University of Glasgow | College of Medical, Veterinary & Life Sciences

Head of College Scholars List Scheme

Summer Studentship 2019

Report Form

This report should be completed by the student with his/her project supervisor. It should summarise the work undertaken during the project and, once completed, should be sent by email to: maureen.bain@glasgow.ac.uk **within four weeks of the end of the studentship**.

1. Student

   Surname: Cunningham Forename: Matthew

   E-mail address: 2297382@student.gla.ac.uk

2. Supervisor:

   Surname: McSharry Forename: Charles

   E-mail address: charles.mcsharry@glasgow.ac.uk

3. Research Project Report

   - Project Title (maximum 20 words):

     Biomarker discovery for investigation and early detection of progressive lung fibrosis in Interstitial Lung Disease (ILD).

   - Project Lay Summary (copied from application):

     Importance: In progressive lung fibrosis, air-sacs become scarred, breathing becomes difficult and life expectancy is 3-4 years. Early diagnosis would greatly improve quality of life. Blood tests to identify people at risk are required.

Aims and Methods: A blood test for protein KL-6 produced by damaged air-sacs will be evaluated as a predictor of fibrosis in at-risk subjects, by comparison with dust exposure, respiratory symptoms and breathing test results.

Outcomes: KL-6 results will contribute to a prospective study evaluating determinants of lung fibrosis. The student will be included in study design, data analysis and presentation, with opportunities for future involvement.

- Start Date: 24/6/19 Finish Date: 8/8/19

  - Original project aims and objectives (100 words max):

Aims: Compare KL-6 with (*i*) symptoms, (*ii*) lung function, (*iii*) IgG antibody, and (*iv*) epithelial tight-junction protein single-nucleotide polymorphisms.

Objectives: Evaluate whether KL-6 can identify (*i*) early sub-clinical disease, (*ii*) early lung function abnormalities particularly in small airways, and whether KL-6 can disclose (*iii*) molecular and (*iv*) cellular mechanisms of pathogenesis associated with immune hypersensitivity and abnormal epithelial permeability respectively.

  - Methodology: Summarise and include reference to training received in research methods etc. (250 words max):

Methods: The levels of KL-6 and anti pigeon-antigen IgG were measured in 47 test samples from pigeon fanciers and 10 control samples using ELISA plates according to the manufacturer's instructions. The samples were pipetted onto the plates in duplicate and a standard curve was also created for each plate. The results were then compared with one another; spirometric data from the participants; and the results of a structured questionnaire, designed to assess the presence of symptoms of Hypersensitivity Pneumonitis (HP) in the participants, to assess the outcomes outlined in the aims section.

The results were processed using Minitab software and several statistical tests (Mann-Whitney, Pearson, Spearman Rho, T-test, Fischer's Exact). A p-value of <0.05 was considered to be statistically significant.

Training: A Health and Safety and basic skills introduction was given at the start of the placement to ensure proper procedure was followed for the various tasks completed in the course of the placement such as pipette technique, preparing controls and standards, and ordering equipment. Instruction was given on how to complete an ELISA test using spare kits from an earlier study and practise under supervision. The final tests were completed independently.

Teaching was also provided by Dr McSharry on data handling and analysis as well as the processes of study design that were outwith the scope of the project. This included areas like statistical analysis, ethics approval, literature review, and the null hypothesis.

- Results: Summarise key findings (300 words max). Please include any relevant tables or images as an appendix to this report:

The levels of KL-6 were compared to the levels of IgG and Lymphocyte proliferation in three different subgroups: healthy controls; an asymptomatic, at-risk group of pigeon fanciers; and a potentially symptomatic, at-risk group of fanciers (as detected by the structured questionnaire). Pigeon fanciers provide a study group for the development of lung fibrosis due to their high risk of inflammation from HP following inhalation of pigeon dust. Figure 1 (Appendix 1) shows the level of serum IgG antibody against pigeon antigens in each of the above subgroups. It shows that IgG is raised in those exposed to pigeon antigens but it cannot distinguish between those who report experiencing symptoms indicative of Hypersensitivity Pneumonitis and those who do not. A similar pattern can be seen in the level of lymphocyte proliferation which is shown in Figure 2.

