The Role of Glutathione Deficiency, NF-κB blockade, and MSIDS Variables in PASC: The Nutraceuticals in Longhaulers Trial ('NIL' trial)
July 31st, 2021, IRB submission, MINIMUM Testing

Richard I. Horowitz, MD
Medical director, Hudson Valley Healing Arts Center
Board certified internal medicine
Member NYS DOH Tick-borne Disease Working Group 2021
Member HHS Tick-borne Disease Working Group 2017-2019
Co-chair HHS Other Tick-borne Diseases and Co-infections subcommittee 2017-2019
Member, HHS Babesia and Other Tick-borne Pathogens 2019-2020

A hypothetical three-part prevention, diagnostic, and treatment approach based on an up-to-date scientific literature review for COVID-19 was published by Horowitz et al. in 2020.[1] The primary hypothesis behind designing an effective prevention and treatment approach was based on the medical literature showing that infection, immune dysfunction, and inflammation (3 I's) cause severe symptoms/clinical manifestations in COVID-19. Pathogenicity of COVID-19 has been linked in some patients to "Cytokine Storm Syndrome" (CSS) with "Acute Respiratory Distress Syndrome" (ARDS), which is the primary cause of death, along with fulminant myocarditis and multiorgan dysfunction. This results from an uncontrolled systemic inflammatory response with an increase in large amounts of pro-inflammatory cytokines (IFN-α, IFN-γ, IL-1β, IL-6, IL-12, IL-18, IL-33, TNF-α, TGFβ, etc.) & chemokines (CXCL9, CXCL10, etc.) by immune effector cells. [2] [3] Recent scientific research has determined that not all patients who succumb to COVID-19 have evidence of a cytokine storm however, and the etiology underlying severe acute and chronic manifestations is still being investigated.[4]

A comprehensive model for chronic disease which can be applied to longhauler research to determine potential underlying etiologies driving chronic illness is the MSIDS (Multi-Systemic Infectious Disease Syndrome) model. The model has been published in both the scientific peer-reviewed literature for chronic Lyme disease, [5] as well as in two scientific books, 'Why Can't I Get Better? (St Martin's Press, 2013) and 'How Can I Get Better? (St Martin's Press, 2017). [6] [7] Post-Acute Sequelae of COVID (PASC) and Lyme disease both share similar symptoms (see below) and both illnesses have been determined to be due to infection, inflammation and immune dysfunction (the 3 I's). The etiology of PASC is still unknown post viral infection, and the 16-point MSIDS model posits that up to 16 potential underlying etiologies may be responsible for both acute and chronic illness. These include the following 16 points and prospective testing strategies:

- 1) **Infections:** multiple infections may be involved in pathogenesis (bacterial, viral, parasitic, fungal)
- 2) **Immune dysfunction**: including cytopenia's, immunoglobulin deficiency, autoimmune reactions
- 3) **Inflammation**: due to cytokines, chemokines
- 4) **Toxicity**: including heavy metals, mold, pesticides, and neurotoxins (internal/external)
- 5) **Allergies**: foods, drugs, environmental sources
- 6) **Nutritional & Enzyme Deficiencies**/ functional medicine abnormalities in biochemical pathways
- 7) **Mitochondrial dysfunction**: free radical stress →fatigue, nerve dysfunction, cardiac problems
- 8) **Psychological disorders:** new onset or exacerbation of underlying illness due to the 3 I's
- 9) **Neurological dysfunction:** leading to neuropathy, dizziness, light and sound sensitivity, memory and concentration problems, cranial nerve dysfunction

- 10) **Endocrine disorders: low hormones** (testosterone, estrogen, adrenal hormones, GH, thyroid hormones, ADH, insulin resistance)
- 11) **Sleep disorders:** insomnia may contribute to chronic symptoms and inflammation (IL-6)
- 12) **ANS dysfunction (Dysautonomia**)+/- Postural Orthostatic Tachycardia Syndrome (POTS)
- 13) **G.I. disorders**: including microbiome imbalance, Leaky gut, parasites, IBD (inflamm bowel d/o)
- 14) **Elevated LFT's**: secondary to infection, inflammation, immune dysfunction, toxins, medication…
- 15) **Pain syndromes**: due to infection, inflammation, immune dysfunction…
- 16) **Deconditioning:** secondary to inactivity from chronic illness.

Table One: The 16 MSIDS Variables and Potential Diagnostic Testing [5]

Table One: MSIDS Variables	Tests/Methods of Evaluation
1. Infections	Laboratory tests for Borrelia, Babesia, Bartonella spp. etc.
2. Immune Dysfunction	Laboratory tests for autoimmune markers (ANA, RF), HLA status, immunoglobulin levels/subclasses
3. Inflammation	Laboratory tests for markers of inflammation, i.e., ESR, CRP, TGFB1, C3a, C4a, and/or VEGF
4. Toxicity	Lab tests for heavy metals, mold toxins, etc.
5. Allergies	IgE levels, food/environmental allergies, histamine, etc
6. Nutritional & Enzyme Def	Lab tests for amino acids, fatty acids, mineral levels (serum, plasma, red blood cell), enzymes
7. Mitochondrial Dysfunction	Clinical evaluation of response to mitochondrial support (NT Factors, CoQ10, L-carnitine), mtDNA mutations
8. Psychological Dysfunction	Clinical evaluation for evidence of depression, anxiety, PTSD…

9. Neurological Dysfunction	Clinical examination, EMG, Small fiber biopsy, MRI brain, etc.
10. Endocrine Abnormalities	Evaluate hormone levels (thyroid, adrenal, sex hormones, Vitamin D) and hormone precursors (DHEA-S, pregnenolone)
11. Sleep Disorders	Clinical evaluation (diet, medication), sleep studies, laboratory evaluation of hormone levels, etc.
12. ANS Dysfunction	Tilt table testing with or without small fiber biopsies and autonomic/electrodiagnostic testing (EMG), clinical evaluation sitting/standing BP/heart rate
13. Gastrointestinal Dysfunction	Endoscopy, colonoscopy, clinical/laboratory evaluation (celiac markers, H. pylori), Comprehensive Digestive Stool Analysis (CDSA) for bacteria (C. difficile), ova and parasites, Candida, etc.
14. Elevated Liver functions	Laboratory evaluation of AST, ALT, Alkaline phosphatase, total bilirubin, etc.
15. Pain Syndromes	Clinical evaluation, EMG, small fiber biopsy, laboratory markers for autoimmune disease (anti-myelin antibodies), etc.
16. Deconditioning	Clinical evaluation and need for physical therapy

Symptoms of Long COVID:
The 16-point MSIDS model can be applied to patients suffering with long COVID (PASC: Post-Acute Sequelae of COVID) who have been found to experience a broad range of symptoms overlapping other diseases. These symptoms include loss of sense of smell and/or taste, parosmia (altered sense of smell), decreased appetite, sore throat, low grade fevers, chronic fatigue, exercise intolerance, excessive sweating, skin rashes, myalgias, arthralgias/joint pain, headaches, tinnitus, brain fog, light sensitivity, blurry vision, hair loss, neuropsychiatric symptoms (primarily anxiety, depression and rare cases of psychosis), insomnia, chest tightness, chronic cough, sputum production, shortness of breath, dizziness, palpitations with an increase in resting heart rates, and rarely gastrointestinal symptoms including dysphagia, nausea, vomiting, cramping and diarrhea. In a 3-month follow-up survey of 538 COVID-19 patients, Xiong and colleagues found that fatigue, cardiovascular-related symptoms such as dyspnea (especially post-activity), and alopecia were more common in women than in men. [8] In that study, demographic, and clinical features among 538 COVID-19 survivors during acute COVID-19 illness showed that 54.5% were female, 78% were between 40 and 80 years old, with 32.9% suffering from co-morbidities, including hypertension, diabetes, cardiovascular disease, COPD, and cancer. These are all known risk factors for severity of illness. In a longer, 6 month follow-up of 1733 COVID patients recently published in the Lancet, [9] patients had a median age of 57 years old and 897 (52%) were men. Fatigue or muscle weakness (63%) and sleep difficulties (26%) were the most common symptoms, followed by anxiety or depression (23%), with 22% to 29% having difficulty with post-exertional fatigue after a 6-min walking distance. Fatigue, dyspnea, muscle weakness with exercise intolerance, insomnia and neuropsychiatric symptoms were therefore the most common complaints among longhaulers, which are symptoms overlapping other chronic illnesses including myalgic encephalomyelitis (ME/CFS), Fibromyalgia and chronic Lyme disease/PTLDS. [10] [6]

A small proportion of patients suffering from PASC in a three month follow-up also complained of increases in resting heart rates (11%) and excessive sweating (24%), with 13% reporting significant cardiovascular symptoms 3 months after discharge.[8] The only etiology to date to explain these symptoms has been post-infectious POTS (Postural Orthostatic Tachycardia Syndrome),[11] an imbalance in the autonomic nervous system, which is part of the 16 point MSIDS model. This has been also published in patients with other chronic illnesses including Myalgic Encephalomyelitis (ME)/FM, [12] apart from those suffering with Lyme disease/PTLDS, [13] [5] where a viral or bacterial infection may result in symptoms of POTS including severe fatigue, dizziness, palpitations, brain fog, anxiety, and sleep disturbances. [14] These are also symptoms which are seen in PASC. Six months after infection with COVID-19, a small proportion of patients still complained of these symptoms (palpitations [9%], dizziness [6%]) as well as other debilitating symptoms. These included a smell disorder (11%), joint pain (9%), decreased appetite (8%), taste disorder (7%), chest pain (5%), sore throat or dysphagia (4%), skin rash (3%), myalgia and headache (2%) with low grade fevers (< 1%). [9] All of the above symptoms would therefore be followed in a longitudinal diagnostic and treatment study of PASC, evaluating different diagnostic and treatment methodologies (listed below), including an evaluation of POTS/dysautonomia using sitting and standing blood pressure and pulse measurements.[15] According to prior published research at the Mayo clinic, approximately half of POTS patients suffer from an autoimmune, neuropathic pathology. [16] This is also an established risk factor in POTS patients suffering with Lyme disease. [6] [7] [5]

A RCT of different diagnostic and treatment methodologies is necessary in PASC since the numbers of patients affected may be significant. In general, this has varied from 10% to 50%, [17] [18] although an Italian study found that, in patients discharged from the hospital after recovery from COVID-19, 87.4% reported persistence of at least 1 symptom, particularly fatigue and dyspnea. [19] In that study, The EuroQol visual analog scale was used to ask patients to score their quality of life from 0 (worst imaginable health) to 100 (best imaginable health) before COVID-19 and at the time of the visit, with a difference of 10 points defining a worsened quality of life. [19] In another study done in China, [9] 75% of those patients who were hospitalized with COVID-19 and then discharged still experienced at least one symptom six months later. This primarily included fatigue or muscle weakness, sleep difficulties, and anxiety or depression. Symptoms of PASC have therefore varied between 10% and 87% based on the population studied and may result in long term disability.

Among those longhaulers who were more severely ill during their hospital stay with abnormal chest imaging (GCO, i.e., ground glass opacities and lines on their Chest X-rays), between 22 and 56% of those patients had more severe impaired pulmonary diffusion capacities 6 months post symptom onset. [9] The disease severity in the acute phase was found to be associated with pulmonary diffusion abnormality and percentage change of CT scores in the multivariable analysis. These abnormal CT patterns with pulmonary interstitial changes (GGOs and irregular lines), were similar to the long-term lung manifestations of SARS or influenza. [20] [21] No etiology to explain the ongoing symptoms has yet been proven, although hypotheses include ongoing low levels of viral infection, immune overactivation, tissue damage and inadequate anti-oxidant protection, including low levels of glutathione. In a published case study by Horowitz et al, high dose glutathione was found to reverse acute symptoms of COVID-19, including dyspnea, [22] and multiple scientific studies have now discussed using glutathione (GSH) in COVID-19. [23] This

may be especially important as an underlying risk factor in pulmonary complications since deficiency in RBC, serum and alveolar GSH has been published in the medical literature for ARDS, as well as viral and bacterial pneumonias, resulting from increased levels of free radical/oxidative stress. [24] [25] GSH in the lower lining of the upper respiratory tract is the first line of defense against oxidative stress, and in the epithelial lining fluid, GSH concentrations are 140X higher than in the serum. Changes in GSH concentration are considered central in the context of inflammatory lung diseases. [26] In one study of ICU patients with COVID-19, [27] the systemic oxidative stress status (OSS) was strongly altered in critically ill COVID-19 patients as evidenced by increased lipid peroxidation but also by deficits in antioxidants (vitamin C, glutathione, thiol proteins) and trace elements (selenium). None of these markers have been evaluated to date in long COVID. As GSH is one of the body's primary anti-oxidants, not only in the lungs but in tissues and organs throughout the body, lack of glutathione may therefore result in increases in free radical stress and organ damage. There is also evidence that GSH deficiency may underlie established risk factors in COVID-19 including hypertension, diabetes and cardiovascular disease, [28] and low GSH levels have been found in aging, healthy adults as well. [29] Its anti-viral properties and ability to act as a signaling molecule enhancing innate immunity may similarly play a role in acute and chronic viral illness. [30] [31] [32] A RCT is therefore required to establish the role of GSH in COVID-19 and whether GSH administration with NAC (N-acetyl cysteine) and Alpha lipoic acid (ALA) can help reverse some of the symptoms of PASC. NAC and alpha lipoic acid both help to increase endogenous glutathione production, [33] [34] and all three nutraceuticals block NFKappa B, [35] [36] [37] [38] a switch inside the nucleus that regulates inflammation and innate and adaptive immune function. [39] NAC and GSH also contain thiol groups which have been shown to have anti-viral effects. [40] [41] [42] [43] [44] The use of NAC, ALA and GSH has been shown to help in other chronic illnesses, including Lyme disease, where it can reduce fatigue, headaches, brain fog, myalgias and arthralgias,[7] which are similar symptoms seen in longhaulers. GSH has also been published in a small case series to help with acute COVID, [22] and in this RCT, we will evaluate the role of ongoing free radical/oxidative stress, NFKappa B activation and glutathione deficiency in those with long COVID.

