National Centre for Neuroimmunology and Emerging Diseases
CFS/ME International Conference: Research Innovation and Discovery

26-27th November 2018, Surfers Paradise, Queensland, Australia

We were privileged to attend this excellent and exciting conference. 2 days were filled with presentations encapsulating the latest research from Griffith University, Queensland, and from other centres around the world.

The conference was opened by **Professor Paul Scuffham** (Director of the Menzies Health Institute, Queensland), who told us that 200,000 Australians are afflicted by ME/CFS, and this equated to a cost of 5 billion$A annually.

The first presentation was by **Dr Elizabeth Unger**, (Atlanta,USA) and her first slide was very appropriate showing us a "Rainbow of Hope". She then outlined some of the focus of the CDC on Public health. With a prevalence of 0.2-0.7%, approximately one million Americans suffer from this disease. In the USA costs equate to $9-$14 billion annually, with a loss in productivity of up to $37 billion.
Regular monitoring of the research and illness should occur, and more ME/CFS experts are needed. Best practices for treatment need outlining.
She described a large telephone survey of 400,000 adults gathering general data on chronic health. Inclusion of ME/CFS has been approved for 2019 as a new optional model.
Details from the latest questionnaire show a lifetime prevalence (ie ever diagnosed) of 1.6% and a current prevalence of 1.2%. 71.4% still have ongoing ME/CFS (this is similar to a Canadian study). 83.4% had another concurrent diagnosis. There needs to be more surveillance, data collection and identification.
Future directions include healthcare provider education. A medical CME has been made with 4 experienced physicians. A pathway towards treatment guidelines is being established. The challenges include: sparse data, lack of experts and a lack of standardised methods.
The latest developments include: multisite clinical assessments, work with clinical experts, protocol development, specialised clinics, added studies looking at exercise, cortisol etc. Measurements are needed to enhance understanding.

Vicki Whittemore, (Bethesday, USA) a programme director at the NIH, was unable to attend but did her presentation by video link. She described the new working group associated with ME/CFS. Funding for the illness has increased to $15million annually, but this is still small compared with other major diseases. There are Intramural and extramural ME/CFS programmes associated with the NIH. Dr A.Nath heads the intramural programme, and patients are being recruited. Extramurally there are 4 centres established. There are opportunities for international cooperation and collaboration.
The NIH will be hosting a ME/CFS conference next April.

Alistair Lynch, a former professional Australian Rules football player, then gave us an overview of his experiences as a top sportsman who succumbed to ME/CFS aged 26. This was a compelling story involving a mental and physiological battle, with frequent relapses. He eventually regained his health and has become a supportive ambassador for recognition of the illness.

Mary MacRae (Australia) is the production manager at Shark Island Institute, an organisation with a team of 8 producing documentary films. In her presentation, she described how by using the film "Unrest" they explored the intersection of philanthropy, film-making and activism to discover how patient advocates are coming together to push for change.
An excellent new Australian mini-documentary, "After Unrest", has just been released. It is the creation of ME/CFS Health Page and patient, Ketra Wooding. Ketra is a patient with ME/CFS who is bedridden and has to live in a retirement home.

Gerald Thiel (Saarland,Germany) opened the afternoon session with a presentation looking at intracellular signalling cascades associated with Transient Receptor Potential (TRP) channels. These are non-selective ion channels that are conserved during evolution, and expressed in many tissues and cell types. They are involved with movement of sodium and calcium in and out of the cells. The TRP channel-induced intracellular signal transduction pathways induce changes in the gene expression pattern of the cells by activating stimulus-responsive transcription factors, and the expression of their delayed response genes. TRP3 activates transcription factor A1. CREB is a major player in transcription coupling.
Stimulation of TRP channels leads to increase in intracellular calcium There is a role of calcium in TRPM3 mediated up-regulation. Protein kinase is essential. There are a number of secondary responses. TRP channels are nociceptors in primary afferent neurons, leading to sensitization. (Nerve fibre initiated inflammation).
In mice TRPM3 inhibitors reduce pain. So some TRP channels are being considered as potential therapeutic targets. eg TRPM5 may be a target for development of drugs for type 1 diabetes. There may also be scope for development of drugs for prostate cancer.

