8. We need to find reliable bio-markers for ME (further details)

Potential Bio-markers

Until the definitions and biomarker issues are resolved there is always doubt that any research properly represents all patients. Differential diagnoses into subgroups should be a high priority for research, and it is also necessary to be aware that it is not known if highly restrictive subgroups could represent a true differential diagnosis or just a subgroup within the spectrum of a disease state. Simply asserting that all patients who meet a definition must have the same disorder is problematic.

A simple illustration may clarify the difficulty. When a person suffers a stroke, a part of that person's brain dies. There are two very different causes though: a blockage in an artery or a burst artery. The symptoms of a stroke can differ greatly from patient to patient, although they all suffer from part of the brain dying. Two people can have similar symptoms, but may have suffered from the different causes of strokes. If we subdivide ME patients into highly restrictive subgroups, could that make it harder to find a common root cause, or are their root causes different? We still do not know.

There are a number of recent potential biomarkers that stand out for both association and indication of severity. These include lipopolysaccharide reactions and post-exercise physiology. The confirmation of efficacy of the drug Rituximab in a phase two clinical trial is also worthy of special mention.

Immunology

Immunological Changes After Both Exercise and Activity in Chronic Fatigue Syndrome: A Pilot Study. *White et al.*

Nine patients with CFS (Fukuda 1994) were tested for post exercise cytokines. TGF-β was constantly elevated in patients. Three days after exercise, fatigue and other symptoms were increased. TNF-α spiked after activity, including travel to the hospital, and persisted for up to four days.
"*The main finding of this pilot study was the elevated median concentration of transforming growth factor beta, which seemed to be related to activity. ... Finally, we found that exercise induced a sustained elevation in the concentration of TNF-α, which was still present three days later, and this only occurred in CFS patients.*" p. 63

In animals TGF-β reduces activity. TNF-α also reduces activity and induces poor concentration and malaise.

While only seven subjects are listed in table 3, (p. 64 TGF-β (pg/ml) before and after travelling to hospital), it is visually obvious that there are two apparently separate groups of patients, with totally different basal TGF-β but similar post-exercise TGF-β. This should have been investigated.

The discussion ends with the following:

"*These preliminary data suggest that "ordinary" activity (i.e., that involved in getting up and traveling some distance) may induce anti-inflammatory cytokine release (TGF-β), whereas more intense exercise may induce pro-inflammatory cytokine release (TNF-α) in patients with CFS . The causal mechanisms involved and the direction of the relationship between these mechanisms remain to be elucidated. Altered cytokine balance, for example, following an infection, may modify the threshold at which cytokine release occurs with exercise or activity, setting up a vicious circle. These processes could contribute to the postexertional malaise, myalgia and the central fatigue that characterise CFS. Future studies should study patients at truly resting baseline levels, over a longer time-course, and should examine gene expression of cytokines, as well as circulating levels.*" p. 64

This study includes authors who are proponents of CBT and GET. Yet they have so far failed to follow up on these findings. Their model presupposes that there are unexplained symptoms, yet their research shows that some symptoms are potentially explained.

Benefit from B-Lymphocyte Depletion Using the Anti- CD20 Antibody Rituximab in Chronic Fatigue Syndrome. A Double-Blind and Placebo-Controlled Study. *Fluge et al.*

This was a small Phase 2 randomised, placebo-controlled double blinded study of 30 CFS patients (Fukuda 1994). Rituximab is a B cell antibody drug that depletes CD-20 B-cells. Ten of fifteen patients received at least temporary reduction of fatigue and non-fatigue CFS symptoms including functional capacity and pain. Two of these were still in recovery at the time of publication.

"*The associations between B-cell depletion and clinical responses, and the time frames for clinical responses delayed 2--7 months after the initial and rapid B-cell depletion, indicate that CFS may be an autoimmune disease, often preceded by an infection, and targeting specific parts of the nervous system.*" p.8

The Rituximab group participants were diagnosed using both Fukuda and the Canadian Consensus Criteria, although two of the placebo group did not fulfil CCC criteria. Response by two thirds of CFS patients to this therapy is strongly indicative of CFS being a biophysical illness, with a primary immunological B-cell component.

"*Thus, we believe that B-cell depletion targets a central player in the pathogenesis of the CFS disease, directly or indirectly.*" p12

A larger trial is being planned.

Biomarkers in Chronic Fatigue Syndrome: Evaluation of Natural Killer Cell Function and Dipeptidyl Peptidase IV/CD26. *Fletcher et. al.*

This paper shows that Natural Killer Cell Cytotoxicity (NKCC) is a potential biomarker for CFS. This topic has been investigated for nearly two decades now and research is being conducted by multiple groups around the world, including Australia and Japan, not just the USA, which makes it one of the more robust findings.