KL-6, however, is significantly raised primarily in those who report HP-like symptoms but substantially remains below the generally accepted cut-off for normal levels in the asymptomatic group as shown in Figure 3. Levels stay below the normal cut-off for all of the healthy control subjects. This is suggestive of the possibility of KL-6 being a diagnostic biomarker of sub-clinical disease in at-risk groups or those suspected of having HP or Interstitial Lung Disease (ILD).

The levels of IgG, lymphocyte proliferation, and KL-6 were compared to Spirometric tests of lung function and no significant relationships were found, suggesting that, even in the possible symptomatic group, there was no permanent reduction to lung function in the pre-clinical stage, reinforcing the potential significance of the test in early detection and preventing development.

KL-6 was, however, found to corelate positively with increases in IgG which could be an interesting mechanistic indication that requires further study.

- Discussion (500 words max):

Biomarkers for subclinical Hypersensitivity Pneumonitis and Interstitial Lung Disease (ILD) are difficult to pinpoint because the majority of cases are sporadic and do not present to the GP until after the development of severe breathing restriction and deposition of fibrotic tissue, at which point treatments are ineffective. However, due to the documented development of HP and ILD in pigeon keepers, there arises an opportunity to look at at-risk and subclinical cases.

KL-6 is a mucin protein produced by the proliferation of Type II Pneumocytes in the alveoli of the lung. Levels of KL-6 have been found to be increased in established ILD but the access to pre-clinical patients allows investigation of an earlier stage of the disease.

The results of the study suggest that the level of KL-6 in the serum is indicative of HP to a greater degree than IgG, a currently accepted biomarker. It is also much more versatile in that, in cases where the stimulating antigen is not known, it is difficult to detect the specific level of IgG to that antigen, whereas KL-6 is not dependent on the source antigen.

The relationship between IgG, exposure, and symptoms is suggestive that the process leading to a raised IgG is a necessary part of the development of symptoms and progressive ILD but that there is another process that must be developing simultaneously to progress to symptomatic disease.

The raised KL-6 indicates both that Type II Pneumocytes are highly active during the process of tissue remodelling in the lung and that permeability of the basal membranes of the alveoli is increased. It is as yet unclear why this is. It is possible that the KL-6 is a signal to damaged Type I Pneumocytes to proliferate, or a mediator in the fibrotic process. Further still it could suggest that in the event of tissue damage to the alveoli, it is the Type II pneumocytes that proliferate to heal the damage and by some mechanism are replaced by or are a precursor of the far more numerous Type I Pneumocytes. Each of which could be a potential hypothesis for further study.

In addition it cannot yet be said if the KL-6 is specific to HP and ILD or if it is indicative of any structural small airways changes so these areas. As well as the potential use as a prognostic marker, the relationship of KL-6 to other factors such as CRP, Leptin, and Adiponectin will be areas of further study into the topic.

The ability of KL-6 to identify symptomatic people as having HP or early ILD is potentially significant in the ability of clinicians to treat early cases of the disease, possibly through screening of at-risk populations.

This study is limited by the quality of, and accuracy of the responses to, the structured questionnaire given to the study participants to identify a potentially symptomatic category.

4. Reflection by the student on the experience and value of the studentship (300 words max):

My experience of the studentship was incredibly valuable. Before it, I had very little lab experience or understanding of the day-to-day life of somebody conducting research. From the moment I decided to apply for funding, I have been learning. I have been taught many new skills both directly and through experience, from finding somebody to work with to analysing data sets.

I began by asking one of my lecturers if they had any contacts who could offer an immunology-focused project. Once I had contacted Dr McSharry we spoke about the studentship programme and potential projects and worked on our application. After being accepted we began with a basic lab introduction. Dr McSharry guided me through ethical approval, and study design, analysis, writing and presentation while Yuan, a PhD student, demonstrated the practical skills I would need before I then generated my own data independently.