Endogenous deficiency of glutathione is considered by some authors to be a likely cause of serious manifestations and death in COVID-19 patients. [28] Glutathione (GSH) is the body's primary antioxidant and has been shown to be deficient in multiple infections with pulmonary complications. [45] Based on the evidence attesting to the ability of glutathione (GSH) to inhibit viral replication and decrease levels of IL-6 in human immunodeficiency virus (HIV) and tuberculosis (TB) patients, as well as beneficial effects of GSH on other pulmonary diseases processes, the use of liposomal GSH has been hypothesized to be beneficial in COVID-19 patients. [23] No clinical studies on COVID patients have been performed to date however to prove the hypothesis.

Dr Horowitz has over a 20-year experience using glutathione in thousands of patients with Lyme disease, where glutathione helps lower inflammatory cytokines produced during Herxheimer reactions. [6] These are similar inflammatory cytokines seen in COVID-19,[3] which is the basis for the Horowitz COVID protocol. The use of glutathione for COVID-19 has also been validated in a small pilot trial of 65 patients with COVID in his clinical practice (personal experience, unpublished data), where patients reported a rapid improvement in symptoms following administration of oral glutathione, glutathione precursors (N-acetylcysteine), nutraceuticals

(alpha lipoic acid, zinc, beta glucan, curcumin, sulforaphane) as well as ivermectin. No patients to date have required hospitalization or gone on to become COVID longhaulers. Two of those cases were published by Horowitz and Freeman in the Journal of Respiratory Case Reports in April 2020, which showed benefit using GSH for COVID-19 pneumonia, relieving dyspnea and associated symptoms, i.e., fatigue, myalgias, headaches.[22] Since that time, the glutathione article by Horowitz et al. has been cited by over 80 authors as a potential treatment for COVID-19 without the benefit of proving the hypothesis via a RCT. Efficacy of glutathione therapy in relieving ... - NCBI - NIH
www.ncbi.nlm.nih.gov › pmc › articles › PMC7172740
by RI Horowitz · 2020 · Cited by 83 — **References**. 1. ... February 2020 doi: 10.1111/**all**.14238. None of those referenced studies have examined GSH with or without an anti-viral medication as a potential treatment in long COVID, and/or its ability to reverse chronic symptomatology.

The scientific reasoning behind a glutathione trial, and using precursors of GSH (N-acetyl cysteine, NAC) is that the primary roles of glutathione (GSH) in the body include antioxidant/anti-inflammatory activity by neutralizing potentially dangerous molecules (Reactive oxygen species [ROS], Reactive nitrogen species [RNS]), xenobiotics, and metals; regulating cell processes of metabolism, proliferation, differentiation, and apoptosis; and regulating our immune response, since the concentration of GSH and its oxidized form, glutathione disulfide (GSSG), mainly determines the redox state of the cell, which plays a direct role in viral pathogenesis [46] [47] [48] The positive effects of N-acetylcysteine (a precursor of glutathione), oral glutathione (GSH) and a novel sublingual form of GSH on oxidative stress markers have been demonstrated in a randomized, controlled comparative crossover study. [49] Glutathione, NAC and alpha lipoic acid all have the ability to block NF-κB, a switch inside the nucleus which increases oxidative stress and inflammatory cytokine production, which accounts in part for its anti-inflammatory and antiviral activity. [50] [51] [52] [53] [54] [55] Many studies have shown that NF-κB plays an important role in the pathogenesis of lung diseases, including a role in COVID-19. [56] Published research has shown that COVID-19 activation of transcription factor NF-κB in macrophages found in the lung, liver, kidney, central nervous system, gastrointestinal system and cardiovascular system leads to production of inflammatory cytokines IL-1, IL-2, IL-6, IL-12, TNF-α, LT-α, LT-β, GM-CSF, and a wide variety of chemokines, which in the elderly and in patients with underlying risk factors such as metabolic syndrome makes this population susceptible to COVID-19 and its worst complications, including higher mortality". [57] This is especially relevant in the elderly, where low blood glutathione levels have been found in healthy aging adults, and the incidence of low blood GSH in the older subjects increased significantly, particularly in the 60- to 79-year-old group.[29] This would potentially explain why the elderly are at higher risk for COVID-19 and why glutathione supplementation and GSH precursors which block NF-κB may be of benefit. Glutathione deficiency would leave individuals susceptible to high levels of ROS and oxidative stress, damaging lung tissue and causing multisystemic dysfunction. The hypothesis examining whether glutathione administration and nutraceuticals that support a healthy inflammatory/immune response can be beneficial in PASC needs evaluation, especially considering the large numbers of individuals with chronic disabling symptoms.

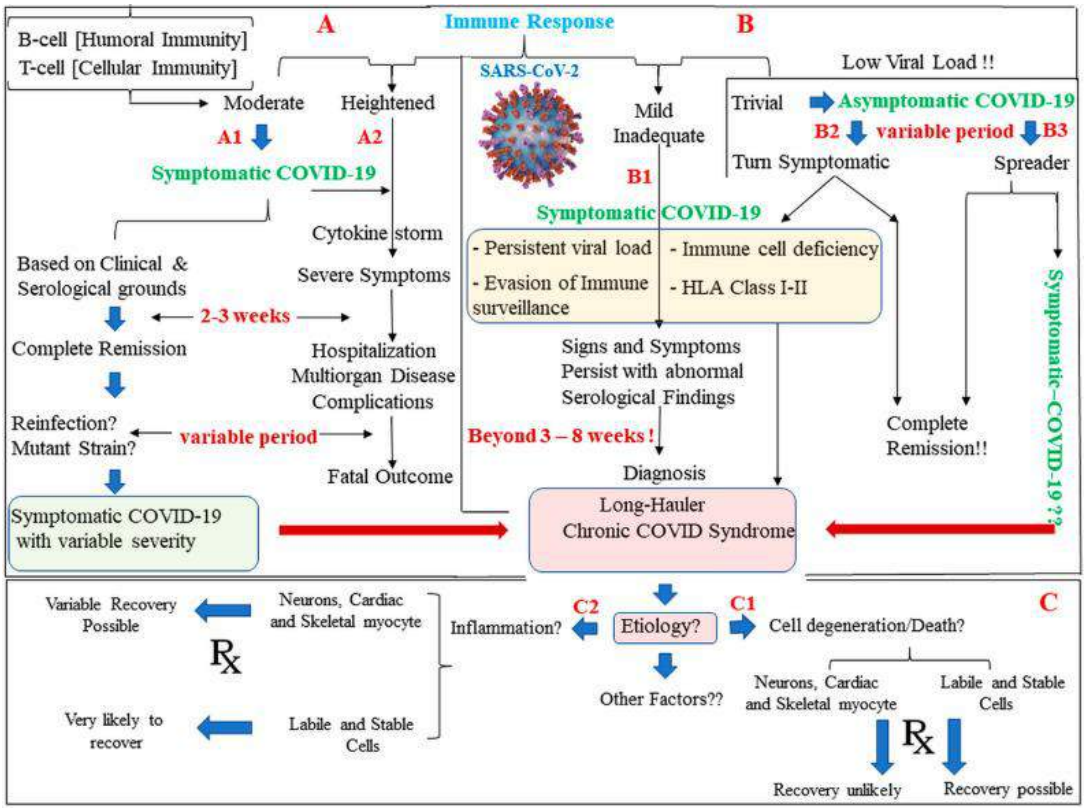
Apart from lowering inflammation by blocking NF-κB and affecting the redox state of the cell, GSH also has direct anti-viral activity, which may help if a chronic viral infection exists with

PASC. [44] [58] [31] Glutathione has been shown to fine-tune the innate immune response toward antiviral pathways in a macrophage cell line independently of its antioxidant properties, [31] and has been shown to inhibit viruses including herpes simplex virus type 1, HIV, and influenza virus. [42] [43] [59]. Loss of glutathione, with associated oxidative stress and decreased intracellular pH have been shown to be sequential steps in viral infection, [60] and some of the antiviral activity of GSH is through a process known as glutathionylation. [61] Glutathionylation is the formation of mixed disulfides between protein cysteines and (GSH) cysteines. Microbial proteins undergo glutathionylation with modification of their functions, and viruses alter the intracellular redox state to pro-oxidant conditions, which is an alteration that contributes to viral pathogenesis. N-Acetyl-L-cysteine (NAC) is a precursor of reduced glutathione (GSH) and both NAC and GSH have disulfide bonds, [33] which may also account in part for their anti-viral effect, as thiols have been shown to block the angiotensin-converting enzyme (ACE) 2 receptor, thereby hampering penetration of SARS-CoV-2 into cells.[62] This is in addition to NAC's mucolytic effects, [63] indirect and direct anti-inflammatory effects, and helping to regenerate glutathione in the body. [64] The efficacy of oral long-term N-acetylcysteine in chronic bronchopulmonary disease has previously been established in a meta-analysis of published double-blind, placebo-controlled clinical trials, and found to prevent acute exacerbations of chronic bronchitis, thus possibly decreasing morbidity and associated health care costs.[65] Patients with PASC who suffer from chronic pulmonary manifestations and dyspnea may therefore potentially benefit. A front-line ER physician with COVID pneumonia, low oxygen saturations and chronic symptoms was treated by Dr Horowitz mid-2020. Post glutathione, nutraceuticals (listed below) and ivermectin X 14 days, his radiological abnormalities reversed, oxygen saturations returned to normal, and debilitating fatigue and dyspnea cleared (unpublished case study).

In order to effectively address increased reactive oxygen species (ROS) and inflammation during COVID and in PASC, postulated to be one of the primary mechanisms responsible for increased morbidity and mortality, we will therefore be evaluating alpha lipoic acid (ALA), NAC and glutathione in our study. Alpha lipoic acid has similarly been shown to block NF-κB inside the nucleus. [54] [66] [38]. Since NF-κB plays a key role in the orchestration of the multifaceted inflammatory response, in the pro-inflammatory phase and later in the resolution of inflammation when anti-inflammatory genes are expressed (ARE) [55] it is potentially a key biochemical pathway in COVID pathogenesis. NF-κB regulates the expression of a large number of genes involved in inflammation and is considered to be the master regulator of the inflammatory response. [37] Activation of NF-κB has also been found to be required for transcription of the genes that increase the pro-inflammatory mediators associated with ARDS.[67] No clinical trials to date have used NAC, GSH and ALA in the treatment of ARDS, and effective treatments for ARDS and chronic pulmonary symptoms post COVID are lacking. A clinical trial of these nutraceuticals is essential in the quest to find more effective treatments and reverse symptomatology.

Despite considerable advances in our knowledge regarding the pathophysiology of ARDS, insights into the biologic mechanisms of lung injury and repair, and advances in supportive care, particularly ventilatory management, there remains no effective pharmacological therapy for this syndrome.[68]Although dexamethasone has been found to be helpful in certain severe cases of COVID, [69] certain authors caution against its immunosuppressive properties. This is due to dexamethasone's ability to inhibit the protective function of T cells, while blocking B cells from

making antibodies, potentially leading to increased plasma viral load that will persist after a patient survives SARS. Moreover, dexamethasone would block macrophages from clearing secondary, nosocomial, infections. Hence, dexamethasone may be useful for the short-term in severe, intubated, COVID-19 patients, but could be outright dangerous during recovery since the virus will not only persist, but the body will be prevented from generating protective antibodies".[70] The variation in viral load and a differential immune response (Figure11) appears to be playing role in different clinical forms and phases of COVID-19.[71]



Glutathione (GSH) may be helpful in this respect. It is the main antioxidant agent in mammalians, and it is the most important antioxidant defense in the lungs as it not only augments innate and adaptive immunity and lowers inflammation, but confers protection against microbial and viral infections. [32] Multiple pulmonary diseases including asthma, chemically induced edema, and Idiopathic Pulmonary Fibrosis (IPF) have improved with glutathione administration. [32] Patients with IPF have a 4-fold lower GSH concentration in the epithelial lining fluid of the normal lower respiratory tract, and the administration of the GSH precursor NAC restores GSH levels. [72] Normal alveolar epithelial lining contains high levels of glutathione [73] which may be lowered by oxidative stress and infection. Those suffering from PASC with pulmonary complications may therefore still be experiencing symptoms due to low GSH levels and elevated oxidative stress. Checking not only GSH serum levels, but markers of oxidative stress including lipid peroxides, may therefore be helpful before and after treatment. [27]

Another biochemical pathway which will be addressing indirectly in our RCT to reverse symptoms of PASC is the Nrf2 pathway. Nrf2 regulates the oxidative stress response in cells and helps lower inflammation. [74] When oxidative stress is present, a molecule in the cytoplasm,

KEAP-1 is released and goes into the nucleus to stimulate antioxidant response element genes (ARE). The redox-sensitive signaling system Keap1/Nrf2/ARE plays a key role in the maintenance of cellular homeostasis under stress, especially inflammatory and pro-apoptotic conditions, which is why it is being considered in our trial as a pharmacological target.[75] Sulfhydryl (SH) group donors, including NAC have been shown to be activators of Nrf2, [76] [77] and ALA may also function as an Nrf2 activator, [78] apart from acting as NF-κB inhibitors, through their regulator molecules KEAP-1 and IκB, respectively. The Nrf2 pathway also regulates GSH homeostasis by affecting de novo synthesis. [79] These three nutraceuticals are therefore potential therapeutic options for SARS-CoV-2 infection. In the NIL RCT, the antioxidants NAC and ALA will be used as our Nrf2 activators, which will both directly and indirectly affect GSH levels.

