6

Conclusion and Future Studies

This chapter summarises the main findings of this thesis. The findings for each project will be examined with reference to the stated Hypothesis and Aims presented in Chapter 2. Finally suggestions for future projects based on the current results will be presented.

6.1 Conclusion

Time Course Study

The key findings of this study can be summarized as follows:

Patients with GOLD stage III-IV disease have higher levels of airway and systemic inflammation in the stable state compared to patients with GOLD stage I-II disease.

When all patients were analysed together, the baseline airway inflammation as measured by the sputum IL-8 level was related to the baseline sputum bacterial load.

At exacerbation onset, there was no significant difference in symptom scores or FEV_1 drop between patients with GOLD stage III-IV and stage I-II disease. However, during the recovery period, patients with GOLD stage III-IV disease showed a trend towards a higher symptom score.

Patients with severe and very severe COPD had smaller changes in sputum IL-8 at exacerbation onset than patients with mild or moderate disease. There were no significant differences in the intensity of change in systemic inflammation and bacterial load between patients with severe to very severe COPD and those with mild to moderate disease.

At exacerbation onset there was a significant increase in systemic inflammation, which was brief as by day seven the systemic inflammatory markers had returned below the baseline levels. This is likely to be in response to the exacerbation treatment. The changes in airway inflammation between baseline and exacerbation onset were more heterogeneous and significant changes were only observed for sputum IL-6. There was no significant change in airway bacterial load between baseline and exacerbation onset.

Patients with more severe disease and those with higher drops in FEV_1 were more often treated with oral steroids at exacerbation onset. There were no differences in total symptom scores, airway and systemic inflammation between those treated with and without oral steroids at exacerbation onset. However, patients treated with a second course of oral steroids at day 14 had higher symptom scores and higher levels of sputum and serum IL-6 at day 14 than those not treated with a second course of oral steroids.

The intensity of the change in sputum IL-8 at exacerbation onset was related to symptom recovery time, this relationship was independent of COPD severity and exacerbation frequency. There was a significant relationship between the resolution of airway inflammation by day 7 and symptom recovery time.

In a mixed linear model analysis, patients with non-recovered exacerbations by day 35 had a persistently higher serum CRP (but not IL-6) concentrations during the recovery period, compared with patients who had recovered.

Patients presenting with symptoms of a common cold at exacerbation onset had significantly higher levels of sputum IL-6 at exacerbation onset and 7 seven days co pared to those without a cold.

There was no significant difference in the changes in airway and systemic inflammation between baseline and exacerbation in those who were frequent or infrequent exacerbators. Frequent exacerbators had a smaller reduction in systemic inflammation (as measured by serum CRP and IL-6) between exacerbation and day 35, despite treatment, compared to infrequent exacerbators.

Twenty two per cent of patients had a recurrent exacerbation within 50 days of the index exacerbation. These patients had higher concentrations of serum CRP at day 14 compared to those without a recurrent exacerbation. A higher serum CRP fourteen days after the index exacerbation was associated with a shorter time to the next exacerbation.

In our study, serum inflammatory markers were better predictors of non-recovery and recurrent exacerbations than sputum inflammatory markers.

Patients requiring hospital admission had significantly higher drops in FEV_1 and higher levels of serum IL-6 at exacerbation onset compared to those treated in the community.

Therefore with regards to the specific questions raised in Chapter 2:

1) **What is the relationship between the intensity of change in airway inflammation at exacerbation and symptom recovery time?**

Our data demonstrated that the intensity of change in sputum IL-8 between baseline and exacerbation onset was directly related to symptom recovery time and that this relationship was independent of disease severity and exacerbation frequency. No similar relationship was demonstrated for the changes in sputum IL-6 levels or

sputum bacterial load. We have also demonstrated that the resolution of the airway inflammation by day 7 was related to the symptom recovery time. The relationship was valid for both sputum IL-6 and sputum IL-8.

2) **What is the relationship between the time course of airway and systemic inflammation after a COPD exacerbation and non-recovery?**

Patients with non-recovered exacerbations by day 35 had higher levels of serum CRP during the recovery period than those who had recovered. No such relationship could be demonstrated with the airway markers.

