Chronic Fatigue Mechanisms in Autoimmune Diseases: Lessons from Primary Biliary Cholangitis and Systemic Sclerosis

by

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Abstract

Systemic sclerosis (SSc) is an autoimmune disease affecting the body's connective tissues, resulting in progressive fibrosis and vasculopathy. In some cases, individuals with SSc may also develop primary biliary cholangitis (PBC), another autoimmune disease characterized by damage to their liver's bile ducts. Chronic fatigue frequently affects many patients with SSc and PBC and is associated with impaired cognitive abilities and reduced quality of life. To date, there are no reliable treatments for these patients' fatigue. Research on myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) - a type of chronic fatigue characterized by post-exertional malaise - has uncovered potential connections between chronic fatigue and abnormalities in the metabolic dysfunction, atypical hypoxia responses, problems with oxygen delivery, and the immune system. Hence, ME/CFS in autoimmune diseases such as SSc and PBC may have a physical basis derived from altered molecular mechanisms. This hypothesis-generating thesis comprehensively summarizes the common molecular mechanisms linking PBC, SSc and ME/CFS with each other. Together, the ideas presented in this thesis may provide additional insights into the common mechanisms linking SSc, PBC and ME/CFS with each other which may lead to designing more effective therapies for chronic fatigue, resulting in improved quality of life for these patients.

Preface

This thesis, in its entirety, stands as an unpublished original body of work by Muhammad Elezzabi, realized through the critical guidance and knowledgeable direction provided by Dr. Mohamed Osman and Dr. Andrew Mason. Their essential contributions in the form of feedback and suggestions played a pivotal role in enhancing the quality of the thesis.

I was responsible for creating the initial draft of the thesis, which underwent extensive reviews by Dr. Andrew Mason and Dr. Mohamed Osman. I took their feedback and revised the thesis accordingly. I also designed and compiled the tables and figures, aiming to augment the clarity and efficacy of the research presented. Notably, the nature of the research in this thesis did not necessitate the use of live subjects.

The Abstract and Chapters 1 to 6 have been adapted from our forthcoming literature review. The purpose of including these sections is to enrich the breadth and depth of the thesis and provide a rigorous exploration of the topic under investigation.

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

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List of Abbreviations

Abbreviation	Meaning
α-KG	Alpha-Ketoglutarate
AMA	Anti-Mitochondrial Autoantibodies
ANA	Anti-Nuclear Autoantibodies
ATP	Adenosine Triphosphate
ARNT	Aryl Hydrocarbon Receptor Nuclear Translocator
CCL2	Chemokine (C-C Motif) Ligand 2
CD62L	L-Selectin
CoA	Coenzyme A
COX7C	Cytochrome C Oxidase Subunit 7C
CyB	Cytochrome B
Cyt C	Cytochrome C
dcSSc	Diffuse Cutaneous Systemic Sclerosis
ETC	Electron Transport Chain
FACIT-F	Functional Assessment of Chronic Illness Therapy – Fatigue
FFSS	Fisk Fatigue Severity Score
FoxP3	Forkhead Box P3
GWAS	Genome Wide Association Study
H^+	Hydrogen Ions
HIF1-α	Hypoxia-Inducible Factor 1-Alpha

HIF1-β	Hypoxia-Inducible Factor 1-Beta
HLA	Human Leukocyte Antigen
HRE	Hypoxia Response Element
IDO	Indoleamine 2,3-Dioxygenase
IFN	Interferon
IL-_	Interleukin
IL12A	Interleukin 12A
IL12RB2	Interleukin 12 Receptor Subunit Beta 2
IRF5	Interferon Regulatory Factor 5
JAK2	Janus Kinase 2
lcSSc	Limited Cutaneous Systemic Sclerosis
ME/CFS	Myalgic Encephalomyelitis/Chronic Fatigue Syndrome
MFI-20	Multidimensional Fatigue Inventory 20 Items
mRNA	Messenger RNA
NAD^+	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucleotide with a Hydrogen
NAM	Nicotinamide Adenine Mononucleotide
ND4	NADH Dehydrogenase 4
NFKB1	Nuclear Factor Kappa B Subunit 1
NK	Natural Killer
NKG2D	Killer Cell Lectin-Like Receptor K1
NQOI	NAD(P)H Dehydrogenase Quinone 1