The referenced supplements will be in MedPax-1 from Xymogen, which will be taken along with liposomal GSH (Essential Pro, Wellness Pharmacy, Birmingham Alabama) at the following dosages:
COVID Study MedPax #1: 28-day trial in Arm A, 14-day trial in Arm B
MedPax #1 contains:
NAC 600 mg – 4 capsules in each am/pm packet (2400 mg BID)
Alamax CR *(alpha lipoic acid-controlled release) - 1 tablet in each am/pm packet (alpha lipoic acid, 600 mg BID)
In each arm of the study, each MedPax-1 will be taken twice a day with/after meals, along with liposomal GSH from Wellness Pharmacy, at a dose of 8 capsules, 250 mg each (2000 mg) two times a day. The liposomal glutathione can be taken at the same time as each MedPax, with breakfast (7-8 am) and dinner (7-8 pm). Arm A will take MedPax-1 for 28 days along with liposomal GSH for 28 days. Arm B will take MedPax-2 for 14 days (multivitamin/mineral) and then cross over to the MedPax-1, with NAC, ALA and GSH arm for 14 days.

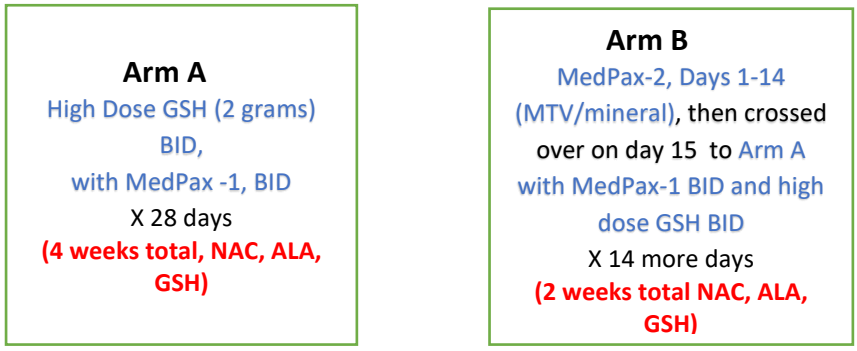
MedPax #2: 14-day trial, Arm B
Each packet of MedPax-2 contains 3 Active Nutrients (multivitamin) without copper or Fe, along with magnesium (OptiMag) 125 mg. None of these have significant anti-viral or anti-inflammatory effects. We will therefore be able to compare these two groups of nutraceuticals and determine whether they can help reverse symptoms of chronic illness and whether a 28-day trial of NAC, ALA and GSH is superior to a 14-day trial.

The proposed RCT will involve 100 patients in the outpatient setting, in two arms of a study. A new clinic is now being established at UCI to evaluate PASC. Considering the large numbers of

longhaulers suffering with chronic symptoms, we may be able to enroll 100 patients within an 8-week time frame in the outpatient setting, however, full enrollment in the study for both arms may take longer, since not all patients will consent to be part of the trial. The details of the two arms of the study and primary and secondary objectives are listed below:

Diagrammatic Outline of Two Arms of The Horowitz Glutathione/Nutraceutical Protocol for COVID-19 Longhaulers (PASC) Figure 1

Arms A + B (Outpatient Setting) 100 Patients, Randomized to Arm A or Arm B



The powered study for the Horowitz 'NIL' (Nutraceuticals in Longhaulers) Hypothesis and Trial will evaluate the following parameters:

1. **Primary Objective: Reversing Symptoms of PASC:** This will involve evaluating symptoms before and after therapy, and determining which, if any symptoms are improved in each arm of the trial. This will be evaluated with a Likert scale (0= no change, +1, mild improvement; + 2= moderate improvement; +3= significant improvement; +4= resolution; -1=mildly worse; -2= moderately worse; -3=significantly worse; -4: debilitating; We will evaluate long COVID symptoms at 2 weeks, 1-, 2-, 3- and 4 months post therapy. **Symptom response will be evaluated with validated screening tools**, **including time to clinical recovery.** Symptom response will be evaluated by several questionnaires which will be filled out by patients during the time frame indicated above.

A. **The SF-36** (Medical Outcomes Trust, Boston, MA) is a multipurpose, short-form health survey with 36 questions. It yields an eight-scale profile of scores as well as physical and mental health summary measures. It is a generic measure, as opposed to one that targets a specific age, disease, or treatment group. Accordingly, the SF-36 has been useful in comparing general and specific populations, comparing the relative burden of diseases, differentiating the health benefits produced by a wide range of different treatments, and screening individual patients. (Ware, J. E., Jr. (1999). SF-36 Health Survey. In M. E. Maruish (Ed.), The use of psychological testing for treatment planning and outcomes assessment (p. 1227–1246). Lawrence Erlbaum Associates Publishers).

B. **COVID Severity of Symptoms Questionnaire:** This will evaluate the initial severity of symptoms (Table 1) and how symptom severity changes over time with treatment (Table 2). Secondary Endpoints: All causes mortality, Frequency of respiratory progression (defined as SPO2≤ 94% on room air or PaO2/FiO2 <300mmHg and requirement for supplemental oxygen or more advanced ventilator support), time to defervescence (in

those with fever at enrolment), frequency of requirement for supplemental oxygen or non-invasive ventilation, frequency of requirement for mechanical ventilation, frequency of serious adverse events as per DAIDS table grade of severity.

C. **COVID Time to Clinical recovery (TTCR).** See Table 3. These measures are based on a recent NIH trial (https://pubmed.ncbi.nlm.nih.gov/33081849/ and defined as the time (in hours/days) from initiation of the study treatment protocol (glutathione + NAC + ALA in arm A, X 28 days, or a multivitamin with minerals in arm B X 14 days, crossed over at day 15 to Arm A with glutathione + nutraceuticals X 14 days) until there is normalization of fever, respiratory rate, oxygen saturation, and alleviation of cough, sustained for at least 72 hours, as well as time to normalize associated symptoms (anosmia, dysgeusia, parosmia, exercise intolerance, fatigue, sore throat, myalgias, arthralgias, headache, brain fog, neuropsychiatric symptoms, diarrhea, etc.). (2) Time to SARS-CoV-2 RT-PCR negative in upper respiratory tract specimen (if positive at enrollment), time to laboratory recovery of each organ involvement. Some patients with PASC may theoretically reactivate their coronavirus viral load or have re-activated other viral infections (HHV-6, EBV, CMV, West Nile, etc.). If PCR +, time to becoming PCR negative will be measured in the outpatient setting as per UCI standard of care (patients will be seen in clinic on day 0, 14, 28). In the outpatient setting, RT-PCR can be measured on days 0, 14 and 28 when repeat laboratory testing is done (see above).

In order to rule out a floor effect (when most of our subjects score near the bottom of the questionnaires) and when there is very little variance because the floor of our test is too high (i.e., inflammatory markers remain high or health outcomes are rated as poor and can't get much worse/higher), and/or to rule out a ceiling effect (all of our subjects score near the top of the questionnaires and get better/low inflammatory markers that can't get much lower), we will have multiple measures evaluated in our study. Rather than depending on one outcome measure like cytokine levels, we will have multiple outcome measures in both arms (biochemical markers of inflammation, change in severity of symptoms, time to clinical recovery, progression to hospitalization). That way even if one measure demonstrates a ceiling or floor effect, our other measures can overcome these potential problems.

2. **Secondary Objective: determine underlying etiologies in PASC using the 16-point MSIDS model**

The etiology of long COVID needs to be determined. The MSIDS model postulates that multiple underlying sources of inflammation may be present increasing underlying illness. **These include primary sources of inflammation**:

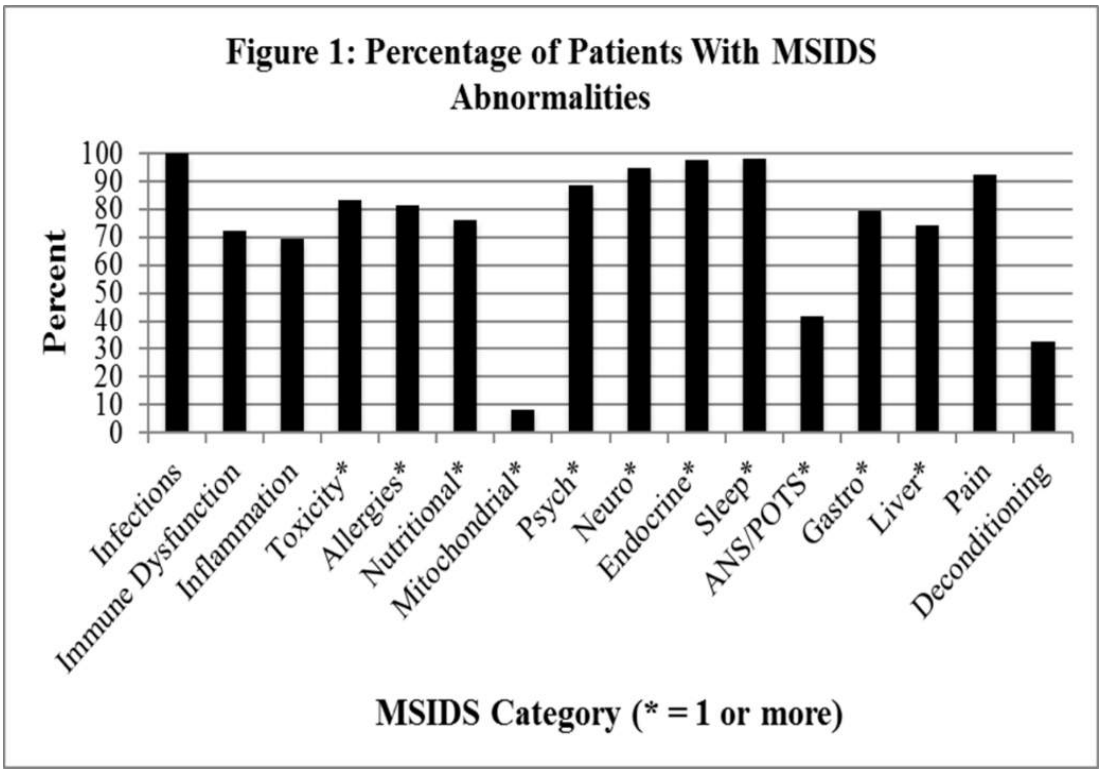
- 1) Chronic infections: bacterial, viral, parasitic, and/or fungal
- 2) Gastrointestinal **(**G.I.**):** Dysbiosis of intestinal bacteria
- 3) G.I.**:** Leaky gut with food allergies and sensitivities
- 4) Sleep disorders: may ↑ IL-6 and contribute to ongoing chronic symptomatology

- 5) Environmental toxins (heavy metals, mold…): can increase free radical/oxidative stress and overlap symptoms seen in other chronic fatiguing/musculoskeletal illness w/neurocognitive deficits
- 6) Nutritional Deficiencies (minerals: RBC and serum zinc, copper, magnesium, selenium, iodine, iron), amino acid/fatty acid deficiencies

Downstream effects of inflammation may include:

- 7) Endocrine disorders: i.e., low Testosterone, low adrenal (f), hypothyroidism, etc
- 8) Neurological dysfunction (headache, cognitive difficulties/brain fog, etc)
- 9) Psychological dysfunction : anxiety, depression…
- 10) POTS/dysautonomia : low blood pressure, elevated resting heart rates, vagal dysfunction of bladder/bowel
- 11) Mitochondrial Dysfunction secondary to free radical/oxidative stress
- 11) Pain Syndromes : arthralgias, myalgias, neuropathy..
- 12) Liver Dysfunction : elevated AST, ALT, alkaline phosphatase, T. bili, GGT
- 13) Kidney Dysfunction : elevated BUN/creatinine
- 13) Autoimmune phenomenon : elevated markers of autoimmunity, including ANA, RF, anti-ganglioside antibodies, GAD 65 antibodies

