

INVEST in ME 14th INTERNATIONAL RESEARCH CONFERENCE

Friday 31st May, 2019

On Friday 31st May 2019, I was privileged to attend the 14th Invest in ME conference in Westminster, London. The conference was attended by participants from all over the globe, and also a number of new young researchers, who had also attended a one-day seminar to present their work. The main conference had also been preceded by a two-day colloquium, with some of the world's leading ME/CFS researchers presenting their research.

The main conference was opened by **Dr Ian Gibson**, who welcomed us with the very positive thoughts that understanding of ME/CFS was advancing rapidly. This can all only lead to better diagnostic and therapeutic opportunities.

The first speaker was **Dr Beth Unger** (Atlanta, Georgia, USA) who is Chief of the Chronic Viral Diseases branch at the CDC. She described 2 large studies being undertaken:

1. MCAM with participants being taken from clinics, where diagnosis had been by expert clinicians. No specific case definition was used. Patients came from 7 clinics around the USA. Data was provided to the Institute of Medicine. There was assessment as to whether patients differed between clinics, and a look at how the experts diagnosed and managed these patients.
2. BRFS – this study potentially involved all states and a phone survey was done of 400,000 adults annually. Data was gathered from 5 states in 2014, and 3 states in 2016. There were 55,000 respondents. Co-morbidities with ME/CFS and other diseases was reviewed. The main concordance was with arthritis, depression and asthma. Most ME/CFS patients did have one co-morbidity, but there was no co-morbidity with cancer and ME/CFS. Comorbidities did have a negative impact on health, involving more doctor visits.

The SF36 was down in ME/CFS on all measures. Both physical and mental functional impairment was different to healthy controls. There were also barriers to health care in all areas for ME/CFS.

The overview was then followed by **Dr Vickie Whitemore** (NIH, Washington,DC) who gave an update on what was happening with research at the NIH. There are 27 institutes at the NIH, of which Vickie represents NINDS (the neurological division). They do fund research worldwide from their extramural division, and there is an ongoing intramural study based at the NIH campus now. This involves 25 ME/CFS patients and 20 healthy controls. She is talking to other countries to stimulate worldwide research, and it is possible for overseas people to apply for research funding.

There are currently 4 collaborative centres doing their own research and also working at collaborative projects. (see www.mecfs.rti.org for details).

The NIH recently hosted a ME/CFS conference to which 350 attended and a further 500 joined on-line. They also had a "Thinking the Future" workshop for researchers, and 60 attended of which 40 were new young researchers. This has provided an opportunity to network, and it could lead to a pathway for a career in ME/CFS. (Funding would be provided).

The NIH now has an advisory working group of clinicians, researchers and lay people. They will report and work towards a full research planning process. There is a need for growth in individual research. Some major studies are underway. Young researchers need encouragement. There should be support for hypothesis driven research, with a loosening up of diagnostic criteria. There should be support for the development of biomarkers.

Details are on the NIH websites.

Prof Maureen Hanson (Cornell) spoke on Immune Dysregulation in ME/CFS. She gave us a much-needed lesson on immunology, explaining how the bone marrow produces CD4 cells, which secrete cytokines, and CD8 cells which cause death of infected and cancer cells. The dendritic cell presents an antigen (T Cell) and signals are interchanged to activate the T cell. T cells are activated in the lab by adding cytokines. This causes a change in metabolism leading to proliferation and glycolysis. The “Seahorse” is used to measure glycolysis and oxidative phosphorylation. The cell gets ATP as a result of these functions.

She used a study population from Incline Village. Patients scored low on all measures of SF36. She measured oxidative phosphorylation. She looked at CD4 cells, and found no significant difference in cells after activation, and similarly none in CD8 cells. She then analysed glycolysis in circulating cells. Both CD4 and CD8 cells had lowered glycolysis in circulating cells, indicating dysfunction. It is possible to inhibit oxidative phosphorylation – compensating glycolysis is down in CD4 and CD8 cells., indicating cells not functioning properly.

She then had looked at memory potential in mitochondria using flow cytometry. Mitochondria can be labelled, and mass and membrane potential can be measured. In the CD4 cells there were no differences, but CD8s were reduced in circulating cells. i.e. there was impaired glycolysis and reduced membrane potential i.e. the immune system is not working properly.