3) **Can the time course of airway and systemic inflammation predict who has a recurrent exacerbation?**

We demonstrate for the first time a relationship between the level of serum CRP 14 days after the onset of an exacerbation and the occurrence of another or recurrent exacerbation. No such relationship was demonstrated with serum IL-6. We did not find any relationships between airway inflammatory markers and recurrent exacerbations.

4) **Is treatment with oral steroids related to airway and systemic inflammation?**

We did not find any significant differences in airway or systemic inflammatory markers between patients treated with and without oral steroids at onset of the exacerbation. However, patients treated with a second course of oral steroids at day 14 exhibited higher levels of airway and serum IL-6 at day 14 than those not treated with a second course.

5) **How does the presence of a common cold at onset of exacerbation affect the time course of airway and systemic inflammation?**

The presence of a common cold at onset of the exacerbation is associated with persistently high levels of sputum IL-6 till day 7.

6) **Are there differences in the time course of inflammatory changes between patients who are frequent or infrequent exacerbators?**

When we compared frequent and infrequent exacerbators, we did not find any significant difference in the intensity of the change in airway and systemic inflammation between baseline and exacerbation onset. However, frequent exacerbators had a smaller reduction in systemic inflammation (as measured by serum CRP and IL-6) between exacerbation onset and day 35, despite treatment, compared to infrequent exacerbators.

Circulating leucocytes and COPD

The key findings of this study can be summarized as follows:

In patients with stable COPD, the percentage predicted FEV_1 was related to the number of circulating B cells and inversely related to the number of circulating neutrophils. The FEV_1/FVC ratio was inversely related to the number of circulating white cells, neutrophils and monocytes. There was no relationship between lung function indices and the number of circulating T cells or the CD4/CD8 ratio.
Patients with COPD who were active smokers had a lower number of circulating monocytes than patients who had quit smoking.

In patients with stable COPD, the sputum bacterial load was inversely related to the number of circulating leucocytes, neutrophils, total lymphocytes and total T cells. The percentage of circulating B cells was directly related to the sputum bacterial load in patients with stable disease. We found no relationship between the blood CD4/CD8 ratio and the sputum bacterial load.

At exacerbation, there was a significant increase in the number of circulating leucocytes, neutrophils and monocytes. There was also a decrease in the number of circulating total lymphocytes, T cells, CD4 cells and CD8 cells. No other significant changes in circulating leucocyte subsets were observed at exacerbation.
The drop in circulating total lymphocytes, T cells and CD8 cells at exacerbation was directly related to the baseline sputum bacterial load.

Therefore with regards to the specific questions raised in Chapter 2:

1) **What is the relationship between the severity of COPD and circulating leucocytes?**

The severity of COPD is directly related to the number of circulating B cells and inversely related to the number of circulating neutrophils. We did not find any significant relationship between the severity of COPD and the number of circulating T, thier subsets or the CD4/CD8 ratio.

2) **Is there a relationship between the circulating leucocytes and the lower airway bacterial load in patients with COPD?**

There was an inverse relationship between the number of circulating leucocytes, neutrophils, total lymphocytes, total T cells and the sputum bacterial load, in patients with stable COPD. No such relationship was found between the circulating leucocyte subsets at exacerbation and the exacerbation sputum bacterial load.

3) **Are there changes in circulating leucocytes at exacerbation and are they related to lower airway bacteria?**

The intensity of the change in circulating total lymphocytes, total T cells and CD8 cells between baseline and exacerbation was related to the baseline sputum bacterial load. No such relationship was observed with the sputum bacterial load at exacerbation

Detection of H influenzae, S pneumoniae and M catarrhalis by real time quantitative PCR

The key findings of this study can be summarized as follows:

The detection rate of any organism was significantly higher by qr-PCR than by conventional cultures. The detection of S pneumonia was significantly higher by qr-PCR than by cultures. No similar relationship was found for the two other organisms.