In a study published in the journal Healthcare in 2018 (Horowitz, R.I.; Freeman, P.R. Precision Medicine: The Role of the MSIDS Model in Defining, Diagnosing, and Treating Chronic Lyme Disease/Post Treatment Lyme Disease Syndrome **and Other Chronic Illness**: Part 2. Healthcare 2018, 6, 129. https://www.ncbi.nlm.nih.gov/pubmed/30400667) the most common MSIDS abnormalities found in chronically ill patients are listed below:



The MSIDS variables will therefore be evaluated in several stages. Stage one will include evaluation of the following variables at the beginning of the study in both arms:
Initial visit: fill out the COVID questionnaires listed above, as well as the Horowitz Lyme-MSIDS questionnaire (HMQ)[80] which is a statistically validated symptom questionnaire to determine the probability of Lyme and associated tick-borne disease overlapping PASC. Symptoms in both diseases overlap and therefore patients exposed to COVID may have reactivated or worsened an underlying tick-borne infection as Lyme and associated tick-borne diseases are now endemic in California. Differences between the two diseases include: loss of sense of smell and/or taste are only seen in COVID, and symptoms coming and going with good and bad days along with migratory pain are highly suggestive of Lyme disease.
Scoring the HMQ[80]:
A score of 63 or above (> 63): high likelihood of exposure to Lyme disease, especially if "migratory" pain is present
Score between 45-62 (probable Lyme disease)
Score between 25-44 (possible Lyme disease)
Healthy individuals scored < 24
Q's 1 +22 on the HMQ indicate possible co-inf w/ Babesia
Differential Diagnosis of Migratory Pain:[80]

- **Acute Rheumatic Fever**: if appropriate history, check ASO, anti-DNAase Ab
- **Crohn's Disease/Inflammatory Bowel Disease:** medical history, last colonoscopy, calprotectin (IBD).
- **Gonococcal Arthritis**: Based on medical history of STD's. Check for triad: suppurative arthritis, tenosynovitis, and dermatitis
- **Hepatitis (A, B, C, D, E):** if elevated LFT's, check viral Abs', PCR, RNA
- **Reactive Arthritis** (Salmonella, Yersinia, Chlamydia exposure…, HLA B 27+): likely if Reiters triad present: conjunctivitis, urethritis, arthritis post infection urogenital/GI tract
- **SLE (Lupus)**: check dsDNA, Smith Ag, specific markers for SLE. Amer Rheum criteria.
- **Lyme Disease**: this is the only disease with migratory nerve pain!

The questionnaire can be found at: https://cangetbetter.com/symptoms/

After evaluating symptoms on the COVID and Lyme questionnaire, the physician at the UCI COVID clinic will do a complete History and Physical with the chief complaints, past medical history, & current symptoms, defining severity and frequency
Social history, Family history
Environmental history (Mold exposure? Chemical exposure?)
Review of Systems & Physical examination
Differential Diagnosis and Testing

The first stage of the trial is therefore evaluating the role of NAC, ALA and GSH in reversing symptoms in longhaulers, and evaluating several of the most important variables on the 16-point MSIDS map that are potentially affecting long term symptoms. In Stage I, there will be no treatment of these variables, just a comprehensive evaluation. According to UCI standard of care, POTS/dysautonomia and laboratory abnormalities can be addressed in treatment trials in Stage II.

Testing during Stage I based on some of the most likely MSIDS variables affecting PASC: Labs will be drawn at time 0 during enrollment. Labs are highlighted in red and listed at table 5 below.

1. **Infections**: 4 Types (Bacteria, Parasites, Viruses, Candida/Fungus): Direct & Indirect Testing
   - **A. Bacterial:** we will focus the first stage of testing on Lyme disease and Bartonella, some of the most common bacterial infections found in TBD patients. These symptoms overlap those in PASC. Initial testing will include a **C6 Lyme ELISA, IgM & IgG ImmunoBlot (IgeneX), Bartonella spp., via Bartonella W Blot and FISH (IgeneX)**. IgeneX will partner with UCI for the tick-borne testing for Lyme, Babesia and Bartonella and provide it at a significantly reduced cost. A **VEGF** will be done as an indirect marker of active Bartonella.
   - **B. Parasitic**: **Babesia IFA (*B. microti, B. duncani/WA-1*), Babesia FISH** (all done through IgeneX), CBC, CMP
   - **C. Viruses**: **EBV (PCR), HHV6 (PCR), W Nile (flavivirus)**: these are viral infection that could potentially reactivate during COVID.
   - **D. Candida/Fungi/Mold**: **Stachybotrys titer**

2. **Immunity/Autoimmunity**: Rheumatology Panel: **ANA, RF, Immunology Panel would include measurements of IgA, IgM, IgG immunoglobulins w/ IgG subclasses, NK cells, CD4/CD8**
3. **Inflammation**: **ESR, hs-CRP, D-dimer, LDH, ferritin; lipid peroxides/F-2 isoprostane** for free radical oxidative stress. The Advanced Oxidative Stress profile will be done through Precision Point Diagnostics, which measures **total glutathione, reduced glutathione, F2-isoprostane, and 8-OH-2deoxyguanosine** from whole blood and urine specimens. These are important oxidative stress markers. We will also check:
   Vitamin D levels: we will check a **1,25/25 OH Vit D ratio**: a ratio greater than 2:1 implies inflammation/active IC infection[81]
4. **Toxicity:** during the initial intake, the physician will check for a history of Chemical Sensitivity, E.I. (Environmental Illness), Heavy Metal and/or Mold exposure, history of pesticide exposure/neurotoxins (working w/chemicals, see OSHA list if exposure)
   Heavy Metal testing to be done: **blood levels of mercury, lead, arsenic**
   Mold testing: initial testing with a **Stachybotrys titer**
5. **Allergies:** foods, drugs, environmental (mold, grasses, trees…)
   Food allergy panel: **IgE levels, IgG with subclasses**, **antigliadin antibody**
   To rule out food intolerances/leaky gut/Candida history, we will check a **zonulin level** in the blood (Precision Point Diagnostics laboratory may partner for testing, using their **Advanced Intestinal Barrier Assessment and 88 serum food allergy test**)
6. **Nutritional & Enzyme Deficiencies/ functional medicine abnormalities:**
   Nutritional testing: mineral levels (**RBC and serum Zinc**)
   Vitamin testing: **B1, B6, B12, MMA, Vitamin D levels (25 OH/,1-25 OH), homocysteine**
   Detox: **Glutathione levels will be checked in the blood before and after treatment. This will be drawn day 0, 14, 28**
7. **Mitochondrial dysfunction:** due to large amounts of free radical/oxidative stress with chemokine and cytokine release in COVID-19, mitochondrial dysfunction may be an important factor in chronic illness. Symptoms/diseases of mitochondrial dysfunction include:
   Brain: migraine, stroke-like events, ataxia, visual & hearing loss, dementia/AD, psychiatric disorders
   Muscle: weakness, cramping, fatigue, pain
   Nerve: Neuropathy, MS-like presentations

Endocrine: diabetes (mtDNA 3243 mutation)
Liver: hypoglycemia, NASH, hepatocellular Ca
Kidney: Fanconi syndrome
Cardiac: early cardiomyopathy, conduction defects
Systemic: CFS-like illness, FM symptoms, PASC?
Mitochondrial dysfunction: can therefore result in resistant fatigue, nerve dysfunction, cardiac problems, arthralgias/myalgias and neurological problems, which are all seen in PASC.
Laboratory: Check blood markers for free radical/oxidative stress: **lipid peroxides (blood), 8-OH d-guanine. The Advanced Oxidative Stress profile from Precision Point Diagnostics measures total glutathione, reduced glutathione, F2-isoprostane, and 8-OH-2deoxyguanosine from whole blood and urine specimens. Also check CoQ10 and carnitine levels (LabCorp)**

8. **Neurological dysfunction**: questionnaires will evaluate symptoms pre-post treatment. May record in the medical history any functional brain imaging: MRI studies, SPECT scans, PET scans (F-18), NeuroQuant? spinal tap. These tests will not be administered in this trial.
9. **Neuropsychiatric dysfunction**: Battery of tests--including the MMPI, PHQ-9, Beck Depression Inventory, MMSE, ADAS-Cog are available, but will not be administered in this trial. If these tests were performed pre-trial, they will be recorded in the initial History and Physical.
10. **Endocrine disorders**: low hormones (sex hormones, adrenal, & thyroid hormones, posterior pituitary hormones ADH, MSH, VIP). In stage 1 of the trial, hormone testing will include: **Hormone testing**: **TFTs (T3/T4, Free T3/T4, reverse T3, TSH)**, **adrenal function (DHEA/cortisol levels (saliva)**, sex hormones (**estradiol, progesterone**, **testosterone, free T, DHT and pregnenolone**). Parathyroid testing (PTH/Ca++) would only be done if hypercalcemia with normal albumin levels is seen on initial screening via a CMP.
11. **Sleep disorders**: multiple etiologies are possible based on medical literature reviews. These include:
    - **A.** Acute or chronic infections with cytokines
    - **B.** Acute/Delayed Sleep Phase Syndrome with Circadian Rhythm Disorders. ? (Obstructive Sleep apnea history, OSA),? history of Restless Leg Syndrome (RLS)→ sleep study would be necessary in stage 3 if there is no improvement in stage 1-2 of the trial. Similarly, if there is a history of Narcolepsy, Cataplexy→ MSLT study would be indicated
    - **C.** If significant nocturia is present (greater than 3x/night): check ADH, check if prostate history of BPH (Benign Prostatic hypertrophy)
    - **D.** Review caffeine intake during the day, amount, timing
    - **E.** ? Shift worker (SWS), evaluate in sleep history (HPI)
    - **F.** ? Environmental noises interfering with sleep, ? significant EMF exposure present with a history of electrosensitivity (check in initial H and P)

    Treatment of sleep disorders depends on the underlying etiologies. During the initial intake, the physician will record whether any of the above medical problems are present based on prior history (OSA, RLS, BPH, SWS, menopause…) and sleep medications (Ambien, Lunesta, Klonopin, Benadryl, etc.) and/or herbal treatments used/failed (melatonin, GABA, L-theanine, Valerian root, etc.). We will consider medication in phase 2 of the trial if still not sleeping, which includes medications to help with stage III/stage IV sleep (doxepin 10 mg, trazadone 100 mg, low dose mirtazapine (7.5-15 mg HS), low dose cyclobenzaprine (5 mg if frequent awakening) with or without herbal therapies (Cerenity PM, Herbsom, GABA-L theanine cream/powder, Valerian root, Honokiol, etc)

12. **ANS dysfunction +/- Postural Orthostatic Tachycardia Syndrome (POTS)**
    Symptoms may include: fatigue, dizziness standing, palpitations, elevated heart rate, anxiety, cognitive difficulties, presyncope/syncope. Vagal dysfunction may also affect the bladder (difficulty starting to urinate) and bowels (gastroparesis, chronic constipation). We will check **Autoantibodies against ganglionic acetylcholine receptors (gAChRs) as they have been associated with POTS, as well as anti-myelin AB's**.

    In the clinic during phase I of the trial: Check sitting and standing BP/pulse 4 x during the initial intake (time 0 = sitting for 5 minutes, check BP/pulse; then patient stands, and BP and pulse rates are checked at 3,6,9 minutes standing)

    A drop in BP of at least 10 points, and/or rise in pulse rate is c/w POTS (mild, mod, severe). [5] Mild POTS = increase in HR by up to 10-19 BPM standing; Moderate POTS = increase in HR by 20-29 BPM standing; Severe POTS = increase in HR of 30 BPM or higher. Treatment in phase 2 would include a high salt diet (one gram salt pills three times a day if no contraindication: i.e., CHF, cirrhosis, nephrotic syndrome), fluids (at least 2 liters a day), TED stockings (if significant edema), midodrine (5-10 mg TID, taken 4 hours apart). Resistant POTS may be seen in EDS, so any patient presenting with POTS/dysautonomia should be checked on physical exam for hypermobility, if present. [82]
    The patient will be given a home electronic BP machine to use to monitor home BP and pulse rates whether initially diagnosed with POTS/dysautonomia or not.
13. **G.I. disorders:** IBS (Irritable Bowel Syndrome), IBD (Inflammatory Bowel, i.e., Crohns, UC), Leaky gut, history of SIBO, parasites, hiatal hernia, ulcer history, esophagitis, etc. These may all affect gastrointestinal symptoms, which can be seen in PASC. In phase I of the trial, only a medical history of GI symptoms and prior testing will be recorded apart from checking for food sensitivities/allergies, leaky gut and mast cell activation disorder with an IgE and IgG expanded food allergy panel (Labcorp, Precision Point Diagnostics), Zonulin (Precision Point Diagnostics). If prior testing (listed below) has been done, they should be recorded in the medical history. These include:
    -Stool for Ova and Parasite testing (local, CDSA), H pylori testing (+breath test), Zonulin, histamine, chromogranin A, tryptase
    -Breath tests for fructose intolerance, leaky gut, SIBO (gas, bloating) with IBS symptoms
    -Antibody tests: Foods, parasites (Entamoeba, Giardia…)
    -Comprehensive Digestive Stool Analysis (CDSA): which may evaluate WBC's, enzyme deficiencies, inflammation, sigA (secretory IgA, low levels can be seen in food allergies), Candida, parasites, and microbiome abnormalities. The initial intake will record a history of an endoscopy, and/or colonoscopy to evaluate GERD, IBD [Crohn's].
14. **Elevated LFT's**: PASC may affect liver (and kidney function). Different tick-borne diseases (Lyme, Relapsing Fever [RF], Ehrlichia, Anaplasma, Rickettsial infections, like RMSF, Q-fever) can also ↑ LFT's, along with a history of hepatitis, hemochromatosis, Wilsons's disease, Alpha-1-antitrysin deficiency and NASH (Non-alcoholic Steatohepatitis). Medical intake should note presence of any of these prior abnormalities affecting the liver (i.e., history of TBD: Lyme, Relapsing Fever [RF], Ehrlichia, Anaplasma, Rickettsial infections, like RMSF, Q-fever). If LFT's are elevated, apart from blood tests evaluating exposure to prior TBD's, the following testing should be performed: test for **Alpha-1 antitrypsin deficiency**, which is a potential cause of cirrhosis, lung disease with emphysema (up to 10% population w/genetic variant). Ferritin will already be checked as an acute phase marker, which also can be a surrogate marker for iron overload and hemochromatosis. The medical history during the initial History and Physical will