Katsuhiko Muraki (Nagoya, Japan) gave an overview of TRP channels in ME/CFS. He described them as being involved with channelopathies .
In ME/CFS single nucleotide polymorphisms (SNPs) have been found in several genes. TRPM3 channels are expressed in NK cells and function as a modulator of the calcium dependant signalling pathway. Pregnenolone sulphate is a potential activator of TRPM3 channels. Other chemicals and steroids have weak stimulatory and inhibitory effects on TRPM3.
There are more than 20 types of isoforms of TRPM3. There are tetra homomeric and tetra heteromeric forms.
When cytokines are released under inflammatory conditions, TRPM3 and TRPM4/C1 expression maybe modified and calcium and sodium dependant signalling pathways are affected.
Changes in TRPM3 cause change in biological function and diseases. Movements of sodium, zinc and calcium ions were discussed. This work is a stepping stone to further exploration looking for targets for ME/CFS treatments.

Helene Cabanas (NCNED, Australia) discussed the characteristics of TRPM3 ion channels on NK cells in ME/CFS patients. NCNED uses NK cells as a model for TRPM3 cation channels for other cells in the body.
TRPs have an important regulatory role in mediating calcium influx to help maintain cellular homeostasis. Significant low NK cell cytotoxicity was noted, of both bright and dim CD56+cells.
TRPM3 current (function) was significantly reduced by pregnenolone sulphate stimulation, and TRPM3 channels were significantly modulated in NK cells by ononetin in CFS/ME patients compared to healthy controls.
Calcium is needed to regulate and there are many functions involved with calcium. In ME/CFS patients compared to healthy controls,. NCNED found significantly lowered expression of TRPM3 which is confirmed by their previous results as well as significant reduction in calcium. With this research, prognostic markers and drug interventions as a result of explanations for aetiology may be possible.

Cassandra Balinas (NCNED, Australia) spoke about the identification and characterisation of TRPM2 Channels and CD38 on NK cells using flow cytometry. She explained that NK cell toxicity is decreased in ME/CFS, and calcium controls NK cell lysis. The TRPM2 receptors are located in the brain, pancreas, adrenal, heart, kidney, immune cells, small intestine and skin. An assay has now been developed for TRPM2 using flow cytometry to measure TRPM2 surface expression and CD38 on immune cells, such as natural killer cells.

Further study of healthy controls showed significant decrease in TRPM3 and CD38 post IL2 stimulation, but no difference in ME/CFS patients ie they were unresponsive indicating impairment in the channel. They are now looking for possible drug interventions.

Natalie Eaton-Fitch (NCNED, Australia) used confocal microscopy and western blotting in addition to flow cytometry to characterise TRP channels including TRPM3 from NK cells. Using human NK cells in this way has helped to show the TRP channel activation and calcium signalling.
These studies help to explain why the drug Rituximab was not shown to be successful for treating ME/CFS.

Andreas Guse (Hamburg, Germany) discussed adenine nucleotide-dependant calcium signalling in T-Lymphocyes. This was a very complex discussion. He related some of the talk to experimental auto-immune encephalomyelitis (EAE). Infected T cells (EAE Cells) were used. EAE is an antigen-driven autoimmune model in which immunization against myelin autoantigens elicits strong T cell responses, which initiate its pathology with CNS myelin destruction. The study showed effects on the brain via the lymph glands, leading to inflammation and degeneration.
Adenine nucleotide 2nd messengers play significant roles in shaping cell calcium signalling, thereby also controlling autoimmunity of the CNS.

Dan Peterson (Lake Tahoe, USA) gave an excellent overview of the diagnosis and management of ME/CFS. He described a genetic predisposition and that the illness usually followed an infectious event. Energy production, ion-channel regulation and hormone dynamics are all likely to be involved. Average age of onset is 33, with a range from 10-70 years. Female: Male ratio is 3:1. ¼ of patients are housebound /bedbound. There is no specific cure, but quality of life can be improved. He compared this to other illnesses where there is an infection-elicited auto-immunity (eg PANDA syndrome). There is an increased risk of lymphomas. Energy metabolism is disturbed leading to the typical post-exertional malaise (PEM). Aerobic energy is disturbed suggesting mitochondrial dysfunction.

A management plan should be established and include development of a good doctor/patient relationship, validation and support, addressing social needs, and gentle physical conditioning. Activities should avoid PEM, be low intensity and paced carefully. Precision medicine was outlined, suggesting a wide array of approaches, specific drug testing, big data and artificial intelligence.

