Diagnosis and management of ME/CFS: understanding the biology and identifying therapeutic targets

Project summary

Although fatigue is a major debilitating symptom in many conditions, we still have no clear insight into its biological basis. This proposal focuses on building the resources to carry out fatigue research in three clinical areas: ME/CFS, lymphoma and stroke. Most fatigue research has been carried out in ME/CFS and this will be our primary focus with the other conditions giving insight into common mechanisms associated with fatigue. Having non-ME/CFS fatigue groups will allow us to focus in on the impact of post exertional malaise on our biomarkers. Using this approach we will be able to identify ME/CFS specific mechanisms which would not be possible if just a healthy control population is used. Our recent metabolomics analysis of two ME/CFS cohorts has emphasised the importance of this approach and the benefit of using a Multiple Sclerosis group as a positive fatigue control. Here we will expand further into two other conditions where fatigue is a major problem for patients.

Our clinical research will involve three European centres and build on existing infrastructure. Pilot treatment interventions will be undertaken in all fatigue groups with blood samples taken for future biomarker discovery projects. Fatigue and movement phone app systems will be established to monitor patients and identify changes in symptoms. We will also study immune dysfunction in ME/CFS in collaboration with pharmaceutical companies. Immune cell function using whole transcriptomic analysis will be carried out on ME/CFS patients with direct in vitro challenges to immune cell function. Importantly SoftCell Biologicals will provide an in-kind contribution to our research (equivalent to $750,000) for whole genome sequencing. This will characterise newly discovered pathogens of chronic illness patients in the blood and provide whole genome sequence data on patients. In addition, pathogenic bacteria and fungi will be cultured from blood samples. We will also carry on our research into disease associated factors in the blood, our current project supported by the ME Association is funded until October 2020. The bio-resources collected during this project will be appropriately stored and made available to future studies, encouraging new investigators and clinicians into the fatigue research area. This is vital if we are to develop ME/CFS and fatigue as a clinical research area.

Key research hypothesis

L-form, wall-less, organisms exist in the blood, cells and tissues of humans potentially evolving with their hosts. Although most of the data on L-forms associated with human disease is unpublished data from our collaborator Softcell Biological Research others have published on the presence of bacteria in the human circulation [1]. We speculate that a set of L-forms organisms likely works in harmony with the host, potentially providing key metabolites and impacting on other currently un-identified cellular processes. Transmission via the placenta maybe possible [2] with L-forms potentially also acquired over time from the environment via the gut [3]. If present in human hosts, levels and type of L-forms will be controlled by a currently unidentified system. With many similarities to mitochondria we

propose that a mechanism regulating L-form bacteria could also impact on mitochondrial/energetic function. L-forms are likely to generate a very different immune response to walled bacteria which are dealt with by complement, phagocytosis and adaptive immunity. Recent studies by Kawai et al (2018) indicated that once engulfed by macrophages bacteria can enter an L-form state evading the bodies defence systems [4].

We propose that the presence of deleterious L-form organisms is the driving force behind many chronic disease states associated with a range of symptoms depending upon the type of organisms present, their location and secretory metabolities. L-forms have been recently identified in urinary tract infections with the ability to convert readily into the walled state [5]. We hypothesise that returning L-form populations to the normal L-form complement will result in the resolution of symptoms. Treatment with antibiotics and antifungal agents specifically targeted to the L-forms present in the patient could also provide a new therapeutic option for a range of chronic diseases including ME/CFS, fibromyalgia, endometriosis, migraine and joint and bone problems. In this project we will thoroughly characterise L-forms in the blood of patients using whole genome sequencing and culturing L-forms from individual patients and controls. Standard antibiotic and anti-fungal agents will be tested in *in vitro* and a data base generated on the drug combinations which are effective for individual L form species. A longitudinal study of L-forms present in fatigue patients will be carried out on over time with blood collected and analysed when patients show improvement or worsening of symptoms. A new research study will be established to determine which blood cells contain L-forms and the impact on immune and blood cell function. L-forms cultured from ME/CFS patients will also be used to infect control human blood cells with a thorough characterisation of the effect on cell function. Recent studies in ME/CFS suggest higher levels of mucosal associated invariant T cells (MAIT cells) in patients which is consistent with a bacterial infection. The T-cell response in ME/CFS patients is also unusual with T-cell reaching an intermediate activation state and not forming mature activated T-cells.

