Wrap up Summary of Biological Research Presentations

IACFS/ME 11th International Conference, Anthony L. Kormaroff, M.D. - Sunday March 23, 2014

Transcript of remarks + highlights of each section (in boxes) + reference to presentation; Numbers at beginning of each section represent time it appears in audio recording available at: http://www.youtube.com/watch?v=nyyjRdbvPj0

Questions addressed by many of the presentations/research
⇨Is there evidence of objective underlying biological abnormalities?
⇨Could these abnormalities theoretically explain the symptoms?
⇨Do these abnormalities correlate to the symptoms?

2:20 I will try to distill down to some simple slides the data and the conclusions from the data that from this meeting impressed me the most. This is one person's view of the highlights of the meeting, Inevitably there will be very good work that I don't mention. I really tried to find themes that spanned one presentation to the next and that influenced heavily my choice of highlights.

I begin by saying, the questions addressed by many of the presentations, even though the work they were doing was entirely different, the general questions addressed were:

Question 1– In an illness that is defined exclusively by a subjective group of symptoms, is there evidence of *objective underlying biological abnormalities*, and why?
Question 2 – Could those biological abnormalities *theoretically* explain the symptoms?
Finally – Do the abnormalities, *in fact*, correlate with the symptoms?

We will come back to those themes repeatedly.

Huge new CFS Databases and Biosample Banks

One of the highlights of the meeting was, for me, not the results of any particular study, but the evidence that we now have a group of different huge chronic fatigue syndrome databases and biosample banks.

- There is the longstanding work by Dr. Klimas and Fletcher, now at Nova Southeastern University and the University of Miami and the Miami VA, a huge database of clinical information and biosamples.
- You have, for the first time at this meeting, the presentation of a new very large effort at Stanford Medical School, down the road, to build a database of clinical information and laboratory samples.

- We heard about the results of the Chronic Fatigue Initiative (CFI), a charitably funded enterprise that created a large multi-centered databank and bio-bank.
- We didn't hear about, but there also exists, as the result of a large NIH multi-center trial that was headquartered at Columbia by Drs. Lipkin and Hornig another multi-centered database with clinical information and bio-samples.
- And then finally, we heard about the UK ME/CFS bio-bank that is being developed.

5:25 ***Immunology***

Elevated levels of allergy-associated cytokines and chemokines (IL-4, IL-6, IL-10, IL-13, IL-17A, CCL5) and other pro-inflammatory cytokines (IL-1α, IL-1β, IL1-RA, IL-8, MCP1, CXCL-10, MIP1α IL-12p40, TGFα) in patients ill for <3 years vs. ill for >3 years: CFI study Ability of multiple inflammation-associated molecules (including leptin) to separate CFS cases from healthy controls: Stanford studies Co-relation of inflammation related molecule levels with illness severity Size of Stanford inflammation studies (numbers studied and number of molecules examined) unparalleled, even outside CFS Elevated levels of interleukin-17 (IL-17) suggests autoimmunity may be playing a role Significant decrease in several miRNAs (miR-146a, miR-106b, miR-191, miR-223): would increase production of pro-inflammatory molecules Interferon gamma (IFN-γ) (thought to be released in response to infection) odds ratios striking

That was the first theme in the program on Friday and the highlights of that to me were the Chronic Fatigue Initiative (CFI) study which **found elevated levels of allergy-associated cytokines and chemokines (**I've highlighted them there) and other inflammatory cytokines and chemokines **in patients who had been ill for less than three years in comparison to those who had been ill for greater than three years.** It validated the sense, by this study and its design, that most of the biological action that you are going to be able to detect is more likely to be there in the earlier stages of the illness than the later stages. [*Allergy-Related Immune Signatures and Duration of Illness in CFS*: Susan Levine, Xiaouyu Che, Ian Lipkin, Nancy Klimas et al reported by **Mady Hornig**, MD. Mailman School of Public Health, Columbia University].

The second point, coming from the Stanford studies, was the ability of multiple inflammation-associated molecules, not all cytokines but even fat-cell produced hormones, (adipokines) to separate CFS cases from healthy controls
Specifically, the Stanford studies that we heard about on the first day included nearly 200 CFS patients compared with nearly 400 age and sex- matched controls. On each of the levels of 51 different inflammation-associated molecules cytokine

family, chemokines, and hormones were measured. To my knowledge, with the possible exception of the CFI study, a study of that size (number of patients) simultaneously assessing that many different molecules is unparalleled, not only in the CFS field but in many fields. Fifteen of the 51 molecules that they focused on, either distinguished cases from controls or correlated with symptom severity or both.