O_2	Oxygen
PAH	Pulmonary Arterial Hypertension
PBC	Primary Biliary Cholangitis
PCR	Polymerase Chain Reaction
PTPN22	Protein Tyrosine Phosphatase Non-Receptor Type 22
ROS	Reactive Oxygen Species
SF-36	Short Form 36 Item
SNP	Single Nucleotide Polymorphism
SSc	Systemic Sclerosis
STAT1	Signal Transducer and Activator of Transcription 1
STAT4	Signal Transducer and Activator of Transcription 4
TBX21	T-Box Transcription Factor 21
TIM-1	T-Cell Immunoglobulin and Mucin Domain 1
Th	T-helper
T-reg	Regulatory T-Cell
TYK2	Tyrosine Kinase 2

1. Introduction

Systemic Sclerosis (SSc) and primary biliary cholangitis (PBC) are life-threatening autoimmune diseases of unknown etiology. Although seemingly different, they share many commonalities (Figure 1 and Table 1). Clinically, the most profound similarity in both diseases is severe debilitating fatigue analogous to that described in patients with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). ME/CFS is an idiopathic severe unresolving form of fatigue characterized by a profound ongoing fatigue lasting more than 6 months in most patients [1]. This fatigue does not improve with rest and can be worsened by physical or mental activity (a phenomenon known as post-exertional malaise). Patients with ME/CFS experience severe muscle fatigue, known as peripheral fatigue, and cognitive dysfunction, or central fatigue. Cognitive dysfunction in patients with ME/CFS is associated with various features which include memory loss, sleep disturbances, and poor concentration. It is also commonly associated with changes in the functions of the autonomic nervous system (e.g. changes in heart rate and/or blood pressure, Raynaud's phenomenon), and diffuse pain [2]. Like SSc and PBC, ME/CFS is poorly understood.

Although extremely common in SSc and PBC, the mechanisms that promote fatigue are not well understood in PBC or SSc. Over half of SSc and PBC individuals suffer from severe and persistent fatigue [3,4]. This fatigue is associated with reduced quality of life and cognitive dysfunction [3,5-10], and are similar to those found in patients with ME/CFS (**Figure 1**) [11]. However, the accepted classification criteria for these three diseases fails to emphasize these similarities (**Table 1**). In this thesis, we will highlight the similar molecular mechanisms between SSc and PBC, and ME/CFS, to provide potential mechanistic insights that promote fatigue in these three devastating diseases.