Cells can release extracellular vesicles (ECVs), exosomes and apoptotic bodies. The latter are the dying parts of cells. ECVs are also released by muscle and brain cells. They can also pass through the blood brain barrier and are also released in the gut.

An ECV study has been done by Dr S Levine on a very severely ill group. There was a significant increase in exosomes. Cytokines were analysed and found to be different in ECVs compared to plasma. She also looked at the connections between cytokines. The ME/CFS network was different in controls because of dysfunction. She looked further into ECVs and looked at microRNAs. The next step will be to look at these things pre and post-exercise. This could lead to potential biomarkers – possibly a “set” of microRNAs. There is potential to attack ECVs with drugs.

Prof Mady Hornig (Columbia, New York, USA) discussed fingerprinting the phenotypes of ME/CFS along the gut-immune-brain axis. She asked the question first “Why phenotype”? She feels it is important to gather as much information as possible on the chemical aspects of the illness, and also to look at the comorbidities. This can give clusters for research, treatment options or biomarkers.

Gastrointestinal comorbidity in brain conditions is very common, e.g. it accounts for 80% of comorbidity in IBS. Also HPA axis activity leads to release of stress hormones (cortisol) and this helps the immune response. Negative feedback is important also. Food may promote inflammation and/or auto-immunity, such as through the leaky gut. There may be an interplay with bacteria. Gastro-intestinal motility also depends on the interaction of multiple cell types. Microbial products affect gastrointestinal motility.

She had done a study looking at the microbiome comparing 50 patients with 50 healthy controls. There were distinct differences in the microbiome of ME/CFS patients with and without IBS, and healthy controls. She then looked at associated genes.

She mentioned that some common herbicides affect the oestrogen system – this may be worthy of a look.

She concluded that ME/CFS is associated with intestinal dysbiosis. IBS comorbidity is a strong driver, and there are different metabolic pathways in ME/CFS with or without IBS. There are also very different metabolomics.

Prof Donald Staines (Gold Coast, Australia) presented work on the transient receptor potential (TRP) ion channels in the aetiology and patho-mechanisms of ME/CFS. He explained how they are now using a patch clamp. Changes in ME/CFS are reflected in NK cells. The illness affects every system in the body. NK cells kill invading cells and also internal cells. NK cell dysfunction is accepted as part of the criteria for making a diagnosis, and this group measured function. SNP studies were done, and they noted SNPs belonging to TRP channels.

The TRP channels are non-specific calcium channels, and threat signals are converted into biological activity. This involves the transport of calcium, magnesium and sodium into the cells. Calcium ions are a thousand times greater outside the cell than within it. They used the patch clamp for this work. Calcium is essential for every cell function, and a store is maintained and used as needed. Calcium maintains the stability of the cell and regulates many systems.

There are many “threats” to the system e.g. chemicals, travel, exercise etc. Then calcium moves into the cell. Abnormal TRP channels lead to depletion of calcium – and this is rather like having a flat battery. He described failed TRP function as a “triplopathy”.

Channelopathies do occur in other diseases and can be genetic, (examples include ALS, diabetes, hypertension etc) or acquired (examples being trauma, EBV etc).

The patch clamp is about 1/12 the diameter of a human hair and is the gold standard for these types of measurements.

TRPM3 is expressed in many neurological channels, (CNS, ANS, PNS) including the eye, which has many symptoms in ME/CFS. TRP function is blocked by ononetin and activated by pregnenolone sulphate and nifedipine. 168 readings were taken from patients and controls in 3 cohorts. Addition of nifedipine (a calcium channel blocker) failed to reactivate. Nifedipine acts in a different part of the receptor.

All patients showed TRPM3 abnormality, and no abnormalities were found in healthy controls. There was an association with acetylcholine receptors. TRPM2 is now being investigated. This is one of the system’s back-up systems.

There is now potential for the patch clamp to be used for drug trials in ME/CFS.

Dr David Andersson (London, UK) went through the pathophysiological changes in Fibromyalgia (FM). He told us that approximately 2% of the world’s population suffer from fibromyalgia. There are many similar and overlapping symptoms with ME/CFS. The female to male ratio is 4:1. FM occurs on 10-30% of patients with a rheumatological diagnosis – therefore there was consideration as to

whether autoantibodies may be involved. There is abnormal pain processing, and therapies tend to be ineffective.