Here is a sample from one of Dr. Montoya's slides, can't see it very well, but this is a group of, I think 14 or 15 different cytokines that plots severity of symptoms against the level of cytokines and I think you can see that for most all of these cytokines **as the symptoms became more severe, the level of the molecule was higher – a linear correlation between the molecule and the symptom**. It doesn't mean that the molecule is causing the symptom but it could mean that. ["*Circulating Cytokines in ME/CFS Patients reveal a Novel Inflammatory and Autoimmune Profile"*: **Jose G. Montoya, MD …** M Davis**,** Stanford Symposium, March 19.]

Another way, and this also doesn't project well, Jarred Younger and Dr. Montoya's center did a study that linked, tried to look for associations between the various cytokines measured, and the startling result, at least to me, was the primacy of **leptin**. Leptin is a hormone discovered, about 1994 that is made by fat cells and that diminishes appetite. It has a powerful role in weight regulation and appetite control in humans and animals. That that molecule should be so closely tied to immune system molecules, was striking and that it should so predominately correlate with the levels of fatigue in the CFS patients was a novel insight, the meaning of which I am sure many groups will be pursuing. Leptin levels were highly correlated with symptom levels, particularly the fatigue. [*Daily Fluctuations of Cytokines in ME/CFS Patients*: **Jarred Younger, MD** Stanford Symposium, March 19.]

Another highlight of the Stanford study, also found by the CFI study that **Dr. Hornig** reported is **elevated levels of interleukin 17.** This is the prime mover of the so-called TH 17 immune cell that has been very strongly linked to many human autoimmune diseases – or more precisely, an elevated level of IL-17 of the TH17 cell, compared to what are called, T regulatory cells makes the risk of many autoimmune diseases worse and it suggests that autoimmunity may be playing a role in CFS.

Significant decreases were reported in several micro-RNAs that control the production of pro-inflammatory cytokines, that is the decrease in the micro-RNA would tend to increase the production of pro-inflammatory cytokines, which is what was found by the studies so it is internal biological confirmation of what you would expect to see if these studies are really valid.

And then finally, there were to me striking results about one particular cytokine, interferon gamma.

I want to take a brief side tour into a statistical term, odds ratios, for those who are not familiar with it. Odds ratio compares the frequency of a finding, any finding, in

one group, like people with CFS, versus the frequency of that finding in a comparison group, like healthy controls. The odds ratio is reported as a number with a confidence interval associated with that number, the lower limit it could be and the upper limit it could be, 95 % confidence ratio. If you have an odds ratio of 1.0 it means that there is no difference in frequency between the two groups that you are comparing. If the confidence interval spans 1.0 it means the difference is not statistically significant. Most of the odds ratios that you see reported in the literature on most conditions and most of the odds ratios you saw at this meeting fall into the range of between 1 and 5. It is not very common to see odds ratios in large studies that go above 5. It means a 5-fold greater chance of some finding in one group compared with the other. So that is by way of background.

What we had presented to us buried in a set of very complex tables in one presentation and found in a poster presentation of another paper, we had two striking odds ratios from Dr. Hornig's group on interferon gamma. In the Chronic Fatigue Initiative study, 200 cases compared those who were ill for less than three years with those who were ill greater than three years the **odds ratio of interferon gamma was 117** with the upper confidence interval being 1,872. I have never seen an odds ratio like that. I have never seen an association that so strongly discriminates one group from another. And then that same molecule interferon gamma was found to correlate with cognitive impairment comparing a highly cognitively impaired subgroup to a low-impairment subgroup with an **odds ratio of 67**. So this is a striking statement to me about this molecule and its possible role in this illness.

Interferon gamma, it is commonly thought, is released by a viral infection or an infection by intercellular bacteria. So it suggests, but by no means proves, that in CFS there may be an underlying infectious agent.