The detection of multiple pathogens was also significantly higher by qr-PCR than by conventional cultures.

We did not observe any significant changes in the sputum bacterial load between baseline and exacerbation by either of the two detection methods.

The absolute bacterial loads detected by qr-pcr were lower than by conventional cultures, raising the possibility of PCR inhibitors in our assay.

Patients with H influenza or S pneumonia detected by qr-PCR in the stable state had significantly higher sputum IL-6 levels than those who patients who did not have these bacteria detected by qr-PCR. At exacerbation, the detection of any of the three pathogens by qr-PCR was associated with higher levels of sputum IL-8. No similar relationships were observed between detection of the organisms by conventional cultures and airway inflammation at baseline or at exacerbation.

Patients with a PPM detected by qr-PCR at exacerbation and then cleared by day 7 showed a trend towards a lower level of sputum IL-8 at day 7 compared to patients who had not eradicated the PPM.

Therefore with regards to the specific questions raised in Chapter 2:

1) **What are the differences in the detection rates of bacteria by the quantitative real time PCR assay and conventional microbiological cultures?**

Our results demonstrate that the detection of any organism is significantly higher by qr-PCR than by conventional cultures. We also observed higher detection rates of multiple pathogens by qr-PCR when compared with cultures.

2) **Is there a relationship between the detection of micro-organisms by PCR and the degree of airway inflammation in patients with COPD?**

We found a significant relationship between the detection of H influenza and S pneumonia by qr-PCR and the sputum IL-6 levels in the stable state. At exacerbation, we found a significant relationship between the detection of either of the three PPMs and the sputum IL-8 level.

3) **Is there a relationship between bacterial eradication by PCR and airway inflammation?**

Our data shows a trend between bacterial eradication detected by qr-PCR and the levels of sputum IL-8.

6.2 Suggestions for future studies

A number of novel findings that have important implications for the clinical management of patients with COPD have been reported in this thesis. The possible ways in which these findings could be explored further are discussed in the next section.

6.2.1 Persistently high CRP and non-recovery

In the present study exacerbation symptoms had not recovered by day 35 in 23% of the patients. These patients had higher serum CRP levels during the recovery.

Exacerbations have a high impact on patients' quality of life, therefore a in depth examination of COPD related patient outcomes with new tools such as the COPD Assessment Tool (Jones et al 2009) or the Exacerbations of Chronic Pulmonary Disease tool (Leidy et al 2011) would give important information in patients with non-recovered exacerbations. A study into the mechanisms leading to non-recovery and persistently high CRP would be important. We could assess if these changes are due to the presence of viruses, bacteria or both during the recovery. Or is it related to the patients underlying immune status?

6.2.2 High CRP at day 14 and recurrent exacerbations

This finding should first be confirmed in a larger study, then the effect of a therapeutic intervention in patients with raised CRP at 14 should be tested with a randomized-controlled study. The therapeutic intervention could consist of a course of oral steroids or other anti-inflammatory drug. The reasons for the increase in serum CRP at day 14 should also be assessed.

6.2.3 Frequent exacerbators have persistently high systemic inflammation during the recovery

There is more and more evidence that patients with frequent exacerbations have a distinct phenotype, is this related to a distinct genotype as well? Or is it related to lung environmental factors such as lower airway bacterial colonization or the inflammatory milieu?

6.2.4 Refining the qr-PCR assay for the detection of H Influenzae, S Pneumoniae and M Catarrhalis in sputum samples

Bacteria play an important role in COPD. Therefore more sensitive, specific and rapid methods for their detection such as qr-PCR are needed. Our pilot results were encouraging but the lower bacterial loads detected by PCR were a major issue. The assay needs to be refined to increase the sensitivity and specificity for the detection of the common PPM from sputum samples. A method to distinguish between live and dead bacteria in the sputum sample is also needed. When such a new assay is optimized, the relationship between lower airway bacteria and inflammation, both airway and systemic could be explored more in depth. PCR also offers the possibility to detect difficult to grow or as yet unidentified micro-organism using conventional cultures.