PREDICTORS OF ME/CFS FOLLOWING EBV & IMPLICATIONS FOR PCS

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Pre-Illness

The key question is why do some people get sick with a viral infection and stay ill versus those who get sick and recover

The best way to answer this question is to examine individuals prior to them getting sick to see if they might have some characteristics that predispose them to long-term illness

For the past decade, our group has been working to identify immune profiles and behavioral domains that existed prior to infection of EBV

Such insights can help us with understanding predisposing factors for ME/CFS as well as COVID long haulers

Method

From 2014 through 2018, we collected data from 4,501 healthy college students and followed them for the development of ME/CFS

About 5% of the students were diagnosed with infectious mononucleosis

Some continued to have severe symptoms at 6-month follow-up

Jason et al. (2021) *Clinical Infectious Diseases*

Case Definitions

Those who met one case definition (i.e., the Fukuda)	ME/CFS
Those who met more than one case definition (Fukuda and either the Canadian and/or Institute of Medicine criteria)	*Severe* ME/CFS or S-ME/CFS.
Those who recovered	Controls

Study 1: Pre-illness Variables

Students who developed ME/CFS versus those who did not following IM did not have significant pre-illness baseline differences in stress, coping, anxiety, or depression

Found baseline pre-illness deficiencies in immune markers of the group that went on to develop S-ME/CFS versus those that recovered following mono

Deficiencies in production of IL-5 and IL-13 prior to contracting IM may influence immune response and immune dysregulation once the virus is contracted

Suggests some predisposing irregularities in the immune response

Study 2: Network Analysis

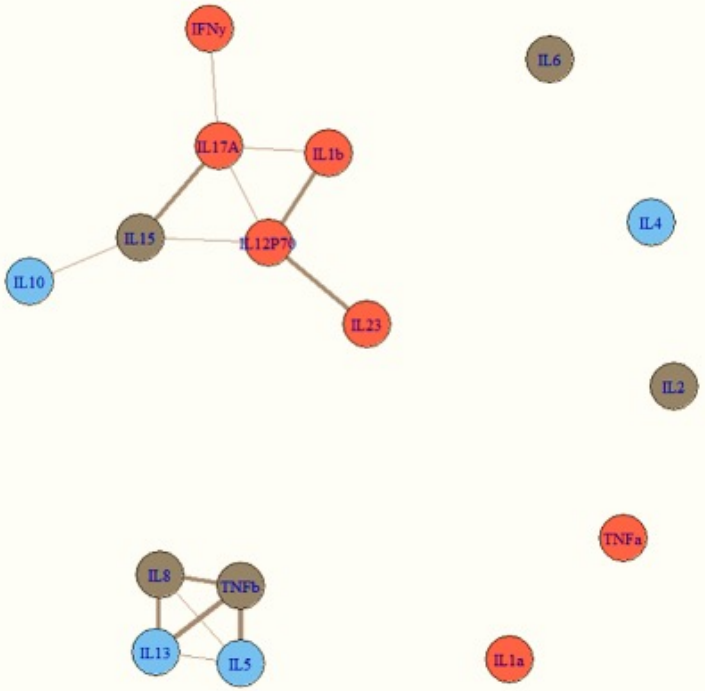
Memberships	How many groups of cytokines exist within each condition
Modularity	Measures the validity of the membership categories
Eigen Centrality	Measure of relative importance of a cytokine in connecting a network
Mean Degree	Average number of connections a cytokine forms with other cytokines

Jason et al. (2021). *Fatigue: Biomedicine, Health & Behavior*

Immune Findings

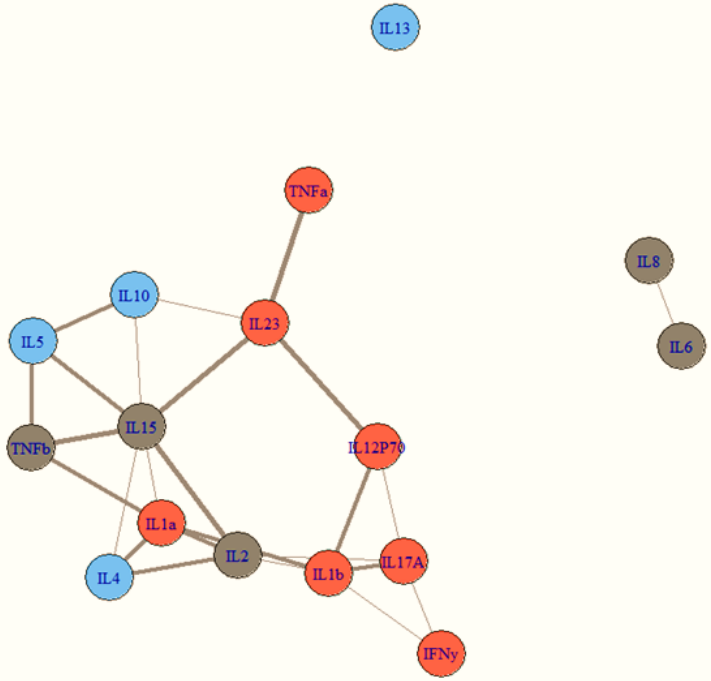
Figures below show intercommunication in the immune system; note the separate groupings in controls compared to highly clustered in those who went on to develop severe ME/CFS