14: 33 ***Basic Biology***

Telomeres in CFS patients shorter than in healthy

We heard another interesting finding, that's sort of basic biology and doesn't fall into any of the categories at the meeting, about telomeres. Telomeres are the ends of the chromosomes, where they all come together to be tethered together at the opposite ends of the chromosomes. Over the last 30 years it has become clear that those telomeres are not just structural elements, that their length affects the viability of the cell. With every cell division the telomere shrinks a little bit and so therefore short telomeres mean this is a cell that has divided many times and is relatively older. When telomeres get to be short enough all of a sudden the cell dies. So short telomeres are a marker for cellular aging. And short telomeres are increasingly being shown to predict an increased vulnerability to a variety of "diseases of aging" including atherosclerosis, neurodegenerative diseases, several malignancies.

One demonstrated association with short telomeres is perceived life stress. That work was done here in San Francisco at UCSF by Elizabeth Blackburn, who won the Nobel prize four years ago for her work on telomeres. She showed that when people are under stress, the group she first studied were parents caring for sick kids, the parents who were under the most severe stress and perceived this as a major stressor were likely to have shorter telomeres.

So Dr Rajeevan from CDC showed us good data that telomeres are shorter in patients with CFS. By no means a finding unique to CFS but it's a finding that suggests that there is an underlying biological process that is making patients with CFS age a little faster. [*A Genome-wide Analysis of Differential Methylation Associated with Chronic Fatigue Syndrome,* **Mangalathu S. Rajeevan**, Virginia Falkenberg, Irina Dimulescu, Elizabeth Unger, CDC]

16:49 ***Virology and Infectious Agents***

CFI study reported no microorganisms *in serum*
More work still to be done to look at circulating white cells, brain and gut
Marked differences in frequency of enterovirus antigens and nucleic acid between CFS patients compared with controls [Entroviral Ag (VP1) CFS 259/314 (82%) vs 9/47 control (19%): Enteroviral dsRNA CFS 198/314 (63%) vs 5/47 control (10%)]
Mice injected with VP1 + gastric tissue samples "indicative of enterovirus" were actually infected
Confirming work needed on all enterovirus work

The Chronic Fatigue Initiative Study reported the results of a really intensive molecular screen for infectious agents. And the first report was of "agents in serum" and they found basically no evidence of microorganisms present in serum. The questions to be addressed in the future are: What about in circulating white blood cells – the cells themselves, not the serum around them? What about in other organs and compartments, particularly the brain and the gut (the microbiome)? Those are studies for future time, several of which are underway.

Dr. Chia reported on enteroviruses again at this meeting as he has in the past. The expansion, the latest summary of data from a remarkable report and a remarkable amount of work, on enterovirus antigen and nucleic acid found in biopsy samples from the stomach in cases and control subjects. He found very marked differences in the frequency of both antigen and nucleic acid in the CFS patients compared with the controls. He then also reported that when you took the biopsy specimens that these tests suggested contained enterovirus and injected them into mice that you found, when you sacrificed the mice, evidence of enteroviral infection -- a virus in the mouse indicating that this thing that lit up looking like it might be an infectious agent on the biopsy tissue actually produced an infection in another animal. To me these results are very impressive but it is also depressing to see that, to my knowledge, no academic enterovirologist has sought to reproduce this, not even in

bulk to take the samples that already have been collected, at enormous effort, by Dr. Chia and test them themselves to see if they get the same results that Dr. Chia does. It is a great shame and I hope it changes.
[*Pathogenesis of chronic enterovirus infection in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) – in vitro and in vivo studies of infected stomach tissues*: **John Chia, MD**, Andrew Chia, David Wang Rabiha El-Habbal. EV Med Research (and a further study)]

19:26 ***Public Health Epidemiology***

CCHS shows 411,500 of 35M Canadians (1.1% have CFS) Chronic Fatigue Initiative (CFI) survey of nearly 1000 patients shows typical illness 15 years, 1/3 experienced a remission, lasting 1 year (median) but which did not normally persist CFI study shows rest/exercise/diet more likely to reduce symptoms than medications or alternative treatments CFI data shows CFS patients more likely than controls to have co-morbid illness

The Canadian Community Health Survey (CCHS) was massaged to try to come to an estimate of the number of Canadians that have CFS and estimated about 1%. That's about 2 to 4 or 5 times higher than in the United States. It may just be that because these data come from survey and patients who weren't then subjected to physical exam and deeper interrogation to see if they might have other conditions that cause fatigue it would have been a lower number. [*Prevalence and Health-related Characteristics of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS), Fibromyalgia (FM) and Environmental Sensitivities/Multiple Chemical Sensitivity (ES/MCS): Results from the Canadian Community Health Survey (CCHS) 2005, 2010 and 2012*: **M. Parlor,** National ME/FM Action Network, E. Halapy.]