Researcher group

The community if the proposal is fully funded will consist of patients, clinicians and researchers from a wide range of areas. Clinicians in ME/CFS include: Professor Angus (Oxford), Dr Kenyon (The Dove Clinic), Dr Zalewski (PZ) (Bydgoszcz, Poland), Dr Martin-Martinez (Valencia, Spain). The group will co-ordinate and discuss ME/CFS patient recruitment (i.e. 150 patients) into the project to give consistency on clinical diagnosis across the three sites. Two non-ME/CFS cohorts with varying degrees of fatigue: minor stroke (100 patients) and lymphoma patients (120 patients) will be recruited from Oxford clinics. Recruitment for these two cohorts will be co-ordinated by Dr Collins (Lymphoma) and Dr Kennedy (Stroke) by LCRN research staff. Participants will be issued with a symptom and movement app (Solve ME) to facilitate re-call when symptoms improve or worsen. A MECFS patient group will be a key part of the research project and involve Andy Devereux Cooke (Science for ME).

Samples generated by the study will be used by Prof Oltra, Dr Morten and Prof Midwood to look specifically into molecular profiles & the immunology of ME/CFS including studying the impact of L-forms on cellular function. An Italian Cohort of five families affected by fatigue

including ME/CFS patients with a clear aberrant cytokine profile will be recruited by Prof Baritussio (Padua) for studies of immune function. These may have a very different underlying biology to ME/CFS patients without clear cytokine profiles. A cryotherapy intervention study (PZ) will provide samples and clinical data helping in both the identification of new biomarkers and finding new strategies to manage the disease symptoms. We will apply for research ethics to obtain blood samples from the ongoing ME/CFS faecal microbiota transplant (FMT) study run by the DoveClinic.

Samples from all three cohorts will be processed using standard procedures to provide a bio-resource sample collection for future investigations. Current collaborators using the samples and data include Prof McCullagh (Chemistry) who will perform advanced metabolomics on blood samples, Prof Wei Huang Raman spectroscopy (Engineering), Prof Smith (University of Brighton) Translatomics on PBMC's and Dr Elson (Newcastle University) mtDNA variant analysis . If funded we will also apply for research grants to recruit patients into MRI muscle (Dr Valkovic, Oxford) and brain imaging studies (Dr Godleswska, Oxford), set up a new trial on specific antibiotics and link to a rodent fatigue blood brain barrier study (Dr Howell Tasmania).

Unmet need

Debilitating fatigue is a common complaint in many illnesses. Currently, little biological explanation as to cause or effective treatments can be offered to seriously fatigued patients. This causes patient dissatisfaction and deters junior clinical staff entering this research field. The necessary infrastructure to run the clinical research into the biological basis of fatigue proposed does not exist in the UK or Europe. Fatigue in other areas including multiple sclerosis, viral infections and endometriosis will be included in follow up studies.

