European Society for Immunodeficiencies
Mijnbouwstraat 23, Delft, the Netherlands
Website: esid.org

ESID End of Fellowship report

1. Fellowship details

- First name: Adriel
- Last name: Roa-Bautista
- Type of fellowship:

☐ **Short-term**

☒ **Medium-term**

☐ **Bridge grant**

- Fellowship's start and end date: January 2023-July 2023
- Hosting institution: Royal Free Hospital
- Supervisor: Dr David Lowe

2. Summary of the work done during the fellowship (max.400 words).

During my medium-term research fellowship at Royal Free Hospital. I worked under the supervision of Dr David Lowe in a project focus on the Clinical features, immunological characteristics and treatment outcomes of Campylobacter spp infections in patients with common variable immunodeficiency (CVID).

Study Design: A retrospective cohort description of CVID patients with *Campylobacter* infection attending the Immunology clinic at a large tertiary hospital in London, UK. Subjects were eligible if they met diagnostic criteria for CVID made by a consultant immunologist following the International Collaboration in Asthma, Allergy, and Immunology (ICON) or European Society for Immunodeficiencies (ESID) definitions and had at least one positive stool PCR for *Campylobacter* spp.

Study Methodology: Flow cytometry (FCM) was used to characterize peripheral blood lymphocyte subsets (frequencies and absolute numbers of CD3+, CD4+, CD8+, CD19+ and CD16+/CD56+ NK cells) and B cell phenotype (Switched memory B cells, $CD21l^{ow}CD38^{low}$, Transitional B cells, Naïve B cells, IgM memory and Plasmablasts)

Long term averages were obtained from all available results from 2000 (or date of CVID diagnosis, if later) to 2022. Values reported as less than a limit of detection (eg <0.1 g/L) were taken as being equal to that limit value for analysis.

Microbiology and Genomics Faecal samples from patients were tested using a commercial enteric bacterial PCR assay for detection of *Campylobacter* spp. PCR-positive faeces are cultured on *Campylobacter* selective agar between 37-42^{O}C under microaerophilic conditions. Positive blood cultures (BACTEC) were sub-cultured on blood and chocolate plates. Colonies cultured were further identified as *Campylobacter* spp. using MALDI-TOF.

Faecal and blood isolates of *Campylobacter* spp. from patients were referred to the national Campylobacter reference laboratory at UK Health Security Agency (previously Public Health England). Isolates on Amies charcoal swabs were cultured overnight on 5% Columbia blood agar, incubated at 37- 42 ^{o}C under specialised atmospheric conditions (5% O_2, 5% CO_2, 3%H_2, 87% N_2) using a Don Whitley VA500 Microaerophilic Workstation.

Genomic DNA was extracted from bacterial cultures using a QIAGEN QIAsymphony, fragmented and tagged for multiplexing with Nextera XT DNA Sample Preparation Kits, followed by rapid-run paired-end sequencing on an Illumina High-Seq 2500 platform to produce 100 bp reads. The 7-loci MLST was determined from WGS data using MOST, a modified MLST typing tool based on short read sequencing. Sequences were assembled using the SPAdes genome assembler in the UKHSA pipeline.

Single nucleotide polymorphisms (SNPs) were identified based on ST-complex specific reference mapping, and cluster detection was performed across the most prevalent ST-complexes. Antimicrobial resistance (AMR) was predicted from the WGS data using a validated in-house bioinformatics pipeline in UKHSA to detect AMR determinants conferring reduced susceptibility

to the following antibiotics/classes: erythromycin (macrolide), ciprofloxacin (fluoroquinolone), gentamicin and streptomycin (aminoglycosides) as well as tetracycline.

Phenotypic antimicrobial susceptibility tests were performed by disc diffusion and E-test according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations.

3. **New skills acquired during the fellowship** (max.200 words).

The competition of this medium-term research fellowship allowed me to acquired multiple skills ranging from laboratory to clinical abilities. My research abilities were enhanced, deepen in multiple aspects related to basic and clinical research, collecting data, analyzing flow cytometry results to finally write and fluently communicate results.

Similarly, I was able to liaise with other world-leading hospital in Clinical Immunology in the U.K. (Great Ormond Street Hospital) increasing my clinical skills in the diagnosis and management of diseases related to the immune system.