therefore include evaluating for exposure to toxins (i.e., carbon tetrachloride, pesticides, mold...), and whether there is a history of NASH, ETOH use, and a history of hyperlipidemia, as well as any history of hepatitis, hemochromatosis, Wilson's disease or Alpha-1-AT deficiency. According to UCI protocols, if significant LFT elevations are present, a hepatitis panel (A, B, C), iron and ferritin levels (hemochromatosis, ferritin is also an acute phase reactant seen in early COVID with Macrophage Activation Syndrome), Ceruloplasmin levels (Wilson's disease, hemosiderosis) would be considered as reflex testing if LFT's remain elevated. An US of the liver would be considered in the differential diagnosis in phase II or III to rule out NASH if above laboratory testing is negative despite elevated LFT's as per UCI standards.

15. **Pain syndromes**: PASC and TBD's may cause myalgias, arthralgias and neuropathy. The initial intake will provide a list of medications (NSAIDS, narcotic use) used for pain, as well as neuroleptics used for neuropathy (amitriptyline, duloxetine, gabapentin, Lyrica [pregabalin]), with dosage, efficacy, side effects.
16. **Deconditioning**: any chronic illness like PASC for several months may lead to deconditioning. A history of physical activities, yoga, weight training, etc. will be noted during the initial 16-point MSIDS intake.

The two arms for phase I of the study are listed below:

Arm 1: As there is no standard of care for treating Long COVID in the outpatient setting, the study design will be to randomize outpatients to one of two protocols. After taking a clinical history and doing the above testing and administering questionnaires, patients will be put in one of two treatment arms. Arm A will be given a COVID MedPack-1 (Xymogen) taken twice a day X 28 days, which contains in total: NAC 2400 mg BID and alpha lipoic acid (ALA) 600 mg BID. This will be taken for 28 days along with liposomal glutathione (250 mg/capsules, Wellness Pharmacy) 8 capsules two times a day (2000 mg BID) for 28 days. The NAC, ALA, and glutathione block NF-κB, these nutraceuticals will stimulate Nrf2 pathways, and they support a healthy immune response. [44] [58] [31]

Arm 2: The other randomized arm of the trial will receive a COVID MedPak-2 (Xymogen) which will contain a multivitamin and minerals (magnesium), taken x 14 days. On Day 15, Arm 2 will be crossed over to the same nutraceuticals and doses in Arm 1 (MedPax-1 twice a day with NAC and ALA) along with 8 capsules of liposomal GSH (Wellness Pharmacy) twice a day. We will therefore evaluate the efficacy of a 14-day vs 28-day trial of nutraceuticals (NAC, ALA, GSH) in PASC.

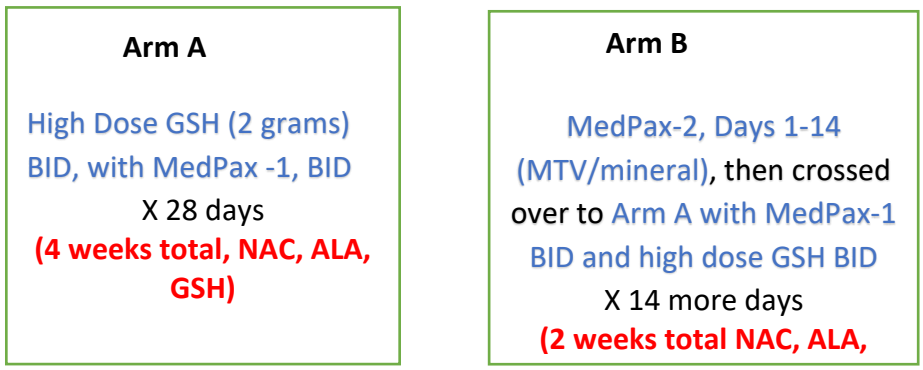
In both Arm A and Arm B, oxygen saturations will be measured with a pulse oximeter given to each study participant, and temperature/fevers and blood pressure/pulse will be measured with a digital thermometer and BP cuff, also provided at the time of enrollment.

Study Calendar: Labs that will be drawn at time 0, 15 and day 28 for each participant in all arms of the study will include a UCI panel with a CBC and lymphocyte count, CMP (electrolytes, LFT's, BUN/creatinine), and laboratory values listed above in the 16-point MSIDS evaluation.

An initial pulse oximetry will be recorded, and ABG will be done if oxygen saturations are less than 90% (as per UCI protocol). An initial CXR will be done at the time of enrollment only if there is ongoing shortness of breath (in all groups) who have tested positive for COVID-19. A repeat CXR in the outpatient group will only be done if COVID pneumonia is present, if there is clinical deterioration (increased cough, shortness of breath) or if oxygen saturations drop below 90%. Any patient with a CXR suggestive of COVID pneumonia with a positive procalcitonin, will receive doxycycline 100 mg BID and zinc 40 mg/day as per early clinical published research, [83] or as per ID protocols at UCI. Probiotics including a broad spectrum acidophilus and *saccharomyces boulardii* will be prescribed BID if patients are on antibiotics to prevent antibiotic associated diarrhea. [84] [85]

Outpatient testing: Patients in Arm A and B will be sent home with their own pulse oximeters, blood pressure cuffs and thermometers, instructing them to check in by phone and video chat when needed with a clinical coordinator if questions arise (9 am – 5 pm PST). Temperature, pulse oximetry and blood pressure will be checked twice a day (i.e., 8 am, 8 pm), and recorded in a patient diary provided at enrollment. (Kranthi Sitammagari, MD et al. Insights From Rapid Deployment of a "Virtual Hospital" as Standard Care During the COVID-19 Pandemic. Annals of Int Med. 11 Nov 2020. https://www.acpjournals.org/doi/10.7326/M20-4076)
Subgroup analysis will evaluate symptom relief, time to clinical recovery (TTCR) and the above secondary endpoints with factors on the 16-point MSIDS model discovered during the initial evaluation. Serum glutathione levels will be checked at enrollment, day 15 and at day 28, evaluating changes in blood levels, which will be correlated with clinical response.
Once each group has finished 28 days of their protocol (arms A + B), they will be evaluated with screening questionnaires at the end of months 1, 2, 3 and 4 of the trial. This will allow us to evaluate the effect of blocking inflammation and supporting glutathione production in the body in Arm A for 28 days, vs simply giving a multivitamin without anti-inflammatory support during the first 14 days (Arm B) and then crossing over to the treatment arm (Arm A) X 14 days. The study schema listed below outlines the three arms of the study.
Study Calendar: Apart from standardized lab testing for all groups on day 0 (beginning of the trial), and day 15 and 28 (listed above), all patients will fill out validated screening questionnaires at time 0 (testing positive in the outpatient setting or testing positive and being admitted to the medical ward), and then again on days 15 and 28 during month one, with follow-up questionnaire to evaluate progression of symptoms of long COVID, at the end of month 2, 3 and 4, which will include questions on active symptoms and time to clinical recovery. This will include the SF-36, HMQ and Questionnaires 1, 2, 3 (Table 1, 2, 3). The follow-up questionnaires will be administered at one-month, two, three- and four-months post trial, to evaluate long term sequelae and potential reversal of COVID longhauler status.
Randomization: The randomization will be done using a secured central computer-based randomization using a secure website using a central, computer-based randomization program in a ratio of 1:1.

Diagrammatic Outline of Two Arms of The Horowitz Glutathione Nutraceutical Protocol for COVID-19 Longhaulers (PASC) Figure 1



Inclusion Criteria: Patients greater than 18 years old, with positive COVID testing (AB, RT PCR) and mild-severe symptoms (see Table 1 for COVID symptom list)
Exclusion Criteria: history of a severe sulfa sensitivity (i.e., anaphylaxis, Stevens Johnson Syndrome), allergy to meat products and/or gelatin (alpha gal allergy) and/or a history of an allergic or adverse reaction to NAC, alpha lipoic acid and/or GSH.

Since glutathione is made in the liver phase II pathways, oral GSH does not usually cause severe reactions, even in those who have a mild-moderate sulfa sensitivity. Zyrtec (cetirizine) 10 mg PO QD and Famotidine 20 mg PO QD (H1 and H2 blockade) can be administered during the trial to those who have a history of mild- moderate sulfa sensitivity to minimize the possibility of any reactions. IV glutathione may cause mild allergic reactions in those with multiple chemical sensitivity and severe allergies to Bactrim (sulfamethoxazole/trimethoprim), but it is not usually seen with PO administration. Therefore, only a history of Stevens Johnson Syndrome would be an absolute contra-indication to use of NAC and GSH.

Potential Side Effects: no significant adverse side effects from the nutritional supplements in the trial based on years of experience prescribing this protocol for other indications. We have successfully treated over 60 COVID patients at the Hudson Valley Healing Arts Center by February 2021 with ivermectin, glutathione and nutraceuticals (arm 1) with no progression of illness and no adverse side effects. Rarely, individuals with significant reactive hypoglycemia may experience an increase in blood sugar swings with alpha lipoic acid 600 mg BID (which was not noted in our pilot study of 60 patients). Participants should be informed that if they have type I or type II diabetes, or a history or reactive hypoglycemia, that strict adherence to a diabetic diet, i.e., Mediterranean/Paleo type diet (40% healthy protein/30% healthy fats [olive oil, avocado], 30% complex carbohydrates) is necessary to avoid increased blood sugar swings, and should adjust diabetic medication accordingly, as per their HCP.

Xymogen will be labeling the MedPax as '1 and 2', as these will be printed on each of the packets/boxes. We can also use a sticker to label the patient number on the packet or box. Xymogen will be contacted immediately once we have a start date so they can begin production. After the patient is randomized to MedPax 1 or 2, they will receive a typed instruction sheet on how to take the supplements (with meals, but one hour away from antibiotics to avoid any minerals interfering with absorption); The liposomal glutathione pills are to be taken one at a time with a sip of water, until reaching the dose of 8 capsules (2000 mg); A compliance sheet can be provided to make sure patients do not miss any doses (check marks/signatures can be placed on the sheet after each time they take their medication/supplements), as illustrated below (dates are appx, patients will fill in the start date):

Arm A: Glutathione + MedPax 1: Nutraceuticals include: NAC, ALA, GSH

14-day Compliance Checklist

Date	Morning Dose	Evening Dose
Day 1, May 1		
May 2		
May 3		
May 4		
May 5		
May 6		
May 7		
May 8		
May 9		
May 10		
May 11		
May 12		
May 13		
May 14		
Until May 28		

Arm B: MedPax 2: Nutraceuticals: multivitamin, multimineral

14-day Compliance Checklist. Will cross over to Arm A on day 15

Date	Morning Dose	Evening Dose
Day 1, May 1		
May 2		
May 3		
May 4		
May 5		
May 6		
May 7		
May 8		
May 9		
May 10		
May 11		
May 12		
May 13		
May 14		

Patients will submit their completed compliance sheets online, as well as fill out the validated symptom questionnaires and submit them online in a HIPPA compliant fashion (unless an app is available through UCI). A patient coordinator for the study will be available to answer questions between 9 am and 5 pm, and an emergency line will also be provided for the study participants.