To address these unmet needs we will develop resources to enable cutting edge research and focus on encouraging new researchers and pharmaceutical companies into the field. We will build infrastructure to collaborate with basic research groups facilitating research projects in clinical areas where fatigue is a major problem. Although, the initial fatigue triggers may differ in different illnesses, it is possible that common pathways exist which drive the fatigue process. Diagnosis of ME/CFS has improved with the inclusion of the Canadian and IOM criteria providing a more homogeneous template for research studies. However, co-morbidity with other conditions and a lack of diagnostic biomarkers makes diagnosis difficult even for experienced clinicians. Our recent research comparing metabolomic profiles in clinically diagnosed patients, compared to those diagnosed using the IOM diagnostic criteria alone, has highlighted differences across sample sets. It is unclear whether these are biomarkers of ME/CFS or fatigue. To make further progress a robust set of homogeneous clinical assessments and standardized diagnostic tests must be developed to facilitate research studies. Using a multi-omic approach in this proposal (i.e. genomics: plasma pathogen levels, patient genome, plasma metabolomics and Immune cell transcriptomics) we will build a data platform to be utilised by future researchers. Our collaboration with Softcell Biologicals will produce a rich source of whole genome sequencing data including pathogens and genetic variants associated with fatigue.

Project Management

This proposal involves three European ME/CFS research groups and Oxford-based clinicians for stroke and lymphoma patients. Patient recruitment using homogeneous symptoms based clinical assessment from ME/CFS international cohorts is crucial for the success of the study and the quality of the collected samples and data for future research. A clinical management group comprising clinicians from the three centres and additional ME/CFS experts from the NIH and Dove Clinic will review clinical data before patients are included in the ME/CFS study. A clinical protocol for ME/CFS patient recruitment will be established at the start of the study. Stroke, Lymphoma and ME/CFS patients will be assessed using SSF 36 fatigue score ratings at the time of baseline blood collection and on follow up. Streamlining SOPs for all the centres and seeking ethical approval for intervention study at Oxford will take between 3-6 months. A data and statistical protocol will be created before starting the study to facilitate integration of data generated from the 3 centres into a data base. Assessment of the data quality will be carried out at 3 monthly intervals during patient recruitment. Oxford will be recalling individual patients when changes are noted in their centrally integrated fatigue and movement App data. PhD projects linked to data and samples collected and new patient MRI projects are planned for long-term sustainability. Future collaborations are envisaged with Softcell bringing in other pharmaceutical companies as therapeutic targets emerge. A fatigue doctoral training programme is proposed for Oxford with opportunities for European Horizon grants to develop research with our European partners.

Summary of budget required

Oxford :

Salary support: 1 X PDRA 4 years (Project Manager, ethics application, researcher and sample processing, NDWRH) (**£221,933)**; 1 x 0.8 FTE Laboratory technician/research nurse (Blood taking, sample processing and storage, NDWRH (**£106,396)**; 1 x PDRA 3 years (Immunology research project, Kennedy Institute) (**£168,858);** 0.2 Data Assistant 3 years (Metabolomics data analysis and remote data capture and)co-ordinator (**£16,970)**; 0.2 FTE 12 months PDRA (Metabolomics Chemisty) (**£10,456)**. 2 year PDRA (Raman Diagnostics Engineering Science) (£101,131)**Total salary support = £625,744**

Non-staff: Blood collection, storage, consumables and metabolomics cost £124,456 .. Data and statistical set up, review sample audits, Statisticians time £20,000. Italian costs £11,000. Translatomics pilot Sussex £45,800.

Poland: Clinical assessments 80 individuals £50,000, Cryotherapy £40,000, consumables and shipping £18,000. Microbiome assessment £20,000. Pupilometry equipment £7,000. Sample collection and processing 100 samples £7,000. Travel costs and miscellaneous £20,000. Translating online diagnostic portal into English and Spanish £3,000.

Spain: Total Salary support £174,000. 1x PDRA 3 years Bioinformatics and PhD student 3 years. Clinical hardware: Taskforce monitor £34,000 and Pupilometry device £5,000. Transcriptomics, validation and exosome consumables £150,000. Transcriptomic outsourcing £180,000.

Project whole genome sequencing costs 750 - 850 samples £65,000 (£76 per sample). Actual cost per sample $1000. Softcell Biologicals contribution to sequencing costs $750,000-850,000).

Total non-staff = £974,256

Total projected project budget = £1,600,000 (plus institutional costs of around 20%)

References

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