IgG antibodies from FM patients were injected into mice and reactions to pain compared to healthy controls. There was significant increase in both pain and pressure after the IgG injection. Onset was rapid. This showed that the sensory profile from the patient can be transferred to the mouse. Pain threshold increased and sensitivity increased markedly also. A similar experiment was done using thigh pressure – tactile allodynia resulted. And further tests were done conclusively on dissected skin. The Fibromyalgia syndrome IgG sensitizes nociceptors.

The conclusion was that fibromyalgia is caused by autoantibodies, and there is sensitisation of nociceptors.

Treatment should include coping strategies and gentle exercise. They will now look for novel mechanism-based therapies.

Dr Jesper Mehlsen (Copenhagen, Denmark) had looked at the characteristics and pathophysiological changes in a large cohort of Danish ME/CFS patients. A number of patients developed many symptoms after HPV immunisation. They were diagnosed initially as suffering from POTS and ME/CFS. There were 845 patients with possible side effects from this vaccine. The age group was 16-26 years and they had multiple symptoms. 80% fulfilled the IOM criteria for a diagnosis of ME/CFS.

Using the autonomic symptom questionnaire (Compass 31) there was a high level of severe autonomic symptoms. Measurements of mental and physical fatigue were as severe as in MS, and worse than post-stroke patients. Looking at autoantibodies, 59% were positive, and these are not usually present below the age of 14. They also looked at the autoantibodies involved in cardiovascular regulation. Many patients had an active stand test. Beta-blockers did reduce heart rate. One patient however using an asthma spray fainted due to very low BP.

The findings of autoimmunity in these patients may represent molecular mimicry or bystander activation via cytokines.

In conclusion, 1 per 1000 patients receiving the vaccine developed serious adverse events resembling ME/CFS. It is likely an autoimmune reaction directed against the autonomic nervous system. These findings will be useful for future research and treatment.

After lunch tributes were paid to **Anne Ortegren**, who died last year and Prof Jonas Blomberg who died suddenly a few months ago.

Prof Stuart Bevan (London, UK) gave the Anne Örtégren Memorial Lecture on Pain and ME/CFS. He specialises in Pain Management. He listed the symptoms in ME/CFS many of which are neurological. He defined pain and said that pain can be good when it acts as a warning. Chronic pain is defined as pain which lasts for longer than 3 months. It maybe due to a disease in its own right (eg fibromyalgia) or secondary to another disease. He described the pain pathway, and also described the range of drugs for conventional pain management.

He made the point that less than 30% patients with chronic pain get more than 30% of relief.

He described the mechanism of neuropathic pain in detail:

1. The initial “lesion” may be varied and cause damage
2. This unbalances the sensory system

3. The system may misread the sensory inputs and there is resultant allodynia or hyperalgesia.
4. Pain then generates spontaneously – shooting, burning etc.

Neurological origins of pain were then discussed at all levels. The key changes driving pain maybe central or peripheral – there is no real consensus on this. The pain changes to secondary sensitivity. Central sensitisation then amplifies the perceptual input.

In inflammatory pain, cytokines are involved. The immune system and the nervous system talk to each other. The position of cytokines is unclear, but there maybe receptors on the nerve fibres. There are lessons to be learnt from CRPS. This is usually triggered by injury. Intense pain may follow often for years. It is usually confined to the limb or other area of injury. There may be skin and temperature changes, and sometimes swelling.

Then ensued discussion as to whether chronic pain is an autoimmune condition. One study has shown that by plasma exchange – using healthy plasma, when pain can be reduced in 30 out of 33 subjects. Much of the plasma exchange works fine in mice.

Fibromyalgia and ME/CFS have so many similar symptoms, that further research in these areas particularly in the area of autoimmunity is warranted.

Potassium channel antibodies were then discussed, these are clustered in the nervous system. They are associated with some proteins, and autoantibodies combine with these. The potassium channel complexes: LGI1 and CASPR2 can be associated with many symptoms. CASPR2 is associated with pain in 50% of patients.

Future pain treatments were considered. These can include: immunotherapy, corticosteroids, IV immunoglobulin exchange, immunoadsorption, and cyclophosphamide and rituximab (for which there is little data).

Treatment mechanisms for both FM and ME/CFS need to be considered. The pain in ME/CFS may not necessarily be “central” but is often widespread. There are variable symptoms therefore likely to be variable mechanisms. The approaches being used in CRPS and FM can and should be used in ME/CFS.