The primary goal of the randomized clinical study will be to evaluate whether the glutathione protocol, along with nutraceuticals is superior to a multivitamin/mineral in reversing symptoms of PASC. The second end point of the study, which will involve all 100 patients, will be to evaluate whether 14 days or 28 days of NAC, ALA and GSH differentially affect longhauler symptoms. The third end point will evaluate which of the 16-point MSIDS variables are present in PASC.
Validated symptom questionnaires evaluating potential exposure to Lyme disease (HMQ), fatigue (SF-36), as well as common ENT and respiratory symptoms along with psychological manifestations (Int J Environ Res Public Health, see below) will be used to follow treatment response, along with the standard of care practiced at the UCI. We added the following 3 symptoms to the ENT questionnaire, which are part of the COVID symptom complex, but not included in the initial version of the ENT questionnaire: chills, vomiting, brain fog/memory problems.
The initial intake: This should contain chief complaints, Past Medical History (PMH), medications, supplements, allergies/intolerances, Review of Systems (ROS) as per UCI protocol. **Risk factors listed in the scientific literature should also be included in the initial intake**: Advanced age (≥ 65), male gender, low T, race (African-American, Hispanic, Native American), ? type O blood (if known) with CCL-2 SNPs, obesity, smoking history, as well as a PMH: HTN, DM, Cardiovascular and respiratory disease (asthma, emphysema), Hemorrhagic or ischemic strokes, immunosuppression, cancer, chronic kidney and liver disease & secondary infections; children however are also at potential risk with MIS (Multi-inflammatory Syndrome).
Rating Severity of Symptoms: when asking patients to rate the overall severity of their symptoms and change from their last report, scoring for each question will allow for both improvement and worsening. The initial questionnaire will determine the severity of symptoms at the beginning of the trial. Follow up questionnaires will evaluate the severity of symptoms since the last report: (0 [no change], + 1 [mild improvement], + 2 [moderate improvement], + 3 [significant improvement], +4 [symptom resolved]. - 1 [mild worsening], - 2 [moderate worsening], - 3 [significant worsening], -4 [debilitating].

Table 1: Initial COVID Symptom Questionnaire, Day of Enrollment, w/ FU days 15 + 28, and at the end of months 2, 3 & 4.
Horowitz, R.I. (modified from Int J Environ Res Public Health)

COVID Longhauler symptom	**None (Not applicable)**	**Mild**	**Moderate**	**Severe**
Loss of sense of smell				
Loss of sense of taste				
Altered Sense of Smell (smelling abnormal odors)				
Decreased appetite				
Sore throat				
Low grade fever		(99.9 F or below)	100 degrees-101.9 F	Greater than 102 F
Chronic fatigue				
Exercise intolerance				
Excessive sweating				
Skin rashes				
Muscle pains				
Joint pains				
Headaches				
Ringing in the Ears (tinnitus)				
Brain fog (difficulty concentrating, memory prob's)				
Light sensitivity				
Blurry vision				
Anxiety				
Depression				
Psychosis (hearing voices, etc)				
Insomnia				
Chest tightness				
Chronic cough				
Sputum Production				
Shortness of breath				
Dizziness				
Palpitations				

Elevated Resting Heart rate				
Dysphagia				
Nausea				
Vomiting				
Cramping (GI)				
Diarrhea				
Hair loss (alopecia)				

Table 2: COVID Symptom Questionnaire, Change in Severity of Symptoms Over Time Day 15, 28, end of months 2, 3, 4. Horowitz, R.I.

COVID Longhauler symptom	None N/A	No Change	+1 Mild Improv	+2 Mod Improv	+3 Signif. Improv	+4 Resol-ved	- 1 Mildly Worse	-2 Mod Worse	-3 Signif. Worse	-4 Debilitating
Loss of sense of smell										
Loss of sense of taste										
Altered Sense of Smell (smelling abnormal odors)										
Decreased appetite										
Sore throat										
Low grade fever										
Chronic fatigue										
Exercise intolerance										
Excessive sweating										
Skin rashes										
Muscle pains										
Joint pains										
Headaches										
Ringing in the Ears (tinnitus)										
Brain fog (difficulty concentrating,										

memory prob's)										
Light sensitivity										
Blurry Vision										
Hair loss										
Anxiety										
Depression										
Psychosis (hearing voices, etc)										
Insomnia										
Chest tightness										
Chronic cough										
Sputum Production										
Shortness of breath										
Dizziness										
Palpitations										
Elevated Resting Heart rate										
Dysphagia										
Nausea										
Vomiting										
Cramping (GI)										
Diarrhea										

Rating Time to Clinical Recovery: Starting on day one of your treatment, if symptoms have improved or worsened, please rate the time it took for symptoms to change since your last treatment: (0 [no change], +1 [improved within 7 days], + 2 [improved within 14 days], +3 [improved within 28 days] + 4 [symptom resolved]; - 1 [worsened within 7 days], -2 [worsened within 14 days], - 3 [worsened within 28 days], - 4 [debilitating last 28 days]

Compared to the last time you filled out the COVID symptom questionnaire (please review your prior questionnaire to ensure accuracy and avoid recall bias)
Table 3: Time to Clinical Recovery: Evaluation of Symptoms Day 15 and 28 (month 1), and at the end of months 2, 3 and 4
Horowitz, R.I. (based on a recent NIH trial (https://pubmed.ncbi.nlm.nih.gov/33081849/) and Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet. 2020;395(10223):497-506. doi:10.1016/S0140-6736(20)30183-5)

COVID Longhauler symptom	None N/A	No Change	+1 Improved in 7 days	+2 Improv in 14 days	+3 Improv in 28 days	+4 Resol-ved	- 1 Worse last 7 days	-2 Worse last 14 days	-3 Worse last 28 days	-4 Debilitating last 28 days
Loss of sense of smell										
Loss of sense of taste										
Altered Sense of Smell (smelling abnormal odors)										
Decreased appetite										
Sore throat										
Low grade fever										
Chronic fatigue										
Exercise intolerance										
Excessive sweating										
Skin rashes										
Muscle pains										
Joint pains										
Headaches										
Ringing in the Ears (tinnitus)										
Brain fog (difficulty concentrating, memory prob's)										
Light sensitivity										
Hair loss										

Anxiety										
Depression										
Psychosis (hearing voices, etc)										
Insomnia										
Chest tightness										
Chronic cough										
Sputum Production										
Shortness of breath										
Dizziness										
Palpitations										
Elevated Resting Heart rate										
Dysphagia										
Nausea										
Vomiting										
Cramping (GI)										
Diarrhea										

https://www.phenxtoolkit.org/toolkit_content/PDF/MGH_CTSM_Symptoms.pdf
Symptoms can be collected via a secure online questionnaire vs app. After initial evaluation (questionnaires from tables 1,2,3), follow-up questionnaires regarding severity and time to resolution will be administered days 15, 28 (one-month post trial), and 2, 3 and 4-months post trial, to evaluate long term sequelae and any reversal of initial symptoms seen in COVID longhauler status. According to a recent JAMA article, accumulating evidence is showing morbidity beyond the initial infection (Datta SD, Talwar A, Lee JT. A Proposed Framework and Timeline of the Spectrum of Disease Due to SARS-CoV-2 Infection: Illness Beyond Acute Infection and Public Health Implications. JAMA. Published online November 18, 2020. doi:10.1001/jama.2020.22717). We will capture data post glutathione with NAC and ALA at time 0, 15, 28, and at the end of months 2, 3 and 4 to evaluate residual symptoms and whether a post-acute hyperinflammatory illness and late inflammatory sequalae still exist.

“Although much of the response to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has focused on acute coronavirus disease 2019 (COVID-19) illness, accumulating evidence demonstrates morbidity beyond acute SARS-CoV-2 infection.1-4 At least 2 other periods of illness appear to be temporally associated with SARS-CoV-2 infection: a rare postacute hyperinflammatory illness and late inflammatory and virological sequelae. These 3 illness periods not only define the temporal course of SARS-CoV-2 infection at the population level but also capture distinct phases of host-viral interaction. A theoretical framework describing illness periods of SARS-CoV-2 infection (including clinical presentations and timing of onset), their pathophysiological underpinnings, and associated key laboratory findings may

contribute to a more inclusive and ordered understanding of the natural history of SARS-CoV-2 infection and enhance research efforts. Within the proposed framework, a patient may experience any combination of these illnesses or may have asymptomatic infection without illness"
The initial validated ENT questionnaire is listed below for reference.

PMC full text: Int J Environ Res Public Health. 2020 Jul; 17(14): 5218.

Published online 2020 Jul 20. doi: 10.3390/ijerph17145218

Severe complications of COVID published in the medical literature include: Novel Coronavirus Pneumonia (NCP) with or without acute respiratory distress syndrome (ARDS) and respiratory failure, organ function damage with cardiac injury and fulminate myocarditis, pneumothorax, liver dysfunction & acute kidney injury. [86] [87] These potential complications will be monitored by the onsite investigator (Dr Alpesh Amin) for outpatients, and recorded if there is any significant worsening of symptoms during the trial. See Table 4.

Table 4: Severe Complications of COVID-19: Clinical Evaluation & Follow-up

Patient #	**Symptom history Which of the following were present?**	Yes/ No	Treated as Out patient	Was Treated as an Inpatient Medical Ward (Y/N)	Was in the ICU (Y/N)	Progression Of Illness Y/N Over Time
	NCP (pneumonia)					
	ARDS/ Resp Failure					
	Cardiac injury					
	Pneumothorax					
	Liver Dysfunction					
	Kidney Injury					

Laboratory markers which will be followed during the GSH and Nutraceuticals Longhaulers RCT are based on a medical literature review of the most common complications reported and potential MSIDS variables affecting long term outcome.

Table 5: Procedures/Laboratory Evaluation for PASC
All labs shown below are drawn on day 0 and 28 except: Day 14 labs include only a CBC, CMP, LDH, hs-CRP, ferritin, D-dimer, fibrinogen, CPK, ESR, serum and reduced GSH levels, lipid peroxides, F2 Isoprostane, 8-OH d-guanine.

Infectious disease testing is only drawn on Day 0 (C6 ELISA, IgM and IgG Immunoblots, *Babesia microti* IFA, *B. duncani* IFA, Babesia FISH, Bartonella W Blot, Bartonella FISH, VEGF, HHV-6 PCR, EBV PCR, West Nile IFA

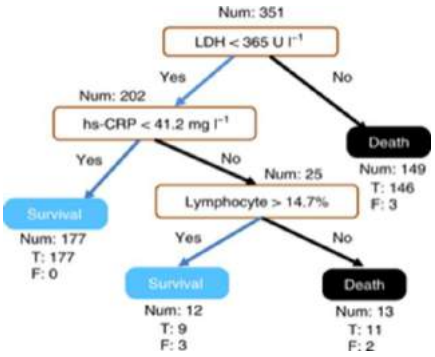
Table 5: Procedures/Laboratory Evaluation for PASC

Patient Number: **Procedures, test time**	**Day 0**	**Day 14**	**Day 28**
Oxygen sat% (pulse oximetry) 0,14,28			
Vital signs (sitting/standing BP and pulse rates) with Temperature 0,14,28			
CBC and diff 0,14,28			
CMP (BUN, Creatinine, lytes, LFT's 0, 14, 28			
LDH 0, 14, 28			
Hs-CRP 0, 14, 28			
Ferritin 0, 14, 28			
D-dimer 0, 14, 28			
Fibrinogen 0, 14, 28			
CPK/ 0, 14, 28			
ESR 0, 14, 28			
Serum Zinc 0			
RBC Zinc 0			
Hg serum level 0			
Pb serum level 0			
Serum selenium level 0			
Stachybotrys titer 0			
Serum GSH 0, 14, 28			
Reduced GSH 0, 14, 28			
Lipid peroxides 0, 14, 28			
F2 Isoprostane 0, 14, 28			
8 OH d-Guanine 0, 14, 28			
25 OH Vit D level 0			
C6 ELISA(IgeneX) 0			
Lyme IgM Immunoblot (IgeneX) 0			

0 Lyme IgG Immunoblot (IgeneX) Procedures/test times			
***Bab microti* IFA (IgeneX) 0**			
***Bab duncani* IFA (IgeneX) 0**			
Bab FISH (Igenex) 0			
Bartonella W blot (IgeneX) 0			
Bartonella FISH (IgeneX) 0			
Tick-borne Relapsing Fever PCR panel (IgeneX) 0			
HHV-6 PCR 0			
EBV PCR 0			
West Nile IFA 0			
ANA 0, 28			
RF 0, 28			
IgM level 0			
IgG level 0			
IgE level 0			
IgG Food panel (88, Precision Point Diagnostics 0			
Zonulin (Precision Point Diagnostics) 0			
B12 0			
Folic acid 0			
Methylmalonic acid (MMA) 0			
Homocysteine 0			
Co Q 10 level 0			
Carnitine level 0			
T3 0			
T4 0			
TSH 0			
DHEA/cortisol saliva test (Genova) 0			
Pregnenolone level 0			
Total testosterone 0			
HbA1c 0			

CD4/CD8 counts 0			
NK cell count 0			

Testing during Stage I is based on likely MSIDS variables potentially affecting PASC.
The laboratory markers of inflammation before and after the trial to stratify risk and outcome, are based on establishment of published host risk scores, [88] hospitalization rates and characteristics of patients hospitalized with COVID-19, [89] clinical course and risk factors for mortality of adult inpatients with COVID-19, [90] Apache II scores, [91] as well as an interpretable mortality prediction model for COVID-19 patients (see figure 1 below). [92] As there is a direct correlation between the lower level of serum testosterone, inflammatory cytokines, disease severity, and poor clinical outcomes among male patients with COVID, [93] we will also check testosterone levels on the initial evaluation.
Figure 1: From 'Yan, L., et al. An Interpretable mortality prediction model for COVID 19 patients. Nature Machine Intelligence. https://doi.org/10.1038/s42256-020-0180-7



Other complications of COVID-19 include secondary Hemophagocytic Lymphohistiocytosis (sHLH), also known as Macrophage Activation Syndrome (MAS). This is a hyperinflammatory syndrome characterized by a fulminant and fatal hypercytokinemia with multiorgan failure. It is seen in approximately 4% of cases triggered by viral infections and/or sepsis.[25 94 95]
Cardinal features of sHLH/MAS include unremitting fever, cytopenia's, low or absent natural killer (NK) cell activity, hepatosplenomegaly (HSM), hepatobiliary dysfunction (HBD), coagulopathy, increased ferritin levels (≥500), fasting triglycerides ≥265 mg/dl with fibrinogen levels ≤150 mg/dl. These markers along with CD4/CD8 counts and Apache scores (inpatient) will therefore be included in data collection for the RCT (if patients were hospitalized), as they can be associated with pulmonary involvement with ARDS (Ground glass opacities [GGO], crazy paving pattern, ill-defined consolidation), which can occur in up to 50% of pts, and in those with fatal complications of COVID-19. [25 96] This is especially important since eighty one percent of critically ill COVID-19 patients develop life-threatening ARDS and hypoxemic respiratory failure. [97]
Therefore, the laboratory markers of inflammation listed above (and previously mentioned levels of hS-CRP, LDH and cytopenias with CD4/CD8 counts, will be included in the study along with D-dimers, ferritin levels, and fibrinogen to evaluate changes in critical lab values over time with treatment.

