Øystein Fluge, MD, PhD | Keynote Address | Clinical Trials and Metabolic Features of ME/CFS
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https://www.youtube.com/watch?v=FKkWb4PsL_8&index=6&list=PLl4AfLZNZEQPxjqF4ojAO3wdCFMeriNBK

Ron Davis:

Last year I tried to get Dr. Fluge here but the timing wasn't right for him to be able to be here. So this year we organized the symposium around his schedule. [laughter] So it's really a delight to have him here. When I read his first paper I was incredibly impressed because Dr. Fluge is an oncologist. What in the world is he doing with chronic fatigue syndrome patients? And it's just simply because, I think, he basically accidentally met one and realized it was a serious problem and he had ideas about how he might help them. So he took that on. And not only that but trying to understand the disease meant that he also had to do a lot of things that were very remotely connected to oncology and he did that. The last meeting in London when I was there I think the most impressive of all the talks came from Norway. And Norway doesn't account for more than 50% of the population of the world. [laughter] They've done a fantastic job. I think it was all because of Dr. Fluge's initial, that Norway has now become a leading country in their research in this field. It's really a great pleasure to have you here. Thank you very much.

[Music]

[Applause]

Øystein Fluge:

Thank you, Ron, for those very kind words and then thank you for inviting me and from the OMF for inviting me. Truly it has been three wonderful days to take part in this working group meeting. For me as a clinician also trying to do some translational research, it's been so interesting to talk to some of the most excellent researchers in the field. Thank you all to the researchers who came to the meeting also to telling me and learning me. I have a lot to think about when we go home on the airplane and when we meet our colleagues at home trying to move the field forward. So what I will do in the next 25 minutes is to tell you a little bit about our clinical trials and a little bit about the laboratory work that we have done, some of the metabolic features of ME/CFS that we see. I'll call it a struggle for energy because that's something I think that the patients described to me. It's like they struggle.

This is just a picture of some of our group on Bergen and to the right you can see the list of the study some trial centers that has been taking part in the clinical studies and some of the groups that we have collaborated on so far. I'm happy to say that I have made several connections

these three days with other researchers that we will continue to work together with. In Bergen we tried to combine work on clinical trials in ME/CFS and trying to combine that with translational work using samples from patients' blood samples mainly taken at baseline and taken at serial intervals throughout the trials to build a biobank. We came to increase the understanding of disease mechanisms and try to be part in the efforts to develop rational treatment. So it's a very tight cooperation between the clinic, the clinical trials, the biobank, and laboratory work in which we have a growing a group of scientists also in Bergen at the universities taking part in this.

The focus for this talk is to give you a brief overview of some of the clinical trials and also a little bit on our translational work. And I must tell you that the Rituximab trial and the cyclophosphamide trial, they have not been published yet so I'm not able to give you details from these studies. I apologize for that but it's not been out and published yet.

So why did I get interested in ME/CFS? That was back in 2004 and the next few years we had observation of patients with longstanding ME/CFS who got cancer or lymphoma and who independently came to us and told us that this is doing something to my ME/CFS disease. And the first time maybe I just registered then, okay. But then it happened again and I really got interested to see if this was something. This was an observation that could tell us something important about the disease.

In the first years we had only two such observations. One was the patient in 2004 with Hodgkin's lymphoma who received chemotherapy, including the drug ifosfamide which is very similar to cyclophosphamide. In 2004 we also saw a patient with non-Hodgkin's lymphoma—2007, sorry—a patient with non-Hodgkin's lymphoma who received chemotherapy with cyclophosphamide together with the monoclonal antibody Rituximab for lymphoma. Both of these patients reported that the major change in their ME/CFS symptoms and we decided to the track of both of these leads. Could it be the chemotherapy? Could it be the B cell depletion therapy with the Rituximab that made this difference to the patients? So we decided to investigate those in separate clinical trials, small trials initially, just to get some thoughts about [whether] this could be something worthwhile pursuing.

