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis (181). • In a murine cigarette-smoking model, IL-1β mRNA has been shown to dramatically increase in lung tissue after lipopolysaccharide challenge (182). • In COPD patients, in the stable state, airway IL-1β relates to exacerbation frequency, is higher in frequent exacerbators (183) and is elevated in patients with bacterial colonisation (184). • At exacerbation, airway IL-1β predicts bacterial aetiology (52) and levels relate to bacterial load (185).

Interleukin-5 (IL-5)	Sputum	- Interleukin-5 is a Th2 cytokine involved in the differentiation, maturation, migration, development, survival, trafficking and effector function of blood and local tissue eosinophils (186) and release of IL-5 from CD4+ cells is increased in stable COPD (187). 28% of exacerbations are associated with sputum eosinophilia (52) and IL-5 correlates with sputum eosinophil counts (188). - IL-5 levels increase during exacerbations of COPD associated with sputum/peripheral blood eosinophilia (52, 189) and are attenuated following treatment with prednisolone (188). Sputum IL-5 levels have also been shown to predict exacerbations after cessation of inhaled corticosteroids in COPD (190).Benralizumab, anti-interleukin-5 receptor α monoclonal antibody, has been proposed as an agent to reduce exacerbation frequency in COPD patients with an eosinophilic phenotype (191), although evidence of benefit is still lacking.
Interleukin-6 (IL-6)	Serum + Sputum	- IL-6 is produced by mononuclear cells, lymphocytes, fibroblasts and airway epithelium. IL-6 is associated with induction of the serum acute-phase response and augmentation of antibody production. - IL-6 is the primary cytokine regulator of both CRP and fibrinogen. - Frequent exacerbators (>3/year) have higher stable sputum IL-6 levels (38). - When stable, patients with high IL-6 have faster lung function decline and frequent exacerbators (> 2.52/yr) have a faster rise over time in sputum IL-6 compared to patients with infrequent exacerbations (< 2.52/yr) (167). - Serum, plasma and sputum IL-6 levels increase from stable values at exacerbation (37, 38, 56, 111) and serum IL-6 levels fall during recovery (56, 112). - The rise in serum IL-6 at exacerbation is greater in viral in comparison to non-viral exacerbations (42). - Exacerbations associated with rhinovirus and *H.influenzae* infection have higher serum IL-6 than exacerbations without both pathogens (48). - During recovery, frequent exacerbators have a smaller reduction in serum IL-6 between exacerbation and day 35, despite treatment, compared with infrequent exacerbators (56).

Interleukin-8 (IL-8)	Serum + Sputum	- IL-8 is produced by neutrophils, macrophages, airway epithelium, T-lymphocytes, endothelium, smooth-muscle cells and fibroblasts. - IL-8 is a neutrophil chemoattractant and activator of neutrophils. - Stable state sputum IL-8 is increased in frequent exacerbators (>3/year) compared to infrequent exacerbators (38). - Sputum IL-8 is increased stable COPD patients colonised with potentially pathogenic microorganisms (105), with sputum IL-8 increasing with increasing bacterial load (129, 192). - At exacerbation, the rise in bacterial load correlates with the rise in sputum interleukin (IL)-8 and fall in FEV_1 (48). - Sputum IL-8 levels fall rapidly following exacerbation treatment during the initial recovery phase (193).
Interleukin-18 (IL-18)	Sputum	- IL-18 is a member of the IL-1 super family and a pro-inflammatory cytokine, synthesized as an inactive precursor requiring processing by caspase-1. The IL-18 precursor is in nearly all cells in healthy humans. - Following cleavage by active caspase-1, mature IL-18 is secreted from monocyte/macrophages. IL-18 induces IFN-γ production from NK cells. - IL-18 increases cell adhesion molecules, promotes nitric oxide synthesis and chemokine production, regulates macrophage/neutrophil accumulation and function, and cellular apoptosis (134). - IL-18/IL-18binding protein levels are increased in plasma, BAL and lung tissue in COPD patients and in mouse models (194-198) - In murine models, IL-18 causes pulmonary inflammation and emphysema, and IL-18 receptor (IL-18R) expression is increased on alveolar macrophages in COPD patients (198). - In COPD patients sputum IL-18 increases at exacerbation compared to paired baseline data, although no significant increase in serum levels are observed at exacerbation (199). - The time course of IL-1β and IL-18 in BAL following LPS exposure in mouse model also suggests promise for these biomarkers in modelling exacerbation recovery (182).

Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) MMP-1, MMP-8, MMP-9, TIMP-1	Serum + Sputum	• MMPs and TIMPs can degrade the extracellular membrane (ECM). Dysregulation of proteolytic MMP activity leads to exaggerated ECM turnover, impaired repair and scar formation, or to ECM accumulation with subsequent tissue fibrosis. • In cystic fibrosis (CF) exacerbations, serum MMP-1/8/9 levels are elevated and decrease in response to treatment (200). • In COPD, sputum MMP-8/9 levels increase and serum TIMP-1 levels decrease at exacerbation (105, 201, 202). Furthermore, in the ECLIPSE biomarker cohort, MMP8/9 levels correlate with GOLD stage and the degree of emphysema (203).
Myeloperoxidase (MPO)	Sputum	• MPO is a strong oxidant stored in primary granules of neutrophils with potent antibacterial and proatherogenic properties (135). Although generation of oxidants by MPO is beneficial in terms of the immune response to invading pathogens, inappropriate stimulation of oxidant formation can result in host tissue damage. • In stable patients with COPD, increased serum MPO levels are associated with rapid lung function decline and poor cardiovascular outcomes (135). • At exacerbation, sputum MPO increases at exacerbation (37) but no increase has previously been seen in plasma levels (111).
Placenta growth factor (PLGF)	Serum	• PLGF is a member of vascular endothelial growth factor family that promotes angiogenesis (204). • PLGF is released from bronchial epithelial cells and may contribute to COPD pathogenesis (205, 206). • PLGF induces apoptosis of type II alveolar epithelial cells and so can cause emphysema when over-expressed in murine lungs and PLGF knock-out mice are protected from elastase-induced emphysema (205, 206). • Higher levels of PLGF have been shown in serum and broncho-alveolar lavage (BAL) fluid of COPD patients and the PLGF levels is inversely proportional to lung function deterioration (207). • In COPD patients, elevated serum PLGF is associated with increased risk of pneumonia (208).

Pulmonary and Activation-Regulated Chemokine (PARC)	Serum	- PARC is constitutively expressed by monocytes/macrophages and dendritic cells and is secreted predominantly in the lungs. - Serum levels are increased in COPD patients compared to controls (209) and plasma levels increase at exacerbation compared to the stable state (111). - PARC levels are decreased by prednisolone (209). - Elevated serum PARC levels were associated with increased risk of cardiovascular hospitalisation or mortality in the Lung Health Study cohort and with total mortality in the ECLIPSE cohort (209).
Serum Amyloid A (SAA)	Serum	- SAA is an acute-phase protein, induced by inflammatory mediators, including IL-6, IL-1b, and TNF-α, that rise acutely in at exacerbations of COPD and is associated with neutrophil accumulation and activation (210). - Bozinovski and colleagues found that SAA increases at acute exacerbation and may have the potential to differentiate exacerbations based on severity of the event: SAA was able to differentiate AECOPD requiring hospitalisation from stable COPD. Higher SAA levels were seen in bacterial compared to non-bacterial exacerbations (132). - When used in a small number of patients during exacerbation recovery, results indicated that SAA may have the potential to study the time course of recovery and recurrence (132). - Recent work also suggests that proinflammatory SAA is disproportionately expressed relative to pro-resolving lipoxins in AECOPD. This imbalance during severe events may represent a fundamental mechanism for prolonging and intensifying inflammation that is associated with AECOPD-related hospitalisation, respiratory failure, and death (210).
Soluble receptor for advanced glycation end-products (sRAGE)	Serum	- sRAGE mediates responses to cell injury mediated by stimuli including oxidative stress. - Serum Amyloid A is a ligand for sRAGE and is expressed in normal lung tissue and treatment with sRAGE can reverse inflammation in animal models. - Plasma sRAGE is elevated in hospitalised exacerbations and falls on discharge (133).

Tumour Necrosis Factor-α (TNFα)	Serum + Sputum	- TNF-α is a potent pro-inflammatory cytokine produced by macrophages, T-lymphocytes and airway epithelium - TNFα is a neutrophil chemoattractant that is elevated in stable COPD patients relative to normal control subjects, when measured in sputum. - Sputum TNFα levels also rise from baseline at exacerbation and resolve during convalescence (36). - Rise in TNFα seen at exacerbation correlates to increase in bacterial load (185)

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