Developments at the Quadram Institute were then discussed by **Professor Simon Carding** (East Anglia, UK). He explained how the microbiome can be abnormal in ME/CFS and asked the question as to whether gut microbes may trigger the illness. The human microbiome has 100trillion microbes: bacteria, viruses, fungi, protozoa and archaea. The gut microbes have been shown to link to diseases such as Parkinsons and Alzheimers.

Environment and lifestyle both have an effect, and ideally controls need to be from the family or same household.

Their focus now tends to be on the more severe ME/CFS patients, who are often ignored. Only 0.5% studies have been on the severe group. Among the severe group there are many differences but some commonalities. 3 particular groups of organisms seem to be out of kilter: eggerthella, faecalibacterium and oscillibacter. Viral bacteriophages affect the bacteria and are common. They attach to bacteria and are 25% identifiable. They continue to track bacteria throughout the body. Could these be a trigger for ME/CFS? Bacterioides are associated with health benefit, and if isolated can help to identify viruses.

He asked the question is the microbiome in ME/CFS unique? In the faecal metabolome, as yet no significant differences have been found in severe patients, there is no obvious signature. But the

virome is different. He then asked the question: Are we looking at a different disease in severe patients?

He then went on to discuss new initiatives at the Quadram Institute:

1. A GP fellowship scheme.
2. A joint UK/Sweden initiative – a PhD studentship
3. “Bacteriotherapy” which was likened to lawncare:
 - a. Plant new seed – eg probiotics – not effective
 - b. Returf
 - c. Replace the lawn. eg faecal transplantation – developed in China using camel dung – history is ongoing.

Faecal transplants may become more useful as a treatment in ME/CFS. One study of 60 patients showed 42 improved and remaining improved still at 7 months. A proper clinical trial is needed. This is planned for Norway and Norwich, looking at safety and efficacy of faecal transplants in ME/CFS. Physical and cognitive outcomes to be assessed. There are however many hoops to still get through.

Prof Oystein Fluge (Bergen, Norway) then brought us up to date with what is happening in their trials. He explained the history of treating lymphoma patients who had ME/CFS with Rituximab. A subgroup did improve. The final trial looking at 151 patients including a placebo group looked at a wide range of outcomes. Many had been long-term ill people. 40% had first degree relatives with autoimmune diseases. The paper was published in April 2019 and is available on line.

There was no significant difference in physical function, and physical activity was the same. There were a small number of adverse serious events, and a few less severe events. The trial was clearly negative. It is hard to say how we are to assess outcomes reliably. We have to consider placebo effects and natural symptom variation.

An open label trial using cyclophosphamide is now being performed. This drug is cytotoxic. Follow up at 18 months is now extended to 48 months. No placebo is being used. No results are available yet. Biobank samples are being used. Considerations will be: whether this is going to be a true drug effect, a placebo effect or natural variation.

Metabolic profiling and associations to clinical data in ME/CFS was presented by **Prof Karl Johan Tronstad** (Bergen, Norway). He has used samples gathered during Oystein Fluge’s trials. The focus is on cell energy metabolism. ATP is used by all cells. Mitochondria are important for ATP productions. He then outlined the mechanism of ATP production from glucose to pyruvate during aerobic work, and how anaerobic work shuts off this process causing increase in blood lactate.

He has been using the blood samples to try to understand. He has looked for clues e.g. measuring amino acids – there are 3 categories depending on entry into the ATP pathway. When metabolism is under stress, they can be down and this is more obvious in females. There is impaired PDH function in ME/CFS – a valve that can open and close and that is particularly important during exercise. Amino and fatty acids are used as compensation.

There may be a metabolic shift to cause a change in fuel consumption, changes in lipid metabolism and redox status. There are similarities in pathways such as hypoxia, starvation and training. He described ME/CFS as being “stuck in a semi-starvation state”.

Prof Nancy Klimas (Fort Lauderdale, USA) broke away from her traditional immunological approach to understanding and managing ME/CFS – and discussed the integrative approach their clinic is taking to management of this illness. She described how their institute has moved into integrative and functional medicine. She gave a brief history of integrative medicine describing how this involves taking care of the “whole” patient and society. The scientific model does not always work for chronic disease. They have set up an educational programme for patients for various reasons: many patients try such a large number of self-remedies, they need to understand the science, and they need to integrate other tools.

“Genetics loads the gun, environment pulls the trigger”.