I can tell you that if you look back now 2004 to 2018, we have seen a total of eight patients who have independently reported benefit from cancer treatment on their ME/CFS – patients with long-standing ME/CFS. And most of these have actually received chemotherapy with cyclophosphamide or ifosfamide. To give you a brief overview of the previous Rituximab studies we first did a small pilot case series in 2008 and 2009. We just gave three patients a single infusion with the Rituximab and followed their clinical course trying to register what happened to them. This had a transient symptom improvement. Then we did a small randomized phase 2 study which was published in 2011. 30 patients, we followed them for 12 months and they had two infusions of Rituximab for the two first weeks and then just followed. In this study the primary endpoint which was at four months, it was negative—there was no difference between the placebo and the Rituximab group at four months. So the study was negative. But there was

separation of the curves later in the study between 6 and 12 months at the secondary endpoints. So we were unsure if this was a sign of clinical activity that we shouldn't [*inaudible*] but we decided to do an open-label phase 2 study with Rituximab maintenance therapy. That was an exploratory study—trying to get more knowledge about the mechanisms and to prepare for a later randomized study.

It's a big limitation when you do an open label study in this disease because you cannot have any proof for a clinical effect. But you can have ideas about the tolerability and the feasibility, look at the response rate, and possible toxicity. But it's no proof of effect. So we did this study with 29 patients and we gave them 6 infusions during the first 15 months and follow them for 3 years. I'll show you in the next slide a little bit just to sum up this. But to give you the history of what we have done so far—we have also studied cyclophosphamide because in fact that was what most of the patients had gotten and had independently reported benefit to us in our cancer clinic. In cyclophosphamide, we first did a case series of three patients—unpublished—I'll tell you a little bit about that. And then an open-label phase 2 study, also without the placebo group which is the limitation and follow them for 18 months, now up to 30 months with 40 patients. This is what I will tell you a little bit.

Just to show you some of the data from the open-label phase 2 study what is called the KTS-2 trial in which patients had the rituximab maintenance therapy. You can see these are the SF-36 which is a questionnaire where they can give you information about physical function. As you can see in the upper panel: vitality, bodily pain, social function, and mental health. The red ones are the patients that really seem to respond clearly. The green ones are the moderate responders and the blue ones are 10 patients with no response at all.

You can see from the curves, I don't know which side to point on, but if you look at the curves the time axis is on the x-axis and you can see that for the first three months there is absolutely no change in this health-related quality of life parameters. But then you have increases especially in the group that seems to respond and in the time window 15 to 30 months they reach high values for this sf-36 course. The group which was half of those included both for physical function, vitality, social function, and bodily pain. So was this a true effect of the drug or Rituximab? We can't say because it's an open label study. There are major limitations such as the outcome measures which were mainly self-reported in this study. Could it be natural variation of symptoms over time, placebo mechanisms, or a selection bias that we had included patients that although fulfilling the Canadian criteria by chance shouldn't be representative for a larger group? You don't know for sure so we were discussing is there a subgroup of M.E. patients that benefit from B cell depletion therapy? And we interpreted the observed responses or improvements are probably related to the intervention.

We have no responses before four months which is not typical for a major placebo effect. But as we know from oncology and also from other clinical trials in autoimmune diseases, such studies have shown that it's very important to do a larger randomized and placebo controlled trial before you can make conclusions—especially maybe in diseases such as a ME/CFS with

subjective and self-reported outcome measures. But no doubt that the patients improved in these studies because these questionnaires, the SF-36 forms, and measurements of physical activity level. For instance by armbands measuring steps per 24 hours all showed the same: patients improved. But what was the cause of the observed improvement?