4. **Your professional plan for the near future and how the fellowship impacted this plan** (max 400 words).

The daily work I engage in is closely related to the field of Primary Immunodeficiencies (PID). As a recently graduated specialty Doctor in Immunology, I encounter patients with suspected or diagnosed PID regularly. This includes conducting clinical assessments, performing diagnostic tests, interpreting results, and developing treatment plans. Being up-to-date with the latest advancements and best practices in the field is crucial to ensure accurate diagnosis, optimal patient care, and improved outcomes. Completing this short-term fellowship allowed me to support my everyday tasks with excellency.

5. **Results obtained from your fellowship project. Please, mention any publications or meeting communications derived** (if applicable, max 800 words).

This study focused on comparing the clinical characteristics and immunological parameters of patients with common variable immunodeficiency disorder (CVID) who had Campylobacter infection to a control group of CVID patients without Campylobacter infection. Here are the key findings of the study:

Clinical Characteristics:

- The median age of CVID patients with Campylobacter infection was 51 years, and there was an equal distribution of males and females.

- All Campylobacter-infected patients received immunoglobulin replacement therapy (IRT) for CVID, either subcutaneously (SCig) or intravenously (IVig).

- Most patients in both groups were on prophylactic antibiotic therapy, with similar distribution and types of antibiotics used.

- Symptoms reported by Campylobacter-infected patients included diarrhoea, weight loss, abdominal cramping, bloating, incontinence, and nausea, although these symptoms were reported by a minority of patients.

- Common comorbidities in Campylobacter-infected patients were autoimmune phenomena and a history of iatrogenic immune suppression.

Immunological Parameters:

- Both Campylobacter-infected patients and the control group had low or undetectable levels of IgA and some Campylobacter patients had long-term low levels of IgG.

- Campylobacter patients showed a decrease over time in the absolute number of CD4+ T cells, CD19+ cells, and NK cells compared to the control group.

- Campylobacter patients also had lower proportions of CD19+ and CD4+ T lymphocytes compared to the control group.

- The Campylobacter group had significantly elevated percentages of CD21LOWCD38LOW B cells and transitional B cells and significantly decreased levels of CD19+ and CD4+ T cells compared to the control group.

Duration of Campylobacter Infection:

- There were four patients in the acute Campylobacter group, and their median infection duration was 20 days.

- Ten patients were in the chronic/relapsing Campylobacter group, and their median infection duration was 113 days.

- No significant differences in immunological parameters were found between the acute Campylobacter group and the control group.

Antimicrobial Sensitivity and Treatment Response:

- Macrolide and fluoroquinolone resistance were common in Campylobacter isolates, while resistance to chloramphenicol and aminoglycosides was rare. One patient showed treatment-emergent resistance to carbapenems.

- Patients with Campylobacter colitis received treatment according to a treatment algorithm, including first-line (azithromycin), second-line (neomycin), and third-line (combination treatment with parenteral antibiotics) treatments.

- Response to treatment varied among patients, with some experiencing prolonged improvement in symptoms and consecutive negative PCR results for Campylobacter.

Overall, the study found that CVID patients with Campylobacter infection exhibited distinct clinical characteristics and immunological changes compared to the control group. The antimicrobial sensitivity of Campylobacter isolates was also evaluated, and a treatment algorithm was proposed based on the patients' response to different treatment options.

6. Any other comments (max.200 words)

Additional information regarding the results previously discus could be found in: https://doi.org/10.1016/j.jaip.2023.06.050

Furthermore, during this medium-term research fellowship I was able to publish a case report of a Combined novel homozygous variants in both SGPL1 and STAT 1 presenting with severe

combined immune deficiency (PMID: 37377976). Finally, I would like to thank the European Society of Immunodeficiencies (ESID), the Royal Free Hospital and the Great Ormond Street Hospital for the astonishing opportunity.

Additional information:

1. The report should be completed within 3 months of completion of the fellowship / bridge grant.

2. Please, note that that once approved, the report will be published on the ESID website, under the Reports section. Bear this in mind and include only information which you would like to make publicly available.

3. Please, complete this report and send it to the ESID Administrative Office at: info@esid.org.