Controls
(did not develop severe ME/CFS)



Red: pro-inflammatory cytokines
Blue: anti-inflammatory cytokines
Grey: mixed or unknown role

Severe ME/CFS Group
(those who went on to develop ME/CFS)



Interpretation of Findings from Study 2

- Those who develop severe ME/CFS following IM had more centrality, or densely interconnected cytokine networks at baseline, at least six weeks prior to the onset of IM
- More differentiated cytokine networks were seen for the recovered controls at baseline
- Using similar network methods, a pediatric community-based sample also found activation of inflammatory mechanisms in am S-ME/CFS group

(Jason et al., 2022. *Chronic Illness*)

Study 3: Predictions

Receiver Operating Characteristic (ROC) statistics were calculated

Two random forest classification analyses were conducted to separate patients with severe ME/CFS from controls

- *one with just the clinical DSQ items*
- *one with just the cytokines*

(Jason et al., 2022)

Findings

Patients with stomach pain, bloating, and symptoms of an irritable bowel at pre-illness baseline, low levels of IL-13 and/or IL-5 at pre-illness baseline, and severe gastrointestinal symptoms when they contracted mononucleosis

had nearly an 80% chance of developing severe ME/CFS six months following IM

Interpretation of findings from Study 3

Those who develop severe ME/CFS 6 months following IM had more baseline gastrointestinal symptoms and abnormal immune markers, and had worse gastrointestinal symptoms at the time they developed IM

It is of interest that the original Fukuda et al. case definition did not include gastrointestinal symptoms among those that qualify for a diagnosis of ME/CFS

Findings Other Data Sets

Adult survey participants reported having developed ME/CFS prior to age 18

- Prior to ME/CFS, 246 out of 617 (40%) experienced gut problems
- Johnson, Torres, Jason (2023)

Factor Analysis with 299 patients with Long COVID

- During first two weeks gastrointestinal factor emerged but not present at 6 months
- Dorri, Jason (2023)

Cytokine Findings from Study 3

IL-13 and IL-5 were implicated as risk factors for the development of severe ME/CFS following IM

There is evidence in human and mouse models that IL-5 and IL-13 contribute to the pathology associated with ulcerative colitis

Study 4: Metabolic Pathways Prior to Mono

Examined baseline levels among those with Severe ME/CFS versus controls

- Non-parametric significance tests were conducted for metabolites with significance levels adjusted using the Bonferroni correction (0.01 / 265 = 0.000038)
- The severe ME/CFS and recovered groups were classified using binary logistic regression with significant features imputed into a series of models with a leave-one-out cross-validation (LOOCV) technique
- A series of binary logistic regressions were conducted to classify the severe ME/CFS and recovered groups

Significant differences in peak area value between the severe ME/CFS and recovered groups were observed for eight metabolites (see next slide)

Table 1. Significant metabolomic results at baseline (prior to IM) in controls who recovered from IM vs participants who went on to develop severe ME/CFS 6 months following IM.

	KEEG	Metabolite	S-CFS M (SD)	Controls M (SD)	*U*	*p*
[a]	C00750	spermine	18.79 (0.13)	19.54 (0.16)	0	0.0000000002
[c]	C00354...C00665	F-1,6/2,6-DP	14.94 (0.43)	13.90 (0.24)	321	0.0000000015
[a]	C00315	spermidine	19.18 (0.66)	17.84 (0.33)	311	0.0000000822
[c]	C00002...C00286	ATP/dGTP	14.57 (1.02)	13.16 (0.54)	301	0.0000012586
[b]	C00169	carbamoyl phosphate	15.67 (0.45)	16.65 (0.12)	23	0.0000012586
[a]	C00127	glutathione disulfide	13.63 (1.00)	11.64 (1.11)	300	0.0000015973
[c]	C00158...C00311	citrate/citrate(iso)	22.90 (0.28)	22.40 (0.30)	290	0.0000135233
[a]	C00112	CDP	10.84 (1.29)	8.88 (0.85)	297	0.0000169794

Note: [a] = identity confirmed, [b] = identity not confirmed, [c] = cannot separate

Note: Bonferroni correction ($p < 0.000038$)

S-adenosyl-L-methionine (SAM) is part of one carbon metabolism and is a methyl donor for epigenetic regulation

Glutathione is part of glutathione metabolism

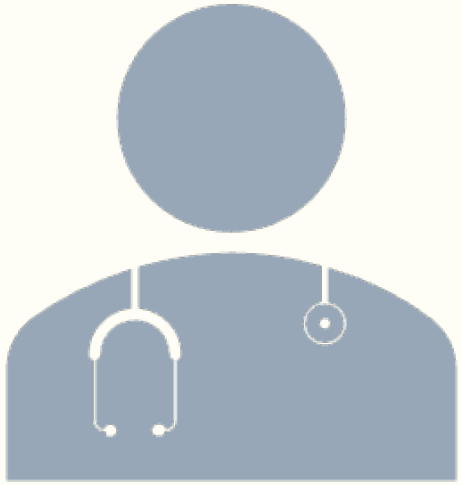
Cysteine is an amino acid that participates in a variety of pathways, including glutathione metabolism