That's why we have performed RituxME which is a multicenter, national, randomized, double blind, and placebo controlled phase three trial with either Rituximab or placebo, six infusions in total during the first 12 months and follow-up for 24 months, 151 patients randomized. And the code for intervention was relieved in October 2017. The inclusion criteria were Canadian Criteria, age 18 to 65, and we had a lot of questionnaires to get several endpoints. The primary endpoint was self-reported based on registrations of symptoms by patients every two weeks. But we also tried to measure physical activity objectively by Sensewear armbands. And we did sub-studies trying to evaluate endothelial function, cardiopulmonary exercise tests, and gastrointestinal function both at baseline and repeat that after 18 months to see. Now as I told you the RituxME trial is being considered by a journal so I cannot give you details of the outcome. But we have stated in public before for ethical reasons that the study was negative, that we couldn't find a significant difference between the Rituximab and placebo groups. And the reason why we did that was because we knew there were off-label treatment in the world patients taking the treatment outside clinical trials. I cannot give you any more details about the study now because it has to wait for final consideration by the journal.

So I will tell you a little bit about the Cyclomide Trial, which is an open-label, phase 2 trial with cyclophosphamide in ME/CFS based on observations of several patients with longstanding ME/CFS who got cancer and reported benefit on the symptoms. We did first a pilot study in 2014. Gave three M.E. patients six infusions with four weeks intervals. One of them was a non-responder, one patient responded for approximately one year, and one severe patient which was bedridden and had less than 300 steps per 24 hours, she had a long lasting response for almost four years now and can still walk five to ten kilometers several times each week. She is not completely healthy but she seemed to improve. So that was a pilot study and we have therefore performed an open label phase two trial. And that's again an exploratory study trying to learn something about the mechanisms and to see for the feasibility so that we can be able to design a new randomized trial. That's our hope.

So this is a limitation. Forty patients were given six cyclophosphamide infusions with four week intervals and been followed now for almost three years. Any questions? This we ask is cyclophosphamide associated with the clinical benefit? What are the side effects and the toxicity and the tolerability? Cyclophosphamide is cytotoxic drug that's used in oncology but it also had broad immunomodulatory properties so it's also used in rheumatology modulating inflammation. So the inclusion criteria were like this: age 18 to 65 years, they had to be disease duration at least two years, and we did not include mild patients. I don't mean that mild ME/CFS is not a big problem also. But we tried to take patients that were either moderate or severe or moderate to severe ME/CFS but not those that were unable to care for themselves at all, not the very severe group. Forty patients and this is an exploratory study. Twenty-six

patients were treatment naive and 14 had received rituximab previously. The mean age was 42 years almost and half of the included patients had been sick for a long time with the disease duration more than 10 years. And the outcome measures were similar to what we did in RituxME except that we measured steps more often: both at baseline and nine, 12, 18, and 24 months.

So the data have been analyzed and the manuscript is in preparation. I can tell you that the response data are interesting but we cannot state that yet because it's unpublished. The toxicity has been acceptable but with discomfort for patients due to the cytotoxic drugs, especially the first four months. So cyclophosphamide is a cytotoxic drug that can affect the fertility. There's a 15% chance of premature menopause in women more than 35 years from this schedule. And there can be rare but severe and not negligible side effects which may occur. We haven't seen this in this study but if you treat a large enough number of patients you can see more severe side effects. So we will have to be very cautious in interpreting the response data. We will see that when we publish it. And I think that cyclophosphamide maybe should not be used outside clinical trials for any CFS until we have done a randomized study and shown if there is a true effect of the drug.

So what we're doing now is the extensive laboratory investigations using serial samples from patients in this trial, trying to strengthen or weaken the case for a new randomized trial where the cyclophosphamide versus placebo in M.E. Is there a true effect or is it a placebo mechanism? Is it natural symptom variation over time? These are very important questions that we need to address. And we have been wondering due to the still unknown pathomechanisms of ME/CFS, an unknown etiology, is it really the more unspecific immune modulation by cyclophosphamide which you know affects different lymphocytic subsets both T-, B-, NK-cells. Is that in fact an advantage in ME/CFS compared to Rituximab which is a very broad B-cell, narrow and specific b-cell depleting agent because we don't know yet the precise disease mechanisms?