The Chronic Fatigue Initiative (CFI) epidemiology study surveyed nearly a thousand patients from multiple centers and ascertained that about a third had experienced at least one remission during what typically was a fifteen year history of illness and that the remissions had lasted, on average, about a year but in most people had unfortunately not persisted. It is the latest study of prognosis in this illness from a long-term database. [*The Natural Course of Chronic Fatigue Syndrome: Evidence from a Multi-Site Clinical Epidemiology Study***: Dana March, Ph.D.,** Assistant Professor, Department of Epidemiology, Columbia University, Mailman School of Public Health.]

The Chronic Fatigue Initiative Epidemiology study also reported that rest, exercise and diet were the interventions that most CFS patients have found helpful in comparison to standard pharmacological care or alternative and complementary treatments. [*What Treatments Alter the Course of Chronic Fatigue Syndrome? Evidence from a Multi-Site Clinical Epidemiology Study*: **Lucinda Bateman, MD**. Adjunct Assistant Professor, Department of Anesthesiology, University of Utah.]

The CFI study finally reported that CFS patients were much more likely to report many co-morbid illnesses than were control subjects. Actually they didn't report that comparison to control subjects but it is true from the CFI data. [*Chronic Fatigue Syndrome and Comorbid and Consequent Conditions: Evidence from a Multi-Site Clinical Epidemiology Study*: **Salima Darakjy, MPH, Ph.D. Candidate**, Department of Epidemiology, Columbia University.]

21:20 ***Case Definitions***

Case definitions need to be precise: the way in which each component is defined needs to be specified – e.g., how severe is "severe" Empirically derived case definitions are superior to consensus derived case definitions as they are better at sub-grouping and predicting end points.

Lenny Jason magnificently led a discussion of what you want in a case definition and was, I think, critical, fairly critical in my view, of the current case definitions that are out there. The main point he made (as I took it) was that case definitions need to be precise, if they are really going the job that you want them to do. You don't want to just list a group of symptoms or physical exam findings. You want to tell the person might use that case definition how you actually define that symptom, when is the severity of that symptom high enough that you want to check the box. That sort of thing needs to be done more. Several groups have attempted it but is its honored in the breach in CFS research.

Empirically derived cases definitions, he argued, are superior to consensus-derived case definitions, such as most of them that exist today are, because they become better at defining sub-groups if you just collect a lot of data very rigorously and use statistical techniques to look for sub-groups. Don't impose your biases on it, just ask do the data tell you anything, are they talking to you? Oh, this symptom and this symptom correlate together very tightly in most patients. That has been done and needs to be done more in defining sub-groups, cause I believe most of us believe CFS, if not a group of different but similar diseases, is a disease with a bunch of sub-groups that may, in fact, respond to different treatments.

And finally, empirically derived case definitions are going to prove to be better at what you want a case definition to do which is to predict some end point like a prognosis or a laboratory finding that is thought to define the pathology of the illness.

23:30 ***Exercise "Provocation" Session***

On a single exercise study CFS patients do not consistently perform below the norm They have abnormal VO2 max the second day, which is very unusual, not only for health controls but also for people with heart disease and lung disease

Some CFS patients have a low peak oxygen extraction rate relative to cardiac output implying a metabolic defect

This was really a session about exercise studies. The main concept behind these studies was that if patients tell you that a particular stressor makes them feel worse then the time to study them to see if you can identify the underlying biology of the illness is at a time when they have been subjected to that stressor and they are likely feeling worse. And in fact, a double dose of that stressor should bring out the underlying pathology even more.