In relation to drugs there is minimal interest from big pharmaceutical companies. It may be that there needs to be repurposing of some already approved/used drugs – eg spironolactone, which can act as an antiviral. (it blocks EBV).

Some of the difficulties include inadequate training of doctors, no available information for primary providers, differences between consensus concepts and clinical guidelines. There is no biological marker, and limited treatment options for this very heterogenous disease. In 2018 there has been a gathering of 12 prestigious CFS physicians in the USA, with a view to producing firm guidelines for management.

He then covered various aspects of symptomatic treatment:
Sleep: Sleep hygiene, OTC medication (eg melatonin), pharmaceuticals and treatment of comorbidities.
Neurocognitive symptoms:
- Non-pharmacological approaches
- Antidepressants, stimulants, tricyclics

Orthostatic intolerance:
- Non pharmacological
- Beta-blockers, fludroncortisone, midodrine, mestinon, modafinil, clonidine

Pain: Osteopathy
- Cannibinoids (endorsed by pain clinics)
- Low dose naltrexone, IVIG, H2 blockers, Ketamine, IV Lidocaine

Immunology:
- Imunovir, IVIG, cytokine blockers, ampligen

Infections:

Antibiotics/antivirals

NB Recurrent herpes – treat long term.

Supplements as appropriate – discuss fully.

There is now no indication to use Rituximab.

Pawel Zalewski (Torun, Poland) spoke about "Whole Body Cryotherapy" as a potential non-pharmacological allied treatment for ME/CFS. He described how this therapy goes back to ancient Greece. This is said to restore the balance of autonomic NS functioning, decrease inflammation and improve cognitive function. Temperatures between -60°C and -140°C are used in a cryochamber. There is no pain and exposure is between 30 seconds and 3 minutes. The therapy has been tried in MS, hypotension, and in athletes, all with some success. Fatigue is improved, and there are effects on the hormones, and CVS (lowering heart rate and increasing stroke volume). It favours post-exercise recovery.

The therapy was tried on 22 ME/CFS subjects, who were exposed to -120°C. Exposure was between 45 secs and 2 minutes. This was followed by some light stretching. Patients were followed up in 4 weeks. Symptoms of orthostatic intolerance, cognitive function and fatigue improved. There were no side effects and the procedure was well tolerated. Follow up at 5 weeks showed ongoing benefit.

Mark Donohue (Melbourne, Australia) discussed POTS in relation to ME/CFS, and has found a higher 24 hour urinary output in the patients who had POTS with ME/CFS as opposed to the non-POTS patients. There may be a loss of autonomic regulatory processes. Urinary volumes in healthy patients can vary from 400ml to 2L daily. The average 24 hour output for the ME/CFS patients was 2.95L – in those with POTS and ME/CFS average volume was 3.3L and the non-POTS patients was 2.5L. The urine was found to be dilute. In the patients who had then recovered the volume went down. The patients did not notice the difference. Aldosterone levels were found to be at the lower end of the range. He thinks a channelopathy may be involved as potassium, sodium and magnesium all tend to be low. He asked the question: "Is POTS a cause or a consequence of this?"

For treatment the following were suggested as possible options: Fludrocortisone, Desmopressin spray, Midodrine, Ivabradine, compression garments.

He suggested this was an opportunity for further research, looking at thirst, HPA axis issues, mitochondrial issues in the renal tubules or maybe other causes.

James Baraniuk (Washington,DC,USA) discussed provocation testing in ME/CFS. His studies involve an intensive protocol, initially using telephone screening. Patients need to answer about 40 on-line forms, covering pain, psychological symptoms etc. Clinical examination is done and a dolorimeter is used instead of thumb pressure to assess pain. Serial bloods are taken to see the effects of exercise MRIs are done on Day 1 and Day 2 following exercise stress tests, some will have a lumbar puncture on Day 2.

1/3 of the patients had POTS after exercise (an increase of 30+ heart rate on standing) and symptoms lasted for 36-48 hour – this is different from true POTS. (True POTS only occurs in about 13% of ME/CFS patients). He asked the question "Is this genuine orthostatic intolerance, or a change in heart rate associated with posture, causing dizziness?" These patients who have the increase of more than 30 beats per minute in the 245 hours after exercise are the "Stress Test Activated Reversible Tachycardia" phenotype. (START). NB (He feels that tilt tables give false positives).