So for the last 12 minutes I will tell a little bit about research focus from the laboratory studies. This was a quick overview of the clinical trials and I think one advantage of doing clinical trials is that we get very carefully characterized and carefully followed patients throughout the study, standardizing of biobank sampling, registrations: everything is put in the system. I think that's it's a good thing. So what we have they have been asking ourselves based on the laboratory data that I will discuss with you. Is there, in fact, an ineffective utilization of glucose as a proper fuel for the citric acid cycle, the TCA cycle, in ME/CFS? And we have been wondering if this includes a reversible and functional inhibition of an important enzyme, the pyruvate dehydrogenase (PDH) enzyme. I'll show you what it is, just introduce you to the thought. This PDH catalyzes the irreversible conversion of pyruvate which is the endpoint of glycolysis, glycolysis to acetyl-coenzyme A which enters the citric acid cycle for fueling. And do the patients try to cope with this inhibition with an increased use of substrates that can fuel the TCA cycle independent of this enzyme, such as specific amino acids and fatty acids? And we also think that maybe the majority of the metabolite alterations that we see are secondary and compensatory to such an obstruction in a way in this central energy pathway.

An important question to me is also: what is the link between the immune system and then this alteration of the central energy pathway? We thought it was the B-cells but the case for the B-cells are at least weakened by the result of our study. So it could be the T-cells? Is it the innate immune system? How is the link between the immune system and these energy metabolic alterations? So this is very simplified overview of the metabolism of carbohydrates to energy. Sugars which is carbohydrates metabolized through the glycolysis to pyruvate and this enzyme PDH is in the mitochondria. With the green square that you can see, we are suspecting that this is impaired probably as a part of a bigger picture some kind of metabolic switch or change that many of the other researchers have also discussed in these few days which is a very interesting. Some kind of modulation of the metabolic pattern. And we know that if you have lack of oxygen, you can have lactic acid and if you have oxygen present the mitochondria, you can have TCA cycle activation and energy, which is ATP. And you can see that the specific amino acids or fatty acids they can fuel the TCA cycle downstream of this PDH enzyme. So a little bit more complex picture of the same thing: the central energy pathway glucose taken up by the cells converted in glycolysis into pyruvate which is converted to acetyl-coenzyme A, entering the TCA cycle and giving energy in the presence of OXPHOS, our oxygen, into ATP. And you can see again amino acids, fatty acids, and ketones or substrates for the TCA cycle which are not dependent on the PDH enzyme.

So we've first focused on amino acids and there has been several previous studies looking at amino acid metabolism in ME/CFS. Several of those were presented at the meeting. We have several studies from the Australian Group showing that you have reduced levels of several amino acids both in the study from 2007, 2012, and 2015 from Christopher Armstrong and his co-workers. Naviaux's paper from 2016 showed that there were reduced plasma levels of branched amino acids, proline and arginine. And we had our paper from 2016. Metabolic profiling indicates an impaired PDH function in ME/CFS. What did we look for first? We looked for all the amino acids in serum and the derived amino acids that we can measure. And we hypothesized that this disturbed central energy metabolism can cause changes in the serum amino acid profile. And we know that serum amino acids, they are a little bit – [they] can fluctuate and they are dependent on diet and diet habits. And a little bit can vary over time so we have also measured them several times during the follow-up in the studies.

But what's a good thing about the clinical trials? We have the data from disease severity, disease duration. We know the physical activity measured by steps. We know the quality of life and we analyzed 153 non-fasting patients and 103 healthy controls. And what we saw was that the sum of all the amino acids that can convert to pyruvate. We looked at how can amino acids be used for energy when they are catabolized. We saw that there were no differences in, especially in female patients, there were no differences for the amino acids that can enter at the pyruvate level but for amino acids that can enter at the acetyl-coenzyme A level or that can replenish the intermediates of the TCA cycle, they were significantly lower in female patients. [This] may be indicating that these female patients use these amino acids as sources for energy. That's what we're thinking. In male patients, we saw that there were increased level of amino acids called 3-methylhistidine which is a marker of endogenous protein catabolism. Men have

usually more muscles than women and may use muscle catabolism as a source for energy. So we hypothesized that there seems to be an increased use of alternative substrates for a TCA cycle, oxidation and energy. And is this, possibly as part of a bigger picture, due to an obstruction in the effective glucose oxidation including a functional and reversible obstruction at the PDH level?