And so what we heard from several groups was that although on a single exercise study CFS patients did not consistently perform below the levels of healthy controls of which there are abundant normative data, **when those patients were exercised 24 hours later again, you saw all sorts of abnormalities popping out**. And you did not see that, not only in healthy control subjects, but you do not see, we were told, that degradation in performance on the second test in people with heart disease and lung disease who are the main people who get this kind of exercise study. So it does not say that this kind of degradation on the second day is unique to CFS but says in their long experience as exercise physiologists they haven't seen it before and that is probably telling us something about this illness. [*Superior Ability of a Two-Day CPET Protocol to Detect Functional Impairment in ME/CFS Compared to Either a Single CPET, A Submaximal Exercise Test, or VO2 Prediction Equation*: **Betsy A. Keller**, Ithaca College, Department of Exercise and Sport Science, Ithaca, NY]

And then finally we heard a report that low peak oxygen extraction relative to an increase in cardiac output occurs in some patients, implying a metabolic defect, possibly a down-regulation of carbohydrate metabolism. [*Oxygen Extraction and Lactate are Low during Cardiopulmonary Exercise Test in Patients with Chronic Fatigue Syndrome*: **RCW Vermeulen**, WG Vermeulen – Van Eck and I.V. de Jong – Medvetska, Head of Research, CVS Centrum Amsterdam]

25:35 ***Pediatric Chronic Fatigue Syndrome***

High prevalence of unrecognized delayed milk protein sensitivity – changing diet may help alleviate some suffering
It appears that widely available/inexpensive laboratory tests can predict who will go on to get post-mono CFS. This may help and allow interventions to prevent.
Depression is only marginally higher in teenagers with CFS than controls

The findings that stood out for me was that there was a surprisingly high prevalence of delayed milk protein sensitivity in kids with CFS that often was unrecognized and was causing symptoms that sometimes were attributed to CFS and by identifying it and changing dietary patterns you might be able to relieve some of the suffering of kids with CFS. [*Increased Prevalence of Delayed Milk Protein Hypersensitivity Among Adolescents and young Adults with Chronic Fatigue Syndrome*: **Peter Rowe, MD.**

Colleen Marden, Samantha E. Jasion, Erica M. Cranston, Marissa A.K. Flaherty, Kevin J. Kelly, MD. Johns Hopkins University School of Medicine, Baltimore]

A second paper talked about a combination of widely available inexpensive laboratory tests that appeared to predict which young adults and teenagers with mono would go on to get post-mono CFS. To me that is interesting, we know from 15 years of research that there is a post-mono CFS and several fancy laboratory studies with sophisticated immune studies done only in certain laboratories seemed to predict who is going to go on to get post-mono CFS but those studies can't really be done reliably in most practices. The types of laboratory tests that were reported here are available to every laboratory and are very inexpensive and this may prove to be a useful finding in kids with mono and intervening in some way to prevent post-mono CFS. [*Tracking Post-infectious Fatigue in Clinic using Routine Clinical Lab Tests*: Alanna Bowie, Jeanna M. Harvey, Ben Z. Katz, Frederick Smith, Maurice R.G. O'Gorman, R. Taylor, Mary Ann Fletcher, Nancy Klimas, **Gordon Broderick**. Institute for Neuro-Immune Medicine, Nova Southeastern University, Fort Lauderdale, FL]

And then finally, the last report in the pediatric session that I thought made an important point was that when they surveyed kids with CFS for depression they found evidence of concurrent depression in only about 25% of the kids even in the months and years after they became ill with CFS. In teenagers particularly, I believe, the background point prevalence of depression is around 15 to 20%, so this is marginally higher, as you might expect, when you are suddenly hobbled with a debilitating illness but it is only in a minority of the patients. [*Does Depression at Presentation Impact on Outcomes for Young People with CFS?* **Katherine Rowe**, MJBBS, M.D. FRACP, Royal Children's Hospital, Murdoch Children's Research Institute, Melbourne, Australia.]

27:50 ***Brain Research***

qEEg changes in CFS allow differentiation from healthy controls (50 CFS patients vs. 50 controls) – reduced alpha (peak alpha wave frequency – PAF significantly reduced over 58% of cerebral cortex, p=0.006) and reduced beta, increased delta particularly in frontal lobes and limbic areas
Degree of abnormality shown in qEEg correlates linearly with degree of the symptom (either pain or fatigue), as assessed by two validated fatigue instruments (For PAF R^2=0.90, p<0.0001)
PET (positron emission tomography) scan shows neuro-inflammation with intensity of signal correlated to cognitive impairment

We heard from the Zinns at Stanford about quantified EEG (qEEG) studies, brain wave studies, that demonstrated a remarkable ability to distinguish CFS patients from healthy control subjects and I'll cite that data in a minute. [*EEG Peak Alpha Frequency is Associated with Chronic Fatigue Syndrome: A Case-Controlled*