The two arms of the treatment protocol will therefore be based on the above scientific review. As previously stated, no harm is to be expected in giving NAC, ALA, glutathione, or a multivitamin. The only rare potential side effect that would normally be seen is nausea with higher dose NAC, and with alpha lipoic acid at a dose of 600 mg BID (in 3 patients in 30 years in Dr Horowitz's practice) which could result in an increase in reactive hypoglycemia. Therefore, a low carbohydrate diet, with healthy protein and fats, and small frequent meals, could be considered as a dietary recommendation for the COVID-19 trial through UCI to help prevent reactive hypoglycemia. If patients are ruled out for reactive hypoglycemia, severe sulfa sensitivity, intolerance to these supplements, and/or multiple chemical sensitivity, no expected harm should be seen.

If the NIL RCT shows positive effects in the long term (i.e., reversing COVID longhauler status), it would allow us to effectively treat and potential reverse the morbidity and disability associated with PASC. Once the initial data is back, we also can design a phase II trial for MSIDS abnormalities found during Phase I, following the UCI standard of care. Please let me know if any other information is required before engaging in the RCT and thank you again for the opportunity to help find answers in those affected with long COVID.

Sincerely,

Dr Richard Horowitz
Medical director, Hudson Valley Healing Arts Center
Board certified internal medicine
Member, NYS DOH Tick-borne Disease Working Group 2021
Member HHS Tick-borne Disease Working Group 2017-2019
Co-chair HHS Other Tick-borne Diseases and Co-infections subcommittee 2017-2019
Member, HHS Babesia and Other Tick-borne Pathogens 2019-2020

Scientific References:

1. Horowitz RI, Freeman PR. Three novel prevention, diagnostic, and treatment options for COVID-19 urgently necessitating controlled randomized trials. *Med Hypotheses*. 2020;143:109851. doi:10.1016/j.mehy.2020.109851

2. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*. 2020;395(10223):497-506. doi:10.1016/S0140-6736(20)30183-5

3. Li X, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. *J Pharm Anal*. Published online March 5, 2020. doi:10.1016/j.jpha.2020.03.001

4. Distinct inflammatory profiles distinguish COVID-19 from influenza with limited contributions from cytokine storm | Science Advances. Accessed November 14, 2020. https://advances.sciencemag.org/content/early/2020/11/13/sciadv.abe3024

5. Horowitz RI, Freeman PR. Precision Medicine: The Role of the MSIDS Model in Defining, Diagnosing, and Treating Chronic Lyme Disease/Post Treatment Lyme Disease Syndrome and Other Chronic Illness: Part 2. *Healthcare*. 2018;6(4):129. doi:10.3390/healthcare6040129

6. Horowitz R. *Why Can't I Get Better? Solving the Mystery of Lyme and Chronic Disease*. 1 edition. St. Martin's Press; 2013.

7. Horowitz R. *How Can I Get Better?: An Action Plan for Treating Resistant Lyme & Chronic Disease*. 1 edition. St. Martin's Griffin; 2017.

8. Xiong Q, Xu M, Li J, et al. Clinical sequelae of COVID-19 survivors in Wuhan, China: a single-centre longitudinal study. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. 2021;27(1):89-95. doi:10.1016/j.cmi.2020.09.023

9. Huang C, Huang L, Wang Y, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. *The Lancet*. 2021;397(10270):220-232. doi:10.1016/S0140-6736(20)32656-8

10. Clayton EW. Beyond myalgic encephalomyelitis/chronic fatigue syndrome: an IOM report on redefining an illness. *JAMA*. 2015;313(11):1101-1102. doi:10.1001/jama.2015.1346

11. COVID-19 and POTS: Is There a Link? Accessed March 21, 2021. https://www.hopkinsmedicine.org/health/conditions-and-diseases/coronavirus/covid19-and-pots-is-there-a-link

12. Karas B, Grubb BP, Boehm K, Kip K. The postural orthostatic tachycardia syndrome: a potentially treatable cause of chronic fatigue, exercise intolerance, and cognitive impairment in adolescents. *Pacing Clin Electrophysiol PACE*. 2000;23(3):344-351.

13. Kanjwal K, Karabin B, Kanjwal Y, Grubb BP. Postural orthostatic tachycardia syndrome following Lyme disease. *Cardiol J*. 2011;18(1):63-66.

14. Wells R, Spurrier AJ, Linz D, et al. Postural tachycardia syndrome: current perspectives. *Vasc Health Risk Manag*. 2017;14:1-11. doi:10.2147/VHRM.S127393

15. Grubb BP. Postural Tachycardia Syndrome. *Circulation*. 2008;117(21):2814-2817. doi:10.1161/CIRCULATIONAHA.107.761643

16. Thieben MJ, Sandroni P, Sletten DM, et al. Postural orthostatic tachycardia syndrome: the Mayo clinic experience. *Mayo Clin Proc*. 2007;82(3):308-313. doi:10.4065/82.3.308

17. Townsend L, Dyer AH, Jones K, et al. Persistent fatigue following SARS-CoV-2 infection is common and independent of severity of initial infection. *PLOS ONE*. 2020;15(11):e0240784. doi:10.1371/journal.pone.0240784

18. Petersen MS, Kristiansen MF, Hanusson KD, et al. Long COVID in the Faroe Islands: A Longitudinal Study Among Nonhospitalized Patients. *Clin Infect Dis*. 2020;(ciaa1792). doi:10.1093/cid/ciaa1792

19. Carfì A, Bernabei R, Landi F. Persistent Symptoms in Patients After Acute COVID-19. *JAMA*. Published online July 9, 2020. doi:10.1001/jama.2020.12603

20. Xie L, Liu Y, Xiao Y, et al. Follow-up study on pulmonary function and lung radiographic changes in rehabilitating severe acute respiratory syndrome patients after discharge. *Chest*. 2005;127(6):2119-2124. doi:10.1378/chest.127.6.2119

21. Bai L, Gu L, Cao B, et al. Clinical Features of Pneumonia Caused by 2009 Influenza A(H1N1) Virus in Beijing, China. *Chest*. 2011;139(5):1156-1164. doi:10.1378/chest.10-1036

22. Horowitz RI, Freeman PR, Bruzzese J. Efficacy of glutathione therapy in relieving dyspnea associated with COVID-19 pneumonia: A report of 2 cases. *Respir Med Case Rep*. 2020;30:101063. doi:10.1016/j.rmcr.2020.101063

23. Guloyan V, Oganesian B, Baghdasaryan N, et al. Glutathione Supplementation as an Adjunctive Therapy in COVID-19. *Antioxidants*. 2020;9(10):914. doi:10.3390/antiox9100914

24. Gadek JE, Pacht ER. The Interdependence of Lung Antioxidants and Antiprotease Defense in ARDS. *CHEST*. 1996;110(6):273S-277S. doi:10.1378/chest.110.6_Supplement.273S

25. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. *The Lancet*. 2020;395(10229):1033-1034. doi:10.1016/S0140-6736(20)30628-0

26. Taís Mazzini Setti (TM) MD, Thiago Setti (TS) MD, Lucas Furtado da Fonseca (LF) MD, Stephany Cares Huber P, Santos (GS)* GS, José Fábio Santos Duarte Lana (JL) MD. The role of Glutathione as an adjunct therapy in the treatment of patients with COVID-19-Related Acute Respiratory Syndrome. *J Med Clin Sci*. 2021;7(02):415-427. doi:10.15520/arjmcs.v7i02.247

27. Pincemail J, Cavalier E, Charlier C, et al. Oxidative Stress Status in COVID-19 Patients Hospitalized in Intensive Care Unit for Severe Pneumonia. A Pilot Study. *Antioxidants*. 2021;10(2):257. doi:10.3390/antiox10020257

28. Polonikov A. Endogenous Deficiency of Glutathione as the Most Likely Cause of Serious Manifestations and Death in COVID-19 Patients. *ACS Infect Dis*. 2020;6(7):1558-1562. doi:10.1021/acsinfecdis.0c00288

29. Lang CA, Naryshkin S, Schneider DL, Mills BJ, Lindeman RD. Low blood glutathione levels in healthy aging adults. *J Lab Clin Med*. 1992;120(5):720-725.

30. Ghezzi P. Role of glutathione in immunity and inflammation in the lung. *Int J Gen Med*. 2011;4:105-113. doi:10.2147/IJGM.S15618

31. Diotallevi M, Checconi P, Palamara AT, et al. Glutathione Fine-Tunes the Innate Immune Response toward Antiviral Pathways in a Macrophage Cell Line Independently of Its Antioxidant Properties. *Front Immunol*. 2017;8. doi:10.3389/fimmu.2017.01239

32. Morris D, Khurasany M, Nguyen T, et al. Glutathione and infection. *Biochim Biophys Acta BBA - Gen Subj*. 2013;1830(5):3329-3349. doi:10.1016/j.bbagen.2012.10.012

33. Aldini G, Altomare A, Baron G, et al. N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why. *Free Radic Res*. 2018;52(7):751-762. doi:10.1080/10715762.2018.1468564

34. Shay KP, Moreau RF, Smith EJ, Smith AR, Hagen TM. Alpha-lipoic acid as a dietary supplement: molecular mechanisms and therapeutic potential. *Biochim Biophys Acta*. 2009;1790(10):1149-1160. doi:10.1016/j.bbagen.2009.07.026

35. Cho S, Urata Y, Iida T, et al. Glutathione downregulates the phosphorylation of I kappa B: autoloop regulation of the NF-kappa B-mediated expression of NF-kappa B subunits by TNF-alpha in mouse vascular endothelial cells. *Biochem Biophys Res Commun*. 1998;253(1):104-108. doi:10.1006/bbrc.1998.9697

36. Lee J-J, Huang W-T, Shao D-Z, Liao J-F, Lin M-T. Blocking NF-kappaB activation may be an effective strategy in the fever therapy. *Jpn J Physiol*. 2003;53(5):367-375. doi:10.2170/jjphysiol.53.367

37. Rahman A, Fazal F. Blocking NF-κB. *Proc Am Thorac Soc*. 2011;8(6):497-503. doi:10.1513/pats.201101-009MW

38. Zhang WJ, Frei B. Alpha-lipoic acid inhibits TNF-alpha-induced NF-kappaB activation and adhesion molecule expression in human aortic endothelial cells. *FASEB J Off Publ Fed Am Soc Exp Biol*. 2001;15(13):2423-2432. doi:10.1096/fj.01-0260com

39. Liu T, Zhang L, Joo D, Sun S-C. NF-κB signaling in inflammation. *Signal Transduct Target Ther*. 2017;2:17023. doi:10.1038/sigtrans.2017.23

40. Sreekanth GP, Panaampon J, Suttitheptumrong A, et al. Drug repurposing of N-acetyl cysteine as antiviral against dengue virus infection. *Antiviral Res*. 2019;166:42-55. doi:10.1016/j.antiviral.2019.03.011

41. Geiler J, Michaelis M, Naczk P, et al. N-acetyl-L-cysteine (NAC) inhibits virus replication and expression of pro-inflammatory molecules in A549 cells infected with highly pathogenic H5N1 influenza A virus. *Biochem Pharmacol*. 2010;79(3):413-420. doi:10.1016/j.bcp.2009.08.025

42. Palamara AT, Perno CF, Ciriolo MR, et al. Evidence for antiviral activity of glutathione: in vitro inhibition of herpes simplex virus type 1 replication. *Antiviral Res*. 1995;27(3):237-253. doi:10.1016/0166-3542(95)00008-a

43. Palamara AT, Perno CF, Aquaro S, Buè MC, Dini L, Garaci E. Glutathione inhibits HIV replication by acting at late stages of the virus life cycle. *AIDS Res Hum Retroviruses*. 1996;12(16):1537-1541. doi:10.1089/aid.1996.12.1537

44. Fraternale A, Paoletti MF, Casabianca A, et al. Antiviral and immunomodulatory properties of new pro-glutathione (GSH) molecules. *Curr Med Chem*. 2006;13(15):1749-1755. doi:10.2174/092986706777452542

45. Pacht ER, Timerman AP, Lykens MG, Merola AJ. Deficiency of alveolar fluid glutathione in patients with sepsis and the adult respiratory distress syndrome. *Chest*. 1991;100(5):1397-1403. doi:10.1378/chest.100.5.1397

46. Dickinson DA, Forman HJ. Cellular glutathione and thiols metabolism. *Biochem Pharmacol*. 2002;64(5-6):1019-1026. doi:10.1016/s0006-2952(02)01172-3

47. Forman HJ, Zhang H, Rinna A. Glutathione: Overview of its protective roles, measurement, and biosynthesis. *Mol Aspects Med*. 2009;30(1-2):1-12. doi:10.1016/j.mam.2008.08.006

48. Aquilano K, Baldelli S, Ciriolo MR. Glutathione: new roles in redox signaling for an old antioxidant. *Front Pharmacol*. 2014;5. doi:10.3389/fphar.2014.00196

49. Schmitt B, Vicenzi M, Garrel C, Denis FM. Effects of N-acetylcysteine, oral glutathione (GSH) and a novel sublingual form of GSH on oxidative stress markers: A comparative crossover study. *Redox Biol*. 2015;6:198-205. doi:10.1016/j.redox.2015.07.012

50. Liao B-C, Hsieh C-W, Lin Y-C, Wung B-S. The glutaredoxin/glutathione system modulates NF-kappaB activity by glutathionylation of p65 in cinnamaldehyde-treated endothelial cells. *Toxicol Sci Off J Soc Toxicol*. 2010;116(1):151-163. doi:10.1093/toxsci/kfq098

51. Cantin AM, Paquette B, Richter M, Larivée P. *Albumin-Mediated Regulation of Cellular Glutathione and Nuclear Factor Kappa B Activation*.