And then we try to measure the enzymes that are known to inhibit the PDH enzyme. We measured that in blood cells at the RNA level. And we saw that these enzymes PDK one, two, and four, they inhibit the PDH enzyme. They were upregulated. So it seems to fit with the hypothesis. So there could be other explanations for this. It could be fasting or diet habits. But we saw that the triglyceride levels they are equal ME/CFS patients indicating that there's no big difference in prandial, postprandial status. And we see similar findings from other research groups also present at the meeting. But it is important to know that this is a cross-sectional study design therefore it's suggesting [a] mechanism. Some people claim that this is just because they are lying down, or such as the rest. But when we look for these associations, for instance, to the physical activity level or disease severity we cannot see a clear association. So it's associated with the disease and it's probably not deconditioning based on our data.

And then just to give you a small snapshot of the metabolism data we have for fatty acids. I will tell you that a little bit about that. Because if there is an impaired PDH function as I told you about, we would then expect that there is an increased conversion of pyruvate to lactate. If you stress the system and put the patients under strain—and that's what patients are telling us—they seem to have sore muscles and quickly a feeling of lactate in the muscles. [There is] reduced ATP production in this energy deprivation. And we would expect that in addition to using amino acids from serum, [they would] also use fatty acids which is a major source for TCA oxidation and energy. And therefore we have analyzed a lot of fatty acids among many other metabolites trying to underpin this data. This is just a snapshot as examples from the saturated fatty acids that we have measured in ME/CFS patients and you can see it divided by women and men. Most of these fatty acids are down regulated, reduced in serum levels, especially the short and medium chain fatty acids in both genders. So we don't have to go into the details. And we looked at the TCA cycle intermediates which are part of the citric acid cycle and these are also reduced. Some of them are equal to healthy [controls] such as citrate. In accordance with that in the model that we are thinking [of], there's a slight increase in pyruvate in the ME/CFS patients that would fit with an obstruction at the PDH level.

So to conclude this, we think that these are features of a struggle for energy by the patients. There is a putative PDH inhibition with change of substrates for how the patients tried to get acetyl-coenzyme A which they take from amino acids and fatty acids. It resembles physiological mechanisms that are activated by fasting or starvation. And it resembles mechanisms that are activated by endurance exercise. I think that in ME/CFS patients this mechanism appeared to be activated at rest or at minimal exertion. It's not a normal physiologic regulation. So this impairment of the central energy pathway seems to be present in both male and female patients. I think that the two genders have different way of coping with this. They have

different compensatory mechanisms which are partly sex specific. So if there is a metabolic obstruction at in the central energy pathway, possibly including the PDH level, it can describe some of the features that patients at least tell me. The devastating lack of energy they feel, this devastating total lack of energy and the lactate accumulation after minimal exertion which has also been measured. It looks like patients use anaerobic metabolism. So somehow it resembles hypoxia which could be real or could be perceived due to signaling disturbances. We are wondering if there is an ineffective glucose utilization switch in the metabolic patterns part of the mechanisms. It's important to understand why this occurs. Somehow it has to do with the immune system, I think, because 70% [of patients with] this disease, [it] develops after infection. This is the last slide. Just the same figure: the lack of energy in effective glucose utilization, lactic acid production, use of alternative substrates to try to keep the energy levels up.

So that's what we are thinking. This is part of a bigger picture. That's the good thing being at such a very nice meeting, hearing from excellent researchers from different groups telling us how they look at this and we find common things. We find out, okay, we will have to adjust this one. And during the next year I really hope that we both can give you more information on the clinical trials. Maybe we are doing new trials and I'm sure at a mechanistic level we will have more information about the pathogenesis. I think so. So thank you

[Applause]

Thank you to the volunteers who helped transcribe the Second Annual Community Symposium.