Observational Study: **Marcie Zinn, Ph.D**., Mark Zinn, MM, Jose Maldonado, MD. FAPM, Jane Norris, PA-C, Ian Valencia, BS] We heard from the Osaka and Kobe, Japan studies of an activation of key brain immune cells microglia and astrocytes using PET scanning that say something about the underlying biology of the illness. [*Neuroscience of Fatigue and CFS/ME by Using PET Molecular Imaging and Functional Neuroimaging*; **Yasuyoshi Watanabe**, Masaski Tanaka. Kei Mizuno, Akira Ishii, et al. RIKEN Center for Life Science Technologies, Kobe, Japan.]

The quantified EEG studies at Stanford, we heard about this morning, involved 50 CFS patients, 50 matched controls and there were three findings that stood out. One was that peak alpha wave frequency was significantly reduced over most of the cerebral cortex in patients with CFS, beta wave activity was also reduced, delta wave frequency was increased particularly in the frontal lobes and the limbic areas of the brain. These **abnormalities were present and clearly distinguished CFS patients from healthy control and the degree of the electroencephalographic abnormality correlated linearly with the degree of the symptom, whether it was fatigue or pain**.

These brain wave abnormalities were thought by the Zinns to indicate likely disruptions of information transfer across cortical networks and inhibition of ascending arousal systems. They are the sorts of things that you see in a whole host of well-documented neurological diseases and you don't see in healthy people.

The PET scans that have been done by the Japanese groups in the past have demonstrated reduced cortical blood flow, reduced glutamate (an important nerve transmitter), reduced serotonin transporter and increased dopamine biosynthesis. At this meeting they used PET scanning for a new purpose. There is, and you have heard it repeatedly in the last three days, a theory that CFS, at least in some people, might reflect ongoing activation of immune cells in the brain, not in the periphery only, but in the brain and that if you could find evidence of a chronic immune activation state in the brain then all of the cytokines that are elaborated(?) as part of that chronic immune activation could easily explain many of the symptoms of CFS.

But how do you measure immune system activation inside the intact brain of a human being without some invasive approach? Their imaging technology included a ligand for a particular protein that is thought to be specific to the activation of the immune system cells, the microglia cells and astrocytes and it showed, in a small study (9 patients, 10 controls), **there clearly was increased signal, evidence of immune activation in these cells in multiple areas of the brain** (not all areas but many areas) areas that have also been found to be abnormal in many other kinds of imaging studies. **The intensity of the signal correlated with cognitive impairment again, not just finding an abnormality but demonstrating that it correlated in degree with the degree of important symptoms.**

32: 15 ***Conclusion***

Biologic abnormalities found in CFS *DO correlate* with symptoms and *DO theoretically* explain symptoms

So in summary, as I said at the beginning many of the studies asked the question, in an illness that is defined entirely by subjective symptoms is there evidence of underlying biological abnormalities? I think the evidence clearly is yes. Could those abnormalities theoretically explain the symptoms? You have heard multiple investigators say, "Yes absolutely they could, from what we know about those abnormalities in other conditions and diseases." And then finally, do the abnormalities that theoretically could correlate to those symptoms actually correlate in patients who are reporting their symptoms at the time they are being imaged or studied? And the answer was, repeatedly, yes.

In Summary Case-control studies comparing patients with CFS to both disease comparison groups and healthy control subjects find robust evidence of an underlying biological process involving: ⇨the brain and autonomic nervous system ⇨immune system ⇨energy metabolism ⇨oxidative and nitrosative stress The illness is not simply the expression of somatic symptoms by people with a primary psychological disorder

33:07 So I would conclude that the last 3 days and the science that has been reported over the last 20 years shows repeatedly that when patients with CFS are compared to various comparison many groups, healthy subjects, other fatiguing diseases what you find is robust evidence for an underlying biological process that involves the brain, autonomic nervous system, immune system, energy metabolism system and although we didn't hear a lot about it at this meeting oxidative and nitrosative stress.

In summary, the illness is not simply the expression of somatic symptoms by people with a primary psychological disorder. It was a fair question 30 years ago to ask whether people with these symptoms might not just be expressing psychiatric stress, amplfying normal body sensations or even fabricating for secondary gain. It was a fair question 30 years ago but today it is no longer a fair question.

Thank you very much.