52. Farid M, Reid MB, Li Y-P, Gerken E, Durham WJ. Effects of dietary curcumin or N-acetylcysteine on NF-κB activity and contractile performance in ambulatory and unloaded murine soleus. *Nutr Metab*. 2005;2:20. doi:10.1186/1743-7075-2-20

53. Paterson RL, Galley HF, Webster NR. The effect of N-acetylcysteine on nuclear factor-kappa B activation, interleukin-6, interleukin-8, and intercellular adhesion molecule-1 expression in patients with sepsis. *Crit Care Med*. 2003;31(11):2574-2578. doi:10.1097/01.CCM.0000089945.69588.18

54. Ying Z, Kampfrath T, Sun Q, Parthasarathy S, Rajagopalan S. Evidence that α-lipoic acid inhibits NF-κB activation independent of its antioxidant function. *Inflamm Res Off J Eur Histamine Res Soc Al*. 2011;60(3):219-225. doi:10.1007/s00011-010-0256-7

55. Gasparini C, Feldmann M. NF-κB as a target for modulating inflammatory responses. *Curr Pharm Des*. 2012;18(35):5735-5745. doi:10.2174/138161212803530763

56. Liao Q-J, Ye L-B, Timani KA, et al. Activation of NF-kappaB by the full-length nucleocapsid protein of the SARS coronavirus. *Acta Biochim Biophys Sin*. 2005;37(9):607-612. doi:10.1111/j.1745-7270.2005.00082.x

57. Hariharan A, Hakeem AR, Radhakrishnan S, Reddy MS, Rela M. The Role and Therapeutic Potential of NF-kappa-B Pathway in Severe COVID-19 Patients. *Inflammopharmacology*. Published online November 7, 2020:1-10. doi:10.1007/s10787-020-00773-9

58. Fraternale A, Paoletti MF, Casabianca A, et al. GSH and analogs in antiviral therapy. *Mol Aspects Med*. 2009;30(1-2):99-110. doi:10.1016/j.mam.2008.09.001

59. Cai J, Chen Y, Seth S, Furukawa S, Compans RW, Jones DP. Inhibition of influenza infection by glutathione. *Free Radic Biol Med*. 2003;34(7):928-936. doi:10.1016/s0891-5849(03)00023-6

60. Ciriolo MR, Palamara AT, Incerpi S, et al. Loss of GSH, oxidative stress, and decrease of intracellular pH as sequential steps in viral infection. *J Biol Chem*. 1997;272(5):2700-2708. doi:10.1074/jbc.272.5.2700

61. Checconi P, Limongi D, Baldelli S, Ciriolo MR, Nencioni L, Palamara AT. Role of Glutathionylation in Infection and Inflammation. *Nutrients*. 2019;11(8). doi:10.3390/nu11081952

62. De Flora S, Balansky R, La Maestra S. Rationale for the use of N-acetylcysteine in both prevention and adjuvant therapy of COVID-19. *FASEB J*. Published online August 11, 2020. doi:10.1096/fj.202001807

63. Brocard H, Charpin J, Germouty J. [Multicenter, double-blind study of oral acetylcysteine vs. placebo]. *Eur J Respir Dis Suppl*. 1980;111:65-69.

64. Samuni Y, Goldstein S, Dean OM, Berk M. The chemistry and biological activities of N-acetylcysteine. *Biochim Biophys Acta*. 2013;1830(8):4117-4129. doi:10.1016/j.bbagen.2013.04.016

65. Grandjean EM, Berthet P, Ruffmann R, Leuenberger P. Efficacy of oral long-term N-acetylcysteine in chronic bronchopulmonary disease: a meta-analysis of published double-blind, placebo-controlled clinical trials. *Clin Ther*. 2000;22(2):209-221. doi:10.1016/S0149-2918(00)88479-9

66. Suzuki YJ, Aggarwal BB, Packer L. Alpha-lipoic acid is a potent inhibitor of NF-kappa B activation in human T cells. *Biochem Biophys Res Commun*. 1992;189(3):1709-1715. doi:10.1016/0006-291x(92)90275-p

67. Moine P, McIntyre R, Schwartz MD, et al. NF-kappaB regulatory mechanisms in alveolar macrophages from patients with acute respiratory distress syndrome. *Shock Augusta Ga*. 2000;13(2):85-91. doi:10.1097/00024382-200013020-00001

68. Horie S, McNicholas B, Rezoagli E, et al. Emerging pharmacological therapies for ARDS: COVID-19 and beyond. *Intensive Care Med*. Published online July 11, 2020:1-19. doi:10.1007/s00134-020-06141-z

69. Ahmed MH, Hassan A. Dexamethasone for the Treatment of Coronavirus Disease (COVID-19): a Review. *Sn Compr Clin Med*. Published online October 31, 2020:1-10. doi:10.1007/s42399-020-00610-8

70. Theoharides TC, Conti P. Dexamethasone for COVID-19? Not so fast. *J Biol Regul Homeost Agents*. 2020;34(3):1241-1243. doi:10.23812/20-EDITORIAL_1-5

71. Baig AM. Deleterious Outcomes in Long-Hauler COVID-19: The Effects of SARS-CoV-2 on the CNS in Chronic COVID Syndrome. *ACS Chem Neurosci*. 2020;11(24):4017-4020. doi:10.1021/acschemneuro.0c00725

72. Meyer A, Buhl R, Magnussen H. The effect of oral N-acetylcysteine on lung glutathione levels in idiopathic pulmonary fibrosis. *Eur Respir J*. 1994;7(3):431-436. doi:10.1183/09031936.94.07030431

73. Cantin AM, North SL, Hubbard RC, Crystal RG. Normal alveolar epithelial lining fluid contains high levels of glutathione. *J Appl Physiol Bethesda Md 1985*. 1987;63(1):152-157. doi:10.1152/jappl.1987.63.1.152

74. Kobayashi EH, Suzuki T, Funayama R, et al. Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. *Nat Commun*. 2016;7. doi:10.1038/ncomms11624

75. Tu W, Wang H, Li S, Liu Q, Sha H. The Anti-Inflammatory and Anti-Oxidant Mechanisms of the Keap1/Nrf2/ARE Signaling Pathway in Chronic Diseases. *Aging Dis*. 2019;10(3):637-651. doi:10.14336/AD.2018.0513

76. Jannatifar R, Parivar K, Hayati Roodbari N, Nasr-Esfahani MH. The Effect of N-Acetyl-Cysteine on NRF2 Antioxidant Gene Expression in Asthenoteratozoospermia Men: A Clinical Trial Study. *Int J Fertil Steril*. 2020;14(3):171-175. doi:10.22074/ijfs.2020.44411

77. Ji L, Liu R, Zhang XD, et al. N-acetylcysteine attenuates phosgene-induced acute lung injury via up-regulation of Nrf2 expression. *Inhal Toxicol*. 2010;22(7):535-542. doi:10.3109/08958370903525183

78. Steele ML, Fuller S, Patel M, Kersaitis C, Ooi L, Münch G. Effect of Nrf2 activators on release of glutathione, cysteinylglycine and homocysteine by human U373 astroglial cells. *Redox Biol*. 2013;1:441-445. doi:10.1016/j.redox.2013.08.006

79. Harvey CJ, Thimmulappa RK, Singh A, et al. Nrf2-regulated glutathione recycling independent of biosynthesis is critical for cell survival during oxidative stress. *Free Radic Biol Med*. 2009;46(4):443-453. doi:10.1016/j.freeradbiomed.2008.10.040

80. Citera M, Freeman PR, Horowitz RI. Empirical validation of the Horowitz Multiple Systemic Infectious Disease Syndrome Questionnaire for suspected Lyme disease. *Int J Gen Med*. 2017;10:249-273. doi:10.2147/IJGM.S140224

81. Mangin M, Sinha R, Fincher K. Inflammation and vitamin D: the infection connection. *Inflamm Res*. 2014;63(10):803-819. doi:10.1007/s00011-014-0755-z

82. Kohn A, Chang C. The Relationship Between Hypermobile Ehlers-Danlos Syndrome (hEDS), Postural Orthostatic Tachycardia Syndrome (POTS), and Mast Cell Activation Syndrome (MCAS). *Clin Rev Allergy Immunol*. 2020;58(3):273-297. doi:10.1007/s12016-019-08755-8

83. A Randomized Trial of Ivermectin-Doxycycline and Hydroxychloroquine-Azithromycin therapy on COVID19 patients. Published online July 15, 2020. doi:10.21203/rs.3.rs-38896/v1

84. McFarland LV. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of Clostridium difficile disease. *Am J Gastroenterol*. 2006;101(4):812-822. doi:10.1111/j.1572-0241.2006.00465.x

85. Castagliuolo I, Riegler MF, Valenick L, LaMont JT, Pothoulakis C. Saccharomyces boulardii protease inhibits the effects of Clostridium difficile toxins A and B in human colonic mucosa. *Infect Immun*. 1999;67(1):302-307.

86. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med*. Published online March 3, 2020. doi:10.1007/s00134-020-05991-x

87. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. 2020;0(0). doi:10.1016/S2213-2600(20)30079-5

88. Shi Y, Yu X, Zhao H, Wang H, Zhao R, Sheng J. Host susceptibility to severe COVID-19 and establishment of a host risk score: findings of 487 cases outside Wuhan. *Crit Care*. 2020;24. doi:10.1186/s13054-020-2833-7

89. Garg S. Hospitalization Rates and Characteristics of Patients Hospitalized with Laboratory-Confirmed Coronavirus Disease 2019 — COVID-NET, 14 States, March 1–30, 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69. doi:10.15585/mmwr.mm6915e3

90. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet Lond Engl*. Published online March 11, 2020. doi:10.1016/S0140-6736(20)30566-3

91. Zou X, Li S, Fang M, et al. Acute Physiology and Chronic Health Evaluation II Score as a Predictor of Hospital Mortality in Patients of Coronavirus Disease 2019. *Crit Care Med*. 2020;Online First. doi:10.1097/CCM.0000000000004411

92. Yan L, Zhang H-T, Goncalves J, et al. An interpretable mortality prediction model for COVID-19 patients. *Nat Mach Intell*. Published online May 14, 2020. doi:10.1038/s42256-020-0180-7

93. Hussain AN, Hussain F, Hashmi SK. Role of testosterone in COVID-19 patients – A double-edged sword? *Med Hypotheses*. 2020;144:110287. doi:10.1016/j.mehy.2020.110287

94. Ramos-Casals M, Brito-Zerón P, López-Guillermo A, Khamashta MA, Bosch X. Adult haemophagocytic syndrome. *The Lancet*. 2014;383(9927):1503-1516. doi:10.1016/S0140-6736(13)61048-X

95. Karakike E, Giamarellos-Bourboulis EJ. Macrophage Activation-Like Syndrome: A Distinct Entity Leading to Early Death in Sepsis. *Front Immunol*. 2019;10. doi:10.3389/fimmu.2019.00055

96. Seguin A, Galicier L, Boutboul D, Lemiale V, Azoulay E. Pulmonary Involvement in Patients With Hemophagocytic Lymphohistiocytosis. *Chest*. 2016;149(5):1294-1301. doi:10.1016/j.chest.2015.11.004

97. Bhatraju PK, Ghassemieh BJ, Nichols M, et al. Covid-19 in Critically Ill Patients in the Seattle Region — Case Series. *N Engl J Med*. 2020;0(0):null. doi:10.1056/NEJMoa2004500