There were some setbacks, including changes to the original plan. However, these were out of our control and so taught me the importance of being adaptable in the research world. I learned the practical techniques fairly quickly, but it took some time for me to feel confident doing them independently.

Overall, the experience has given me insight into the practical side of science that we aren't always fully exposed to in medicine. I have always considered being involved in research but now I feel even more like it is something I would like to pursue at some point in my career.

If I were to repeat the experience, I would try to be more proactive and ask more questions about the other things going on around me without worrying that I would just be in people's way. I discovered that everyone I did interact with ended up being more than happy to speak to me.

5. Dissemination: (note any presentations/publications submitted/planned from the work):

Inclusion in the publication of the paper entitled *"Krebs von den Lungen-6 is a molecular biomarker of subclinical hypersensitivity pneumonitis among pigeon fanciers".*

Presentation of the findings of the above study to the *'Respiratory Research Group'* at the Glasgow University Institute of Infection, Immunity and Inflammation.

6. Signatures:

Supervisor  Date 28/8/19

Student  Date 28/8/19

Appendix 1 – Results Graphs

Figure 1

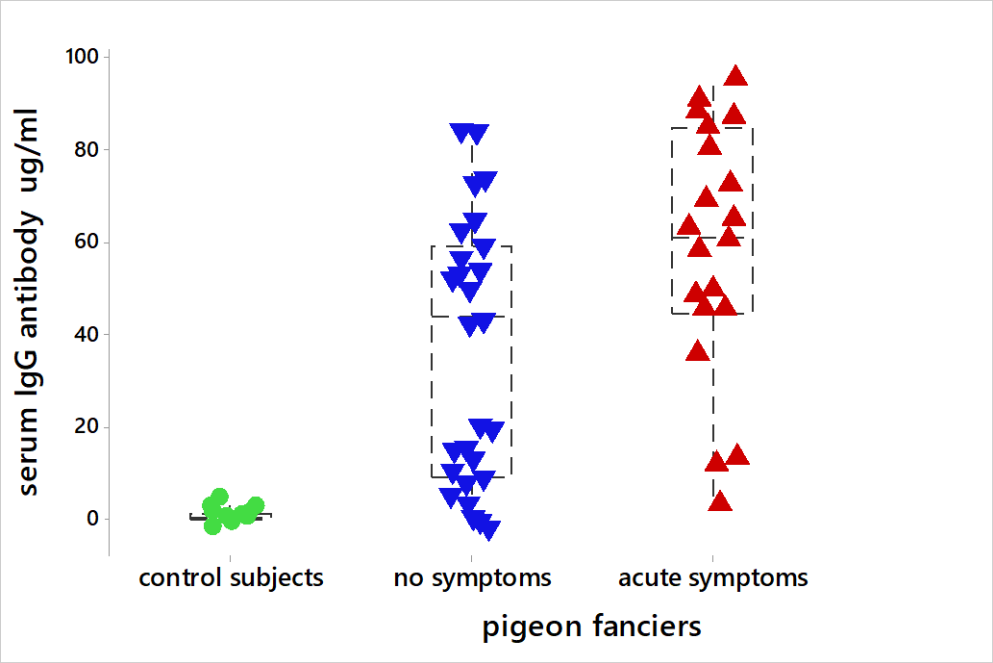


Figure 2

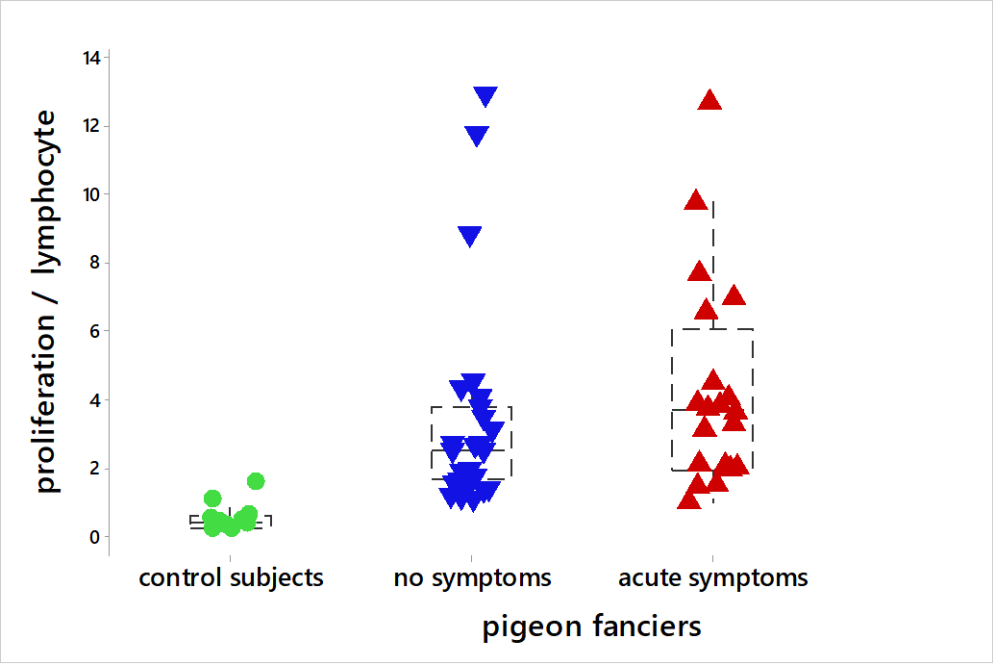
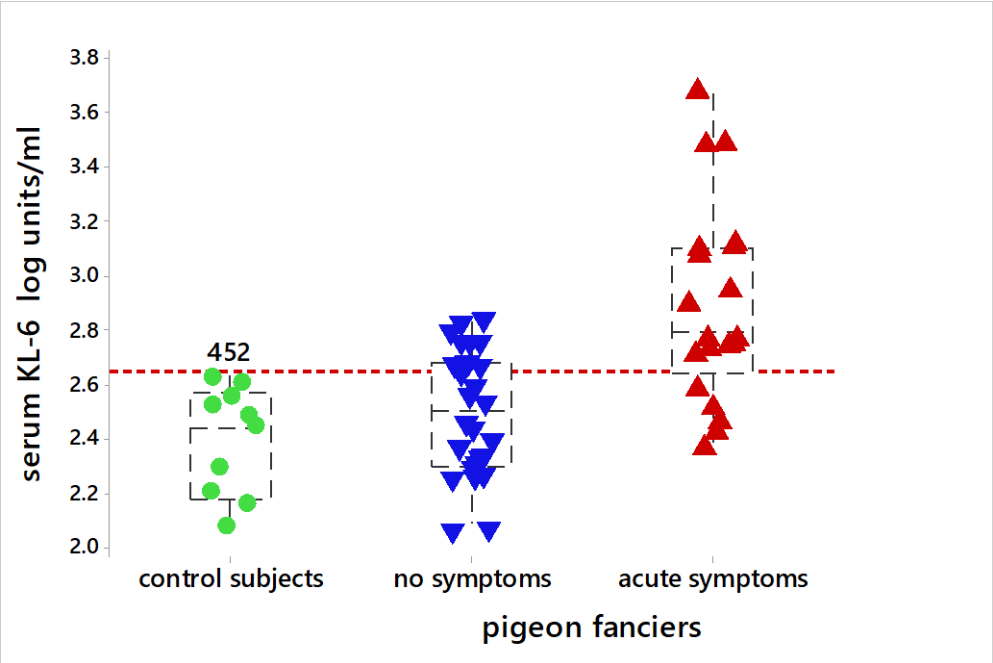


Figure 3



The serum IgG antibody concentration(Figure 1), Lymphocyte proliferation (Figure 2), and KL-6 concentration (Figure 3) against pigeon antigens in healthy control subjects with no significant exposure to pigeon antigenic dusts and in pigeon fanciers categorised according to history of acute HP. Student's *t*-test comparing pigeon fanciers with and without a history of acute symptoms, $p<0.05$ represents statistical significance.