34.43 ***Questions***

35:33 Nancy Klimas -- Do you think that we are at the point where we can use biologic markers for this disease clinically?

AK – I don't think we have evidence yet that anyone of these biologic markers is sufficiently sensitive or specific to constitute a diagnostic test. Several are close and one of those EEG figures showed nearly a perfect separation, if I remember, between cases and controls, so maybe that day is coming but I am not sure that we have yet something we can call a diagnostic test.

36:12 Nancy Klimas – How about clinically to monitor or to pick a drug? How about for managing and following, therapeutic decision-making?

AK – I think for several including dopamine being (downregulated) I believe could lead to a trial but it would have to be a randomized trial of interventions that ramp up dopamine production (for example). I think where you find a result that has a therapeutic implication that ought to be tested in a randomized trial. I'm less comfortable with saying the individual clinician who has available dopaminergic agent should use it in an uncontrolled setting to treat patients but I hope it would lead to controlled studies.

37:29 Q. Given the fact that Japanese group found neuro-inflammation in the hippocampus, hypothalamus and the pons, which is anatomically part of spinal cord -- neuro-inflammation, not classic inflammation, but it is still inflammation, isn't that encephalomyelitis?

AK – Yes, if it were confirmed by multiple other investigators it would for me say, there is a low-grade chronic encephalitis in these patients. That the image we as clinicians have of encephalitis as an acute and often dramatic clinical presentation, that can even be fatal, may have blinded us to the possibility that there may be an entity of long-lasting, many years long, cyclic, chronic neuro-inflammation and that that is underlying the symptoms of this illness. It is entirely plausible, these data are consistent with it but I would feel more strongly if other labs using the same technology came to the same result.

38:47 – This question may be more for Dr. Klimas – in terms of the cytokine changes that we are seeing consistently over various labs all over the world, as well as NK cell function how can we interpret the studies in Norway with Rituximab? How does that make sense, from a bio-clinical point of view, the improvement in certain patients?

NK answers – So I think we have all the evidence we need for NK cells to call that a biomarker of this illness and need not continue to hammer down on that one. Tony has a paper in 1987? (AK interjects, I thought it was in the Jurassic era) other papers since …rest of answer pretty inaudible .. [do not rely on it] 40:43 …

biomarkers I think are reasonable places to define subgroups particularly as we move for therapeutics and very specific …. We have a reliable way, through separate measures to confirm our observations to be certain we have the right group…

41:25 Q. An advocacy type question – Thank you, Dr. Kormaroff, for your summary. …We can't seem to declare we have biomarkers. Until we accept a universal case definition for this disease that may always be the case. We might not have as many subgroups for this illness as we think because we are not replicating science and we'll never find targeted drugs until we all admit that we need to adopt a single case definition for example Canadian Consensus Criteria would be a good start. So I would like you to address that and also, you are so wonderful in all your summaries of biological abnormalities on the CDC website, here and everywhere else and advocates were astonished two or three days ago with your interview that appeared in the Monterey Herald that talked about wonderful things about the biological abnormalities but it ended with you saying CBT and GET are really good treatments. So I wonder if you could respond to those.

AK – I wonder if I could just respond to the "you are so wonderful" part. {laughter} I don't want to get dragged into a discussion of the role of CBT because I know it is a polarized issue. It was not discussed at this conference. I will be happy to talk with you individually about it.

In terms of whether all investigators should use a common case definition. Yes that would be the ideal. I think the problem is not all investigators agree on what is the best and easiest to implement case definition. And part of the problem of choosing any one is that if there is a lot of literature already that uses another case definition besides the one that you regard as currently best that it will be hard to compare the results of any studies going forward with the published results of any research that already exists. So I do not think that there is a good answer to that question. And if there is I don't have it.

Q. 44.24. That was an excellent summary and I would love to have this summary available to present to various people that were not able to be here who might find it interesting, various committees, countries. Is it possible to get your presentation put up online?

AK -- We have two phones here that are digitally recording my remarks. I am going to make pdfs of my slides available for the CFIDS Association to sync to my voice, so I hope on their site, I hope on the IACFSME site and any other sites that would like to have it, it's made available.

Q. 45:23 (Last two questions, somewhat inaudible but deal with clinical trials (one question referring to poster presentation using material from a bio-bank asked by Mary Anne Fletcher and another on animal models).