Cerebro-spinal fluid biomarkers are significantly different comparing those who had a lumbar puncture after an overnight rest with those who had the lumbar puncture after exercise. When looking at 1200 metabolites in the CSF,

89 were significantly different in ME/CFS and Gulf War Illness (GWI). They were not identified specifically, but some which were down in ME/CFS were up in GWI. Looking at microRNA, there were some reductions after exercise in the ME/CFS patients.

Leighton Barnden (NCNED, Gold Coast) looked at MRI changes in the brainstem in ME/CFS. He explained how there are multiple nuclei associated with the cortex which release excitory hormones and inhibitory fibres. The fibres "talk" to each other. The vasomotor centre involved controls heart rate, BP, thermo-regulation, respiration etc. Impaired signals cause many symptoms.
The relationship between the MRI and BP was found to be abnormal in ME/CFS. He had also looked at myelin vs ME/CFS severity, myelin in ME/CFS vs healthy controls, and compared the brainstem and sensorimotor areas. fMRI detected altered transmission within the brainstem. Brainstem connectivity is impaired in ME/CFS, and this may explain many symptoms.

He asked the question: "What causes the damage to the brainstem?"

Zack Shan (NCNED, Gold Coast) described how a unique brain structure may be identifiable in ME/CFS. Many symptoms suggest that the brain may play a key role in ME/CFS. The cerebral white matter shows shrinkage in the left posterior inferior fronto-occipital fasciculus longitudinally, and in the medial prefrontal cortex, which is associated with sleep quality. More brain regions tend to be recruited in ME/CFS in a compensatory role.

These brain imaging results could be potentially used as a diagnostic biomarker, and help with understanding of the pathophysiology.

Richard Kwiatek (Adelaide, Australia) In ME/CFS both ventricular CSF lactate and occipital cortex glutathione have been shown to be elevated and suppressed respectively, and other recent studies have shown ventricular lactate is elevated in fibromyalgia syndrome. Kwiatek has found that cortical glutathione levels are normal in fibromyalgia syndrome.
The drug milnacipran has been shown to lower the elevated lactate in fibromyalgia, leading to reduced pain, by attacking glial inflammation.

Nicholas Cherbuin (Canberra, Australia) discussed the planning and implementation of longitudinal cohort studies. He said that these could be unethical, impractical or costly. In ME/CFS one could look at epidemiology, course of illness, risk factors etc. He is currently doing a Path Through Life study of dementia. Patients are assessed at age 20, 40and 60, with then follow up at 80. Many things are being measured.
Epidemiological studies are designed to detect "associates". For example, when diabetes is followed, great brain shrinkage is detected. There follows cognitive decline and this can then be checked against smoking, BP etc. They looked at BMI, cortical thinning and found that plasma glucose affects the thinning rate. They can work out what "normal" shrinkage should be – and then find it is increased in diabetics. Brain inflammation can affect cognitive decline.

When doing long-term cohort studies, the study needs to be clearly identified. Other issues to be considered include budget, type of measure, length of follow up, management structures etc. Short or long term goals should be recognised. There needs to be good governance in conducting a successful longitudinal study. There should be plans for disagreement – which will occur! Look for collaborations and consortia, and learn from experience.

Heidi Nicholl (Melbourne, Australia)is an ethicist and the CEO of EMERGE. And she presented results of a 102 question survey of 610 ME/CFS patients. 80% were female and all had a diagnosis of ME/CFS. 62% had had the illness for more than 20 years. 10 categories were covered. Results are detailed on their website

https://emerge.org.au/wp-content/uploads/2018/09/Emerge-Australia-Health-and-Wellbeing-Survey-of-Australians-with-MECFS-2018.pdf.
60% who had been part-time employed prior to illness, and 57% fulltime were now unemployed and 34% had no income. 13% received a carers allowance. 68% needed some care at some time. Carers were usually family members.
Strategies that worked included: rest, pacing, medication for sleep. GET and GATusually made things worse. Among key findings were naming the condition and options were given to choose. There is a need to lock onto one diagnosis. There was a need for improved GP education. 29% rated their GP as good, 44% as poor. Early diagnosis was potentially helpful.