She then outlined a sensible approach:

1. Clinicians are partners
2. Mind, spirit, community and body
3. Conventional and alternative medicine.
4. Interventions should be natural and less invasive ideally
5. Integrative does not reject conventional
6. Good medicine = good science
7. Health promotion and prevention is paramount
8. Practitioners of integrative medicine should exemplify principles and care of self.

ME/CFS is a complex multisystem illness, and integrative medicine integrates diagnosis and treatment-based with broad knowledge of physiology.

Treatment approaches should include: diet and suitable supplements, sleep management, appropriate exercise, pain relief, detoxification, and emotional support.

Developing evidence suggests a new approach to research using new modelling approaches. Personalising approaches should be used as much as possible, and should go hand in hand with sophisticated research.

Nancy paid tribute to her colleague **Prof Mary Ann Fletcher**, one of the earliest pioneers in ME/CFS research, who is finally retiring.

Dr Ronald Tompkins (Harvard, Boston, USA) talked about Harvard’s plans for clinical research. They now have a team of vibrant young researchers. He explained how many researchers have been working independently for years and are keen to collaborate. The Open Medicine Foundation have funded 3 centres, Harvard being the third. The others are Stanford, USA and Uppsala, Sweden. A number of research thrusts are in the pipeline. There is much interest in getting a centre of excellence established at Harvard. They need to establish a clinical infrastructure

There is interest in the issue that after serious injury, there is hypermetabolism, protein synthesis is upregulated, and there is reprogramming of energy metabolism. There is activation of liposomal protein degradation. They have looked also at what happens with exercise. All this may relate to post-exertional malaise. And they are looking at structure and function of skeletal muscle in ME/CFS, and relating this to age, sex and lifestyle. All collagens seem to be downregulated. Samples will be taken

before and after CPET. They will look at the biology of immobility gene changes and calcium signalling.

The talk by **Dr Michael Van Elzaker** (Harvard, Boston, USA) was titled "Physiological and fMRI measures before and after symptom provocation by invasive cardiopulmonary exercise testing". He told us neuro-inflammation is synonymous with ME/CFS. The brainstem is central to pain processing. The vagus a mixed cranial nerve detects peripheral catecholamines. This can be the initiation of central glial illness response. If the vagus nerve is cut in rats, illness response does not occur. Glial response may occur with injury too. The microglia are the resident macrophages in the brain. They change shape and release chemicals equivalent to immune signalling. The microglia magnify signals rather like an amplifier. But this is not a specific diagnostic tool.

Efferent vagus function is associated with failure of the anti-inflammatory reflex, parasympathetic autonomic control, POTS and it is measurable with invasive CPET.

fMRI is associated with reduced cerebral perfusion.

The final speaker of the day was **Prof Ron Davis** (Stanford, USA). He presented work relating to ongoing search for a pathogen in 20 severely ill patients. There were less DNA viruses in patients than in controls. No parasites have been found yet. The search for RNA viruses is expensive, but in development. Search for fungi and bacteria are also in the planning. Searching for metabolites, there have been shown to be 63 out of 292 which are significantly changed.

The nanoneedle (very small) is used for diagnostics, and measurements are at 200/sec. Salt can be added early on, and this stresses the cells, and many changes are noted within an hour or two. But there is a need to screen for other diseases for comparison, and ME/CFS diagnosis needs to be compared to controls. A design is needed for a better cheaper chip.

Deformability of red blood cells is impaired in several diseases, but in CFS there is less deformability than in controls. There was some discussion also about the effects of adding ME/CFS cells to healthy plasma and vice versa.

The next stage is to screen for FDA approved drugs. Prof Davis mentioned 2 which might have potential: one of which can repair mitochondrial membrane.

As always, the conference concluded with a lively Q and A session and much animated discussion and excitement about all the research in the pipeline. There has been enormous development in research and management over the years, leading to greater understanding of this complex disease, which seems to become more complicated as we learn more! The answers are getting closer and there is so much hope now for the potential biomarkers to be confirmed, and treatment options to be forthcoming. I think meanwhile Nancy Klimas thankfully has brought us all down to earth with her now wide-ranging approach to whole patient care, and we can all take many useful messages home.

I should like to thank ANZMES and Invest in ME for making it possible for me to attend this conference once again, and also the preceding 2-day colloquium.

ROSAMUND VALLINGS MNZM