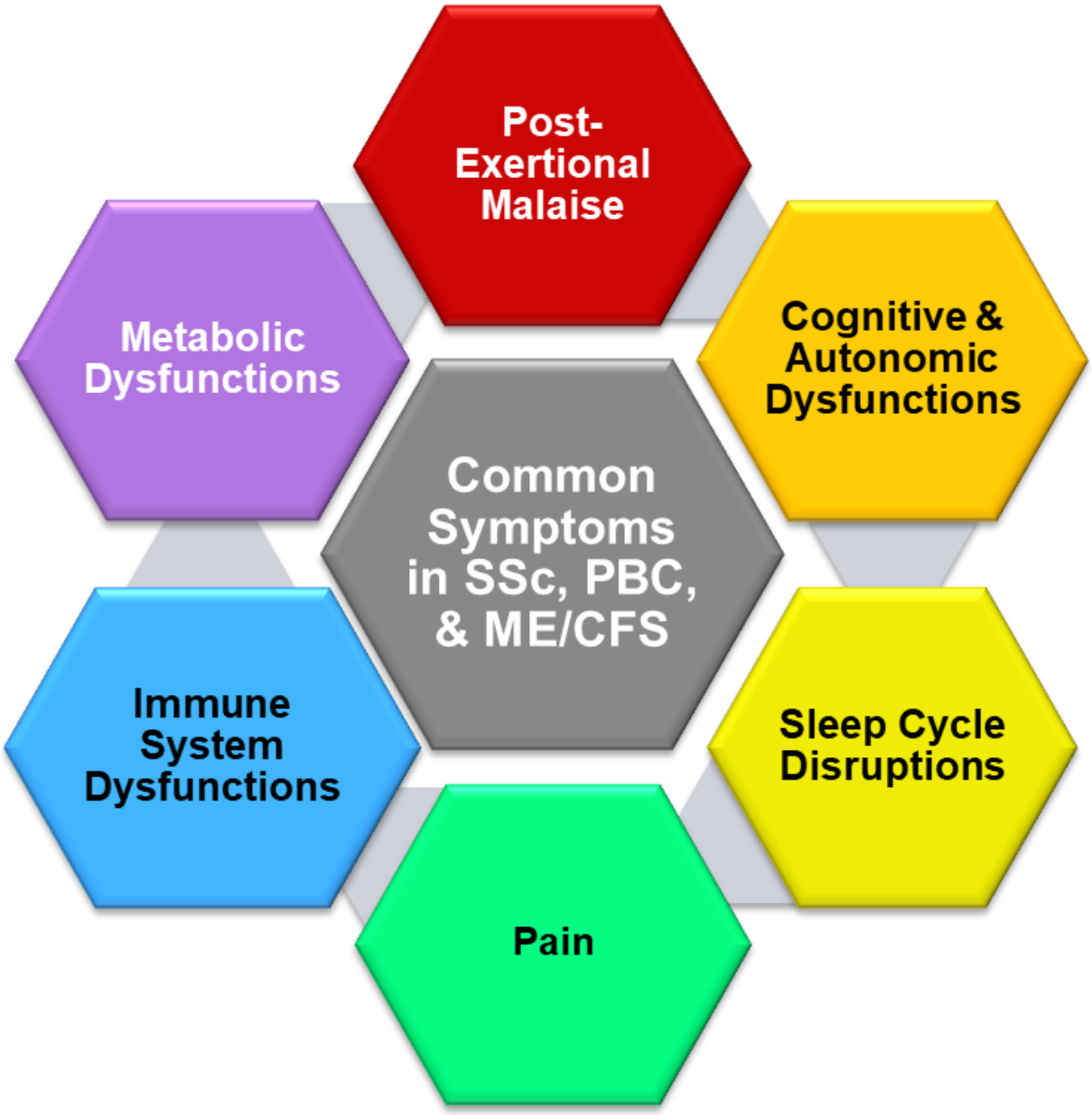

Figure 1. A depiction of some of the similar symptoms in the diseases primary biliary cholangitis (PBC), systemic sclerosis (SSc), and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). These symptoms include post-exertional malaise, cognitive & autonomic dysfunctions, sleep cycle disruptions, pain, immune system dysfunctions, and metabolic dysfunctions.

Table 1. The Most Widely Used and Accepted Classification Criteria for Primary Biliary Cholangitis (PBC), Systemic Sclerosis (SSc), and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS).

Disease	Primary Biliary Cholangitis	Systemic Sclerosis			Myalgic Encephalomyelitis/Chronic Fatigue Syndrome		
Classification Criteria	(1) EASL Clinical Practice Guidelines. (2) 2018 Practice Guidance from the American Association for the Study of Liver Diseases	2013 ACR/EULAR Classification Criteria for Systemic Sclerosis			Canadian Consensus Criteria		
Criteria Components	(1) EASL • EASL recommends that in adult patients with cholestasis and no likelihood of systemic disease, a diagnosis of PBC can be made based on elevated ALP and the presence of AMA at a titre >1:40 (III, 1). • EASL recommends that in the correct context, a diagnosis of AMA negative PBC can be made in patients with cholestasis and specific ANA	Item	Sub-item(s)	Weight	Item	Sub-Item(s)	Requirement
		Skin thickening of the fingers of both hands extending proximal to the metacarpophalangeal joints (sufficient criterion)	-	9	Fatigue	The patient must have a significant degree of new onset, unexplained, persistent, or recurrent physical and mental fatigue that substantially reduces activity level.	Required
		Skin thickening of the fingers (only count the higher score)	Puffy fingers	2	Post-Exertional Malaise and/or Post-Exertional Fatigue	There is an inappropriate loss of physical and mental stamina, rapid muscular and cognitive fatigability, post-exertional malaise and/or post-exertional fatigue and a tendency for other	Required