Thiamine is modified and used as a cofactor in several TCA cycle enzymes

N-acetyl-alanine may have a role in protein signaling and post-translational modifications

Results

- The models produced correctly classified those with severe ME/CFS from recovered controls with an accuracy of 97%, sensitivity of 94%, and specificity of 100%
- We identified potentially dysregulated pathways that are essential for proliferating cells, particularly during a pro-inflammatory immune response, and are thus consistent with irregularities in cytokines that have been reported



6-7 Year Follow-Up Now Occurring

- Received additional NIH funding to follow up on our student sample
- Comparing their baseline data—behavioral and immune—from 6-7 years ago to their current state
- Also recruiting patients now with pediatric Long COVID to compare our sample to those with ME/CFS

Implications for Long COVID

In our work over the past decade with this prospective study

We have learned lessons about the ME/CFS case definition that can be instructive for those working with Long COVID

Critical Tasks for Any Illness

Decide	Develop	Specify
Decide on which symptoms are contained in a case definition for a disease (IOM and CCC)	Develop valid instruments to reliably assess agreed upon symptoms (DePaul Symptom Questionnaire)	Specify thresholds regarding whether or not a particular symptom is severe and frequent enough to meet criteria

Importance of Criterion Variance

- Criterion variance involves differences in the formal inclusion and exclusion criteria used by clinicians to classify patients' data into diagnostic categories

  - *accounts for the largest source of diagnostic unreliability*

- Criterion variance is most likely to occur when operationally explicit criteria do not exist for diagnostic categories, or when there are varying criteria for contrasting case definitions
- When diagnostic categories lack reliability and accuracy, the validity (i.e., usefulness) of a diagnostic category is inherently limited

  - *problems of criterion variance have plagued PASC case definitions*

We can't just ask patients if they have ME/CFS

- In working with ME/CFS, we learned it is important to use methods that can rely on more than just asking patients whether or not they have this illness

- In adult and pediatric community-based studies, 90 to 95% are not even aware they have ME/CFS (Jason et al., 1999; 2020)

Assessing ME/CFS In Long COVID Samples

Recent study with a sample of 465 with Long COVID

- Of respondents who reported that they had ME/CFS, 29% did not meet criteria for ME/CFS
- Of those who did not report they had ME/CFS, 40% nevertheless did meet criteria for the disease
- Both over-diagnosis and under-diagnosis were evident on self-report - *Jason et al. (2023)*

Indicates the need to rely on designating key symptoms

- Provided by a case definition

Second Critical Task for Any Illness

Use valid instruments to reliably assess symptoms to elicit information in similar ways across studies

DePaul Symptom Questionnaire assesses symptoms and whether a person has ME/CFS

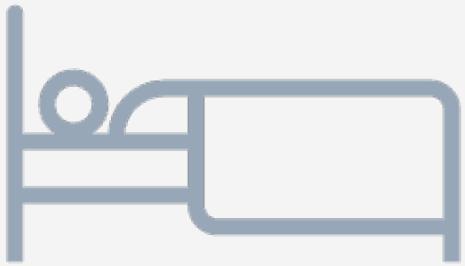
US, Canada, Mexico, Asia (e.g., Japan), the Middle East (e.g., Iraq), Great Britain, Ireland, and Europe (e.g., Spain, Germany, France, Poland, Belgium, Netherlands, Finland, Denmark, Latvia, and Norway) and South Africa

Short Form has 14 items and can assess patients in 5 min

Can determine if a person meets the criteria for an ME/CFS case definition

Symptoms	*Frequency:* Throughout the past 6 months, how often have you had this symptom? For each symptom listed below, circle a number from: 0 = none of the time; 1 = a little of the time; 2 = about half the time; 3 = most of the time; 4 = all of the time					*Severity:* Throughout the past 6 months, how much has this symptom bothered you? For each symptom listed below, circle a number from: 0 = symptom not present; 1 = mild; 2 = moderate; 3= severe; 4 = very severe				
28) Chest pain	0	1	2	3	4	0	1	2	3	4
29) Bloating	0	1	2	3	4	0	1	2	3	4
30) Abdomen/stomach pain	0	1	2	3	4	0	1	2	3	4
31) Headaches	0	1	2	3	4	0	1	2	3	4
32) Muscle twitches	0	1	2	3	4	0	1	2	3	4
33) Muscle weakness	0	1	2	3	4	0	1	2	3	4
34) Sensitivity to noise	0	1	2	3	4	0	1	2	3	4
35) Sensitivity to bright lights	0	1	2	3	4	0	1	2	3	4
36) Problems remembering things	0	1	2	3	4	0	1	2	3	4
37) Difficulty paying attention for a long period of time	0	1	2	3	4	0	1	2	3	4
38) Difficulty finding the right word to say or expressing thoughts	0	1	2	3	4	0	1	2	3	4
39) Difficulty understanding things	0	1	2	3	4	0	1	2	3	4
40) Only able to focus on one thing at a time	0	1	2	3	4	0	1	2	3	4
41) Unable to focus vision and/or attention	0	1	2	3	4	0	1	2	3	4
42) Loss of depth perception	0	1	2	3	4	0	1	2	3	4
43) Slowness of thought	0	1	2	3	4	0	1	2	3	4
44) Absent-mindedness or forgetfulness	0	1	2	3	4	0	1	2	3	4
45) Bladder problems	0	1	2	3	4	0	1	2	3	4
46) Irritable bowel problems	0	1	2	3	4	0	1	2	3	4