The results are promising but the problem with it is that the highest levels of NKCC assayed for patients is higher than the lowest levels of NKCC for controls. This implies the test is confirmatory at best, but it still has promise if combined with other tests. It was however a highly significant finding, with $p < 0.001$.

Several other markers were also looked at but they have the same issue and the best were slightly less predictive than NKCC.

Immunological abnormalities as potential biomarkers in Chronic Fatigue Syndrome/Myalgic Encephalomyelitis. *Brenu et. al.*

This study also showed reduced cytotoxic capacity in both NK cells and CD8+ T cells. A new finding was that numbers of a subset of NK cells labelled CD56 bright CD16- were decreased in CFS.

This study also showed elevated lymphocyte VPACR2 receptors and elevated cell counts for CD4+ CD25+ regulatory T cells and FoxP3+ regulatory T cells. These did not overlap with controls, indicating they may be much stronger biomarker candidates.

Increased IgA responses to the LPS of commensal bacteria is associated with inflammation and activation of cell-mediated immunity in chronic fatigue syndrome. *Maes et. al.*

Maes et. al. showed that for a subgroup of patients symptom severity is associated with an increased response of Immunoglobulin A and cell-mediated immunity. They could also differentiate between ME/CFS and chronic fatigue. Symptoms that could be explained include flu-like symptoms, neurocognitive and other symptoms. Further research is needed in this area. This is a development of earlier work by Maes.

Normalisation of leaky gut in chronic fatigue syndrome (CFS) is accompanied by a clinical improvement: effects of age, duration of illness and the translocation of LPS from gram-negative bacteria. *Maes & Leunis*

In this paper Maes shows that the symptoms can be reduced by addressing the proposed pathophysiology, including the use of dietary factors such as NAC, glutamine and zinc. Since a reduction in LPS associated biomarkers leads to a reduction in symptoms, it strongly suggests that LPS is an important factor in ME and CFS pathophysiology for a subset of patients.

Neuropsychology

EEG spectral coherence data distinguish chronic fatigue syndrome patients from healthy controls and depressed patients-A case control study. *Duffy et. al.*

Coherence is a measure of how much two different regions of the brain are coupled.

The patients were diagnosed with CFS using the Fukuda (1994) criteria.

Duffy et. al. were able to identify all depressed patients and from 60% to 92.4% of CFS patients, depending on the specific subgroup tested. The 60% was for males on psychoactive medications, and the best result was for females on no psychoactive medication.

This test is potentially diagnostic with some extra development work. Most importantly it can discriminate between healthy controls and CFS patients, as well as depressed controls and CFS patients.

The other important finding is that most of the discriminatory parameters reflect temporal lobe impairment, which might be related to the memory problems in CFS.

Does hypocortisolism predict a poor response to cognitive behavioural therapy in chronic fatigue syndrome? *Roberts et. al.*

Roberts et. al. have identified a potential biomarker for poor or non-response to CBT. They found that those who responded better had higher cortisol, but that low cortisol was not sufficient to explain non-responders. Instead, a blunted circadian variation in cortisol release was the greatest predictor.

This study used the Fukuda definition and was written by proponents of CBT and GET. It shows that they have looked at biomarkers and recognized that they can be significant. Yet further studies did not follow up on cortisol and other biomarkers.

Exercise Physiology

Diminished Cardiopulmonary Capacity During Post-Exertional Malaise. *VanNess et. al.*

This research has firmly established that physical exercise in excess of capacity decreases functional capacity in female CFS (Fukuda 1994) patients. Using full gas analysis they can show that mild to moderate CFS patients do not show significant difference from healthy controls due to exercise on day one. A second exercise test the following day shows marked functional decline. This is the only disease for which this result is known. The accuracy of classification as CFS or control was 91.7%.

Post Exertional Malaise (PEM), which has been recently relabelled under the ICC as post exertional neuroimmune exhaustion (PENE) is not the complete story. Physiological capacity is substantially impaired in just one day post exertion.

Such decline can be caused by low intensity physical activity.

Metabolic capacity post exercise is well understood and is typically not changed the following day. Even pulmonary hypertension and cardiac patients return very similar results two days running.

This was a very small study but large numbers of patients have been tested since the study and research is continuing. These changes are also measured only for patients still capable of intense exercise. We have no idea how strong the response might be for those whose functional capacity have declined to the point where participating in such testing is impossible - other methods are needed to investigate this.