	immunofluorescence (nuclear dots or perinuclear rims) or ELISA results (sp100, gp210) (III, 1). • EASL recommends against liver biopsy for the diagnosis of PBC, unless PBC-specific antibodies are absent, co-existent AIH or NASH is suspected, or other (usually systemic) co-morbidities are present (III, 1). • AMA reactivity alone is not sufficient to diagnose PBC. EASL recommends following-up patients with normal serum liver tests who are AMA positive with annual biochemical reassessment for the presence of					associated symptoms within the patient's cluster of symptoms to worsen. There is a pathologically slow recovery period – usually 24 hours or longer.	
			Sclerodactyly of the fingers (distal to the metacarpophalangeal joints but proximal to the proximal interphalangeal joints)	4	Sleep Dysfunction	There is unrefreshing sleep or sleep quantity or rhythm disturbances such as reversed or chaotic diurnal sleep rhythms. Note that patients without sleep dysfunction can still meet the diagnostic criteria if their illness began with an infection.	Required
		Fingertip lesions (only count the higher score	Digital tip ulcers	2	Pain	There is unrefreshing sleep or sleep quantity or rhythm disturbances such as reversed or chaotic diurnal sleep rhythms. Note that patients without sleep dysfunction can still meet the diagnostic criteria if their illness began	Required

	liver disease (III, 1). (2) American Association for the Study of Liver Diseases: • Biochemical evidence of cholestasis based on ALP elevation. • Presence of AMA, or other PBC-specific autoantibodies, including sp100 or gp210, if AMA is negative. • Histologic evidence of nonsuppurative destructive cholangitis and destruction of interlobular bile ducts.				with an infection.	
			Fingertip pitting scars	3	Neurological / Cognitive Manifestations	Two or more of the following difficulties should be present: Confusion Impairment of concentration and short-term memory consolidation Disorientation Difficulty with information processing, categorizing and word retrieval (word-finding problems) Perceptual and sensory disturbances (for example spatial instability and disorientation and inability to focus vision) Ataxia, muscle weakness and fasciculations are common. There may be overload phenomena: cognitive overload, sensory overload (for example photophobia and hypersensitivity to noise) and/or	Two or More of the Sub-Items

				emotional overload, which may lead to crash periods and/or anxiety.	
		Telangiectasia - 2	Autonomic, Neuroendocrine, Immune Dysfunctions	Autonomic Manifestations: • Orthostatic intolerance (either neurally mediated hypotension, postural orthostatic tachycardia syndrome or delayed orthostatic hypotension) • Light-headedness • Extreme pallor • Nausea and irritable bowel syndrome • Urinary frequency and bladder dysfunction • Heart palpitations with or without cardiac arrhythmias • Exertional dyspnea Neuroendocrine Manifestations: • Loss of thermostatic stability	At Least One Symptom from Two of the Three Sub-Items

			(subnormal body temperature and marked diurnal fluctuation) • Sweating episodes, recurrent feelings of feverishness • cold extremities (cold hand and feet) • Intolerance of extremes of heat and cold • Marked weight change (anorexia or abnormal appetite) • Loss of adaptability and worsening of symptoms with stress Immune Manifestations: • Tender lymph nodes • Recurrent sore throat • Recurrent flu-like symptoms