DePaul Symptom Questionnaire (DSQ)



Fatigue

Fatigue affects about 25% of the population

– *Not specific enough just to ask whether the person has experienced the occurrence of fatigue*

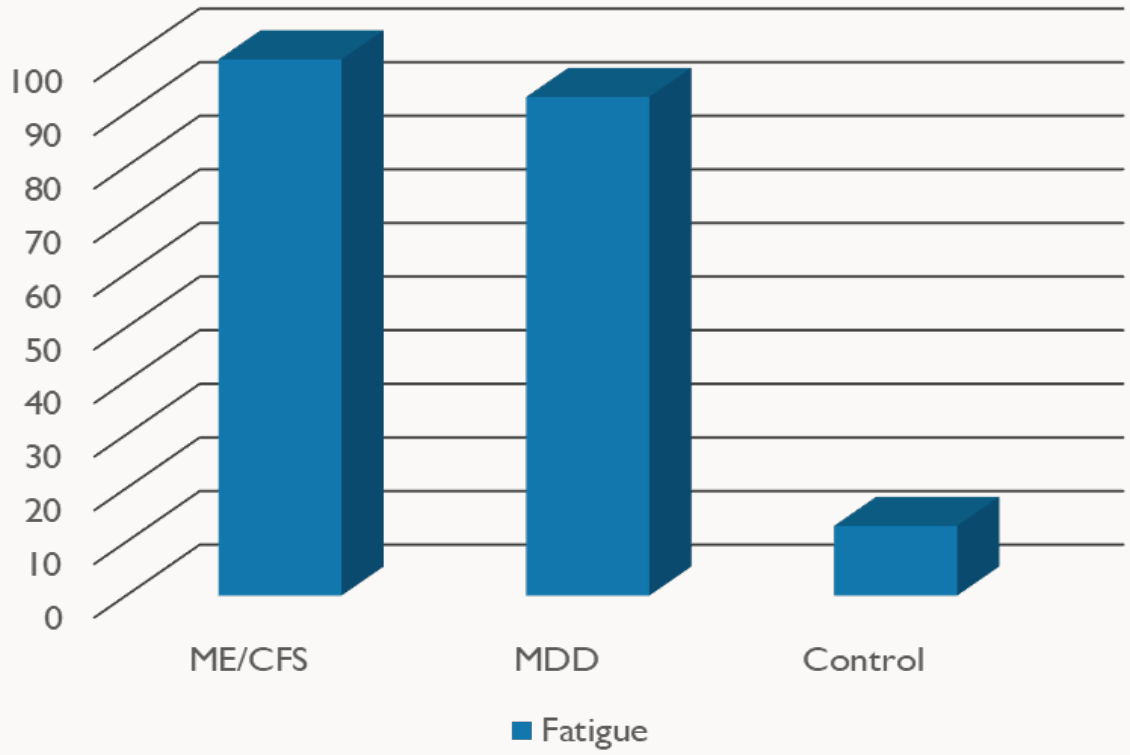
– *Learned that just asking about the occurrence of this type of symptom will not differentiate ME/CFS from other conditions*

Frequency

Many questionnaires switched from the occurrence of symptoms to the frequency

Problems still occurred with trying to differentiate ME/CFS from psychiatric conditions like Major Depressive Disorder (MDD)