Gene expression alterations at baseline and following moderate exercise in patients with Chronic Fatigue Syndrome and Fibromyalgia Syndrome. *Light et. al.*

Light et. al. have demonstrated several post-exercise biomarkers correlate with fatigue, pain, immunological and hemodynamic problems in Chronic Fatigue Syndrome (Fukuda definition). Furthermore, these biomarkers seem to divide patients into two subgroups which may require different treatment. These markers are very important as they may demonstrate the molecular basis of why exercise therapies for CFS patients can fail. These markers do not change just a little; the change is massive compared to controls.
NIH are funding further studies into this.

Proteomics

Distinct Cerebrospinal Fluid Proteomes Differentiate Post-Treatment Lyme Disease from Chronic Fatigue Syndrome. *Schutzer et. al.*

This study looked at cerebrospinal proteins in CFS, neurological post treatment Lyme disease (nPTLS) and controls. Both CFS and nPTLS have a large number of proteins that are candidate biomarkers, as well as a moderate number in common that are not shared by controls. This research is very early and relied on pooled cerobrospinal fluid samples. What it establishes is that proteomics may be just as useful in establishing biomarkers as genomic studies.

The 738 proteins that were unique to the CFS patients almost certainly include many that are irrelevant as diagnostic markers. However many of these proteins are involved in immune and signalling pathways, including adrenergic pathways.

Cardiovascular

Impaired cardiac function in chronic fatigue syndrome measured using magnetic resonance cardiac tagging. *Hollingsworth et. al.*

This paper is one in a long list of papers showing cardiac problems in CFS. Magnetic resonance cardiac tagging was on CFS patients and showed substantially reduced heart function using several measures including a 25% reduction in cardiac output.

The authors concluded: "*Patients with CFS have markedly reduced cardiac mass and blood pool volumes, particularly end-diastolic volume: this results in significant impairments in stroke volume and cardiac output compared to controls. The CFS group appeared to have a delay in the release of torsion.*"

Why are people with reduced heart function being persuaded to do graded exercise (GET) when their cardiac function is neither adequately tested nor monitored?

Abnormal impedance cardiography predicts symptom severity in chronic fatigue syndrome. *Peckerman et. al.*

This study suggests patients with more severe CFS may have diminished cardiac output, which may affect ability to carry out activities of daily living and possibly compromise blood flow to vital organs. Significant predictors of reduced cardiac output included PEM, joint pain, and other important signs and symptoms of ME/CFS.

Combinations of Bio-markers

The issue of diagnostic criteria is important in determining if a biomarker is reliable, although to some extent a good biomarker could reinforce or undermine any particular definition. There is a need for old bio-markers to be re-validated under any new definition, and new bio-markers are required. It is likely that eventually a model of ME pathophysiology will dominate and this will lead to examination of key pathways. One or more highly reliable biomarkers might then be identified that could lead to a diagnostic test and several candidate diagnostic tests are currently being investigated.

One interesting direction for advancing biomarker research could be to examine clustering of biomarkers. While reliability of current biomarkers is just short of acceptable criteria to be diagnostic it may be that subsets of biomarkers taken together are diagnostic for specific subgroups. This needs further research.

Another issue that affects ME research is the problem of definitions and biomarkers. Without adequate bio-markers the development of good definitions is problematic. The solution might be iterative refinement of biomarkers and definitions, with an emphasis on distinct subgroups until it can be shown that certain subgroups are just variants of the same disease state.

A combination of assays could be highly diagnostic of CFS, but we don't know which assay combinations would be optimal at present. Using biomarkers that are presumed to relate to different mechanisms it should be possible to construct a combined diagnostic test. For example, NKCC and the Stevens protocol could be combined to increase diagnostic accuracy.

What is not clear however is what subgroups are involved. If specific biomarkers cluster together they would be strongly indicative of specific subgroups, and might qualify as a subgroup diagnostic biomarker combination. Other subgroups would then have to be evaluated for alternate bio-markers, as the pathophysiological mechanisms might well be different.

It is of interest that the Fletcher study again found that one biomarker (CD26) exists in **both** rheumatoid

arthritis (RA) **and** CFS, which indicates that markers that discriminate between these two illness may be important. The other biomarker found in RA is 37 kDa RNase L. This RNase L variant is found in MS, RA and CFS. Only in CFS is it associated with elastase.

This implies that additional bio-markers may be required to differentiate CFS from other immune disorders; the immune bio-markers being investigated are at least sometimes not sufficient to be diagnostic by themselves, even in principle. A secondary implication is that the research is still not focusing on any primary causal mechanism.