Sara Close (Darwin, Australia) discussed the personal economic costs of ME/CFS , as no cost analysis had been undertaken in Australia recently. She had surveyed 353 participants, of whom 52 were eligible and 30 of these had ME/CFS. Direct medical expenses worked out at $1100 per month and support costs were $360 per month. The total overall cost including economic loss (74%) per month was $5576. This equated to an average annual cost of $66900. As approximately 190,000 individuals have this illness in Australia, the broad total estimate would be greater than $12billion. This represents a large economic burden for Australia.

Daniel Dahdah (St Ives, Australia) spoke of the benefits of recording capacity and severity of symptoms in electronic format on line, to gauge progress and wellbeing. 17 ME/CFS patients participated. It was found that subjective recordings and patient centred outcomes empowered patients and validated their suffering, while providing a good visual tool for engendering hope as they saw positive progress.

Geoffrey Hallman (Gold Coast, Australia) Discussed disability insurance issues in relation to claims for ME/CFS. The National Disability Iinsurance Agency does not have an established policy for claimants with ME/CFS, and people are getting turned away from this scheme. The main reason for this is a misinterpretation of the Dubbo study. ME/CFS is not currently considered a permanent condition.

Paul Fisher (Melbourne, Australia) spoke of his work in specific mitochondrial respiratory defects and compensatory changes in ME/CFS patient lymphocytes.He asked the question: “Is respiratory function impaired in ME/CFS?” There is generally a reduction in respiratory function, and mitochondria are affected in muscles. The functions are normally regulated by exercise. In their laboratory, they are usually looking at “dying” lymphocytes. The genes coding mitochondrial proteins are upregulated in ME/CFS.
For subsequent studies, they used lymphoblasts, which are easy to obtain and can grow.
Mitochondrial function, genome copy number, RNA expression and glycolysis are unchanged in the lymphoblasts in ME/CFS. ATP synthesis is less efficient in ME/CFS lymphoblasts. The basal respiratory rate is not significantly changed. Proton leak and non-respiratory oxygen consumption are elevated in ME/CFS lymphoblasts. Mitochondrial respiratory capacity and membrane potential are elevated in ME/CFS according to ME/CFS severity. There is dysregulation of proteomes in ME/CFS lymphoblasts – 8 are downregulated and some are upregulated (those involved in amino acids and fatty acids).

Stress protein signalling may be responsible for the upregulation of ME/CFS mitochondrial proteins. Cause/effect is yet to be determined.

Stanley Du Preez (NCNED, Gold Coast) presented work on enteric dysbiosis in ME/CFS. Enteric dysbiosis is not part of the criteria included for a diagnosis of ME/CFS, but gut symptoms are recognised. There is substantial diversity in the microbiome, and this is subject to various factors. Pertubations give rise to chronic gastrointestinal diseases. ME/CFS may have a bacterial or viral basis. Dysbiosis can affect the bloodstream via the leaky gut, leading to nervous system and gastrointestinal disruption.

They did a literature overview looking at 989 manuscripts. 7 main studies were evaluated: one randomised controlled trial and six observational studies. 6 studies found differences in the microbiome. No two studies showed the same differences. Changes were notes after use of probiotics. Specific associations with the microbiome composition were not made. None of the studies reported illness severity. Faecal samples only were used and this represented only luminal flora. Culture is less accurate than sequencing.

The conclusion was that dysbiosis is inconsistent and limited in ME/CFS. Dysbiosis needs to be checked in the severely affected patients. There is no support yet to use gastro-intestinal flora as a treatment.

Eiren Sweetman (Otago, NZ) looked at the changes in the transcriptions of circulating immune cells of a NZ cohort with ME/CFS. She raised the issue that this illness is amenable to precision medicine. She had studied isolated peripheral blood mononuclear cell transcriptomes of a small patient group compared to healthy controls. The levels of 27 gene transcripts increased 1.5-6 fold and with 6 transcripts decreased 3-6 fold. Further analysis of these altered gene transcripts indicated inflammation, dysregulated circadian clock function, oxidative stress responses and mitochondrial dysfunction. The RNA-seq analysis of the ME/CFS transcriptome alone provides novel insights into the molecular changes in ME/CFS. This contributes to more understanding of the mechanisms of this disease.

The conference was closed by **Mr Takeshi Tanabe**, Acting Consul-General of Japan

The organisers, **Professors Sonya Marshall-Gradisnik and Donald Staines** (NCNED, Griffith University) must be thanked for the enormous effort in organising such a prestigious event.

Drs Rosamund Vallings and Sarah Dalziel, New Zealand