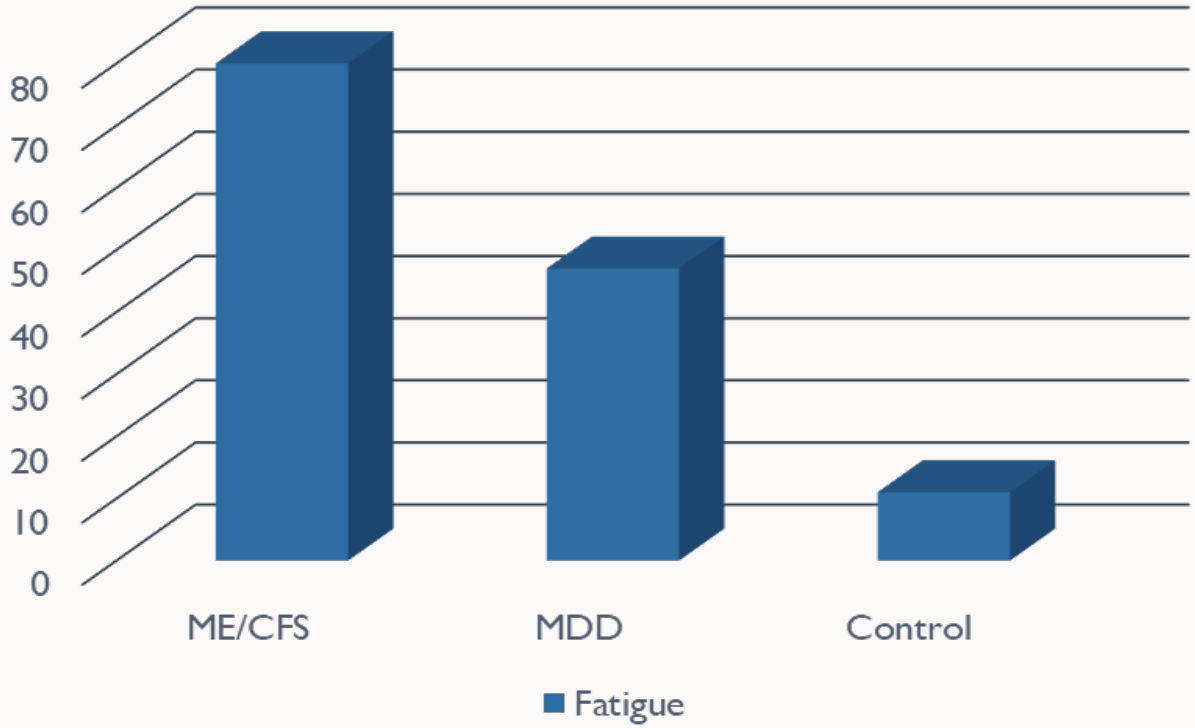
Reporting Occurrence of Fatigue 6 Months or Longer



Severity

Investigators in the ME/CFS field moved to adopting severity measures due to limitations with frequency measures

Reporting Severity of Fatigue for 6 months or longer

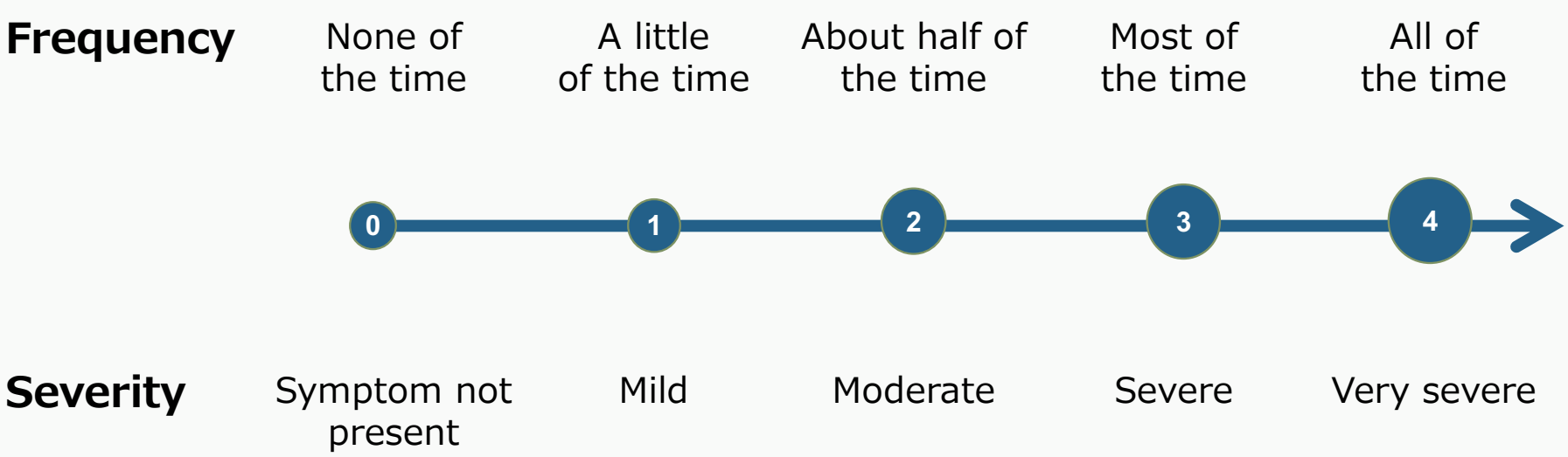


Use of Frequency and Severity

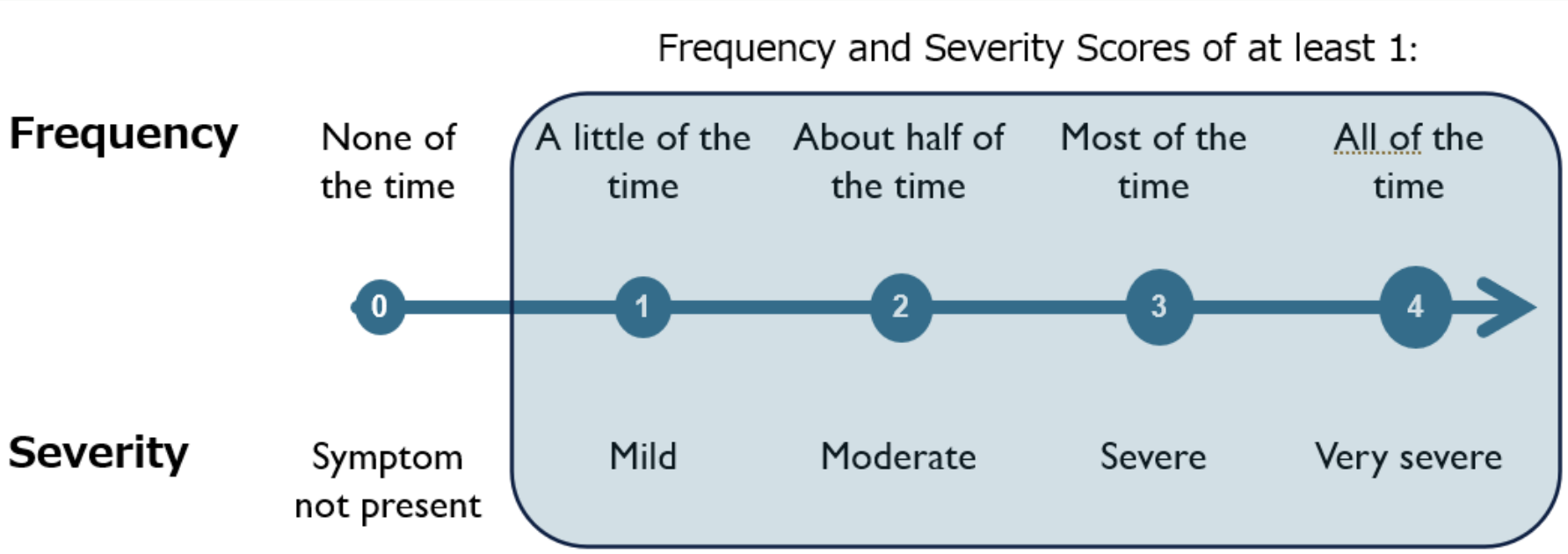
Some somatic symptoms occur at a high severity (migraine) but only infrequently (once every two months)

So, just severity will not capture the burden of a symptom for a patient

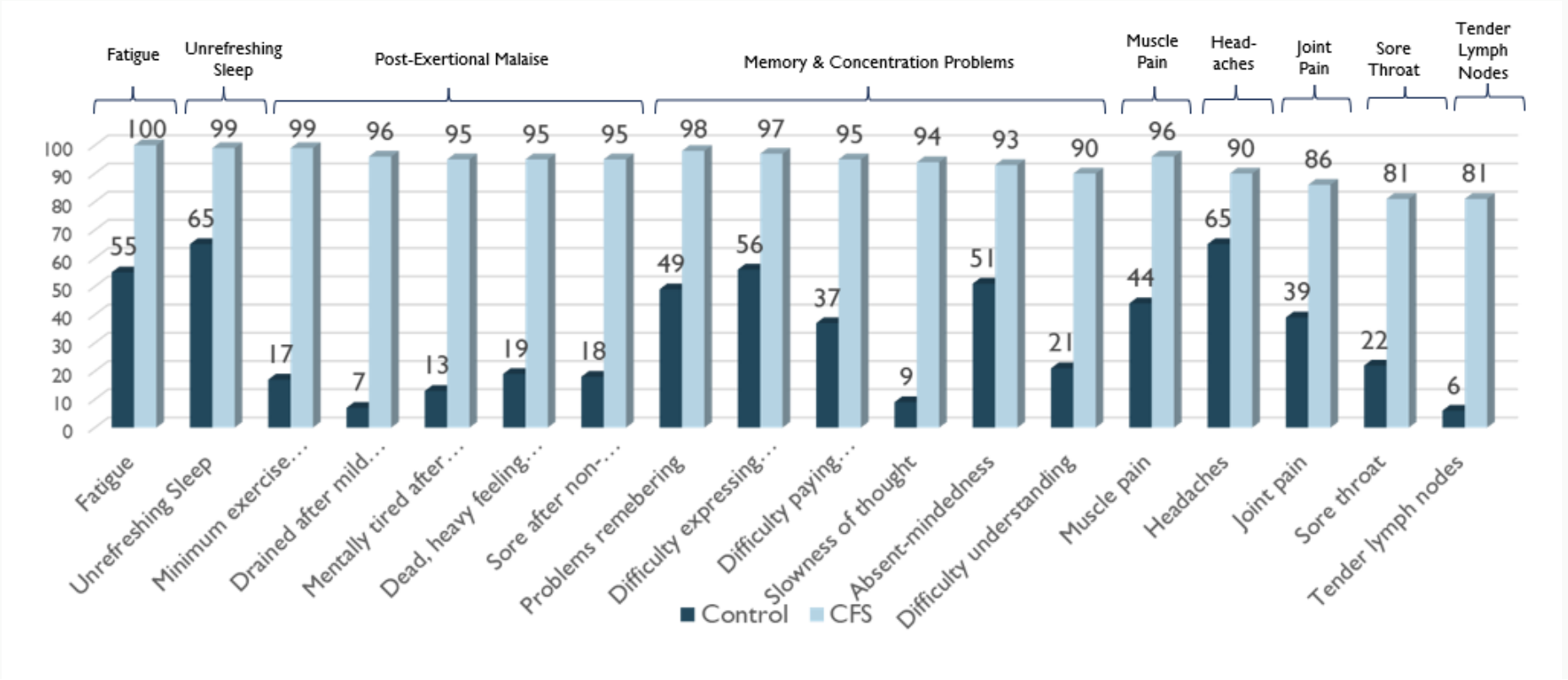
DePaul Symptom Questionnaire:
Frequency and Severity Scales for Each Symptom



DePaul Symptom Questionnaire: Frequency and Severity Scales for Each Symptom



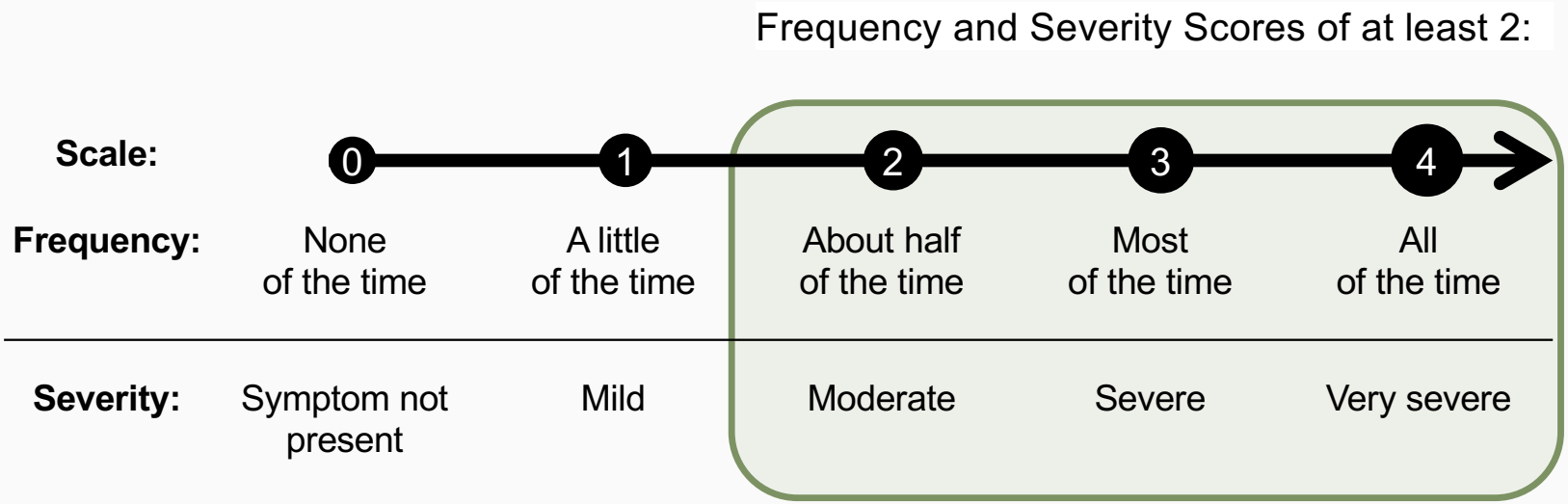
Percentage of CFS and Controls with Frequency and Severity Scores ≥ 1



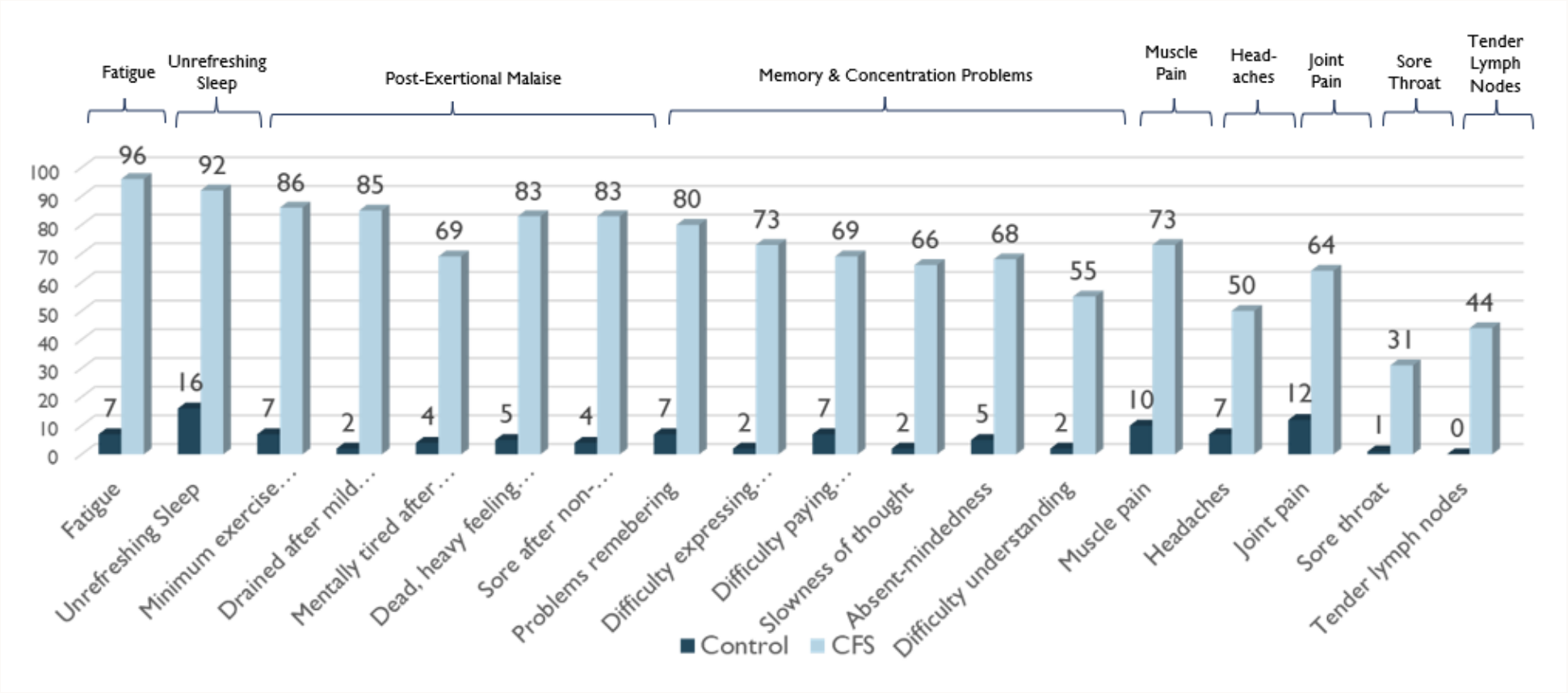
33.7% of controls would meet Fukuda symptom requirements when including participants who report frequency and severity scores of 1 or greater

Misclassifications of
Fukuda et al. (1994) CFS criteria

Frequency and Severity Scores ≥ 2



Percentage of CFS and Controls with Frequency and Severity Scores ≥ 2



Three Tasks Facing Long COVID Investigators

Specify Symptoms	Develop Instruments	Specify Thresholds	Examine Studies
Specify Symptoms as has occurred with ME/CFS (hundreds of possible symptoms that could be assessed)	Develop psychometric sound instruments with adequate reliability and validity	Specify thresholds for when a symptom can be considered a burden	Examine some studies I have been involved in over the past few years that have tackled these conceptual criteria challenges

ME/CFS versus Long COVID

Oliveira et al. (2023) used the DSQ-SF with patients with ME/CFS and Long COVID over a year period of time

At baseline, those in the Long COVID group had similar symptom scores as patients with ME/CFS

Five symptoms improved significantly over the course of 1 year for PASC patients including fatigue, post-exertional malaise, brain fog, irritable bowel symptoms and feeling unsteady

In contrast, there were no significant symptom improvements for ME/CFS patients over time

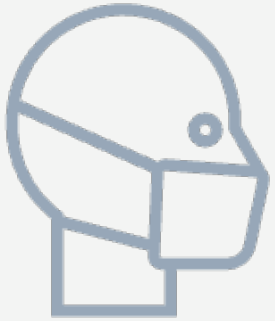
Second Study

McGarrigle et al. (2023) examined those with ME/CFS and Long COVID

- They trained a single three-class and multiple binary random forest models to assess the predictive accuracy, sensitivity, and specificity of the DSQ-SF in discriminating between Long COVID and ME/CFS groups
- They ran 30 iterations for each classifier, and the data was partitioned into an 80:20 split between training and out-of-bag sets

Two DSQ-SF items related to neuroendocrine and immune dysfunction—"Cold limbs" and "Flu-like symptoms"—were found to be the most important features differentiating the groups

- Neither symptom on most COVID Questionnaires



Third Study

- **Hua et al. (2023) built predictive models based on a random forest algorithm using the participants' symptoms from the initial weeks of COVID-19 infection to predict if the participants would go on to later meet the criteria for the development of ME/CFS**
- **Early symptoms, particularly those assessing post-exertional malaise predict the development of ME/CFS**

  - *accuracy of 95%*

Clinical versus **Research**

- When classifying patients into broad (clinical) versus narrow (research) ME/CFS case definitions

- Significant differences between those with narrow research ME/CFS criteria vs controls:

- Saliva biomarker of fatigue concentrations of 2 peptide fragments in a pediatric sample (Jason, Kalns, et al., 2021)
- More dense interconnected cytokine networks at least six weeks prior to the onset of infectious mono (Jason, Cotler et al., 2021)
- More gastrointestinal distress and autonomic symptoms, along with several immune markers (Jason, Cotler, et al., 2022)
- Metabolite differences with 97% accuracy differentiate groups (Jason, Conroy et al., 2022)

Final Comments

- Considerable work needs to be done in the Long COVID area involving specifying symptoms, developing sound psychometric instruments to measure the symptoms, and establishing thresholds that symptoms need to meet to signal it is a burden
- Insights from ME/CFS research could help in the development of research with Long COVID

    -ultimately improve our efforts to understand its pathophysiology, early diagnosis, and prognosis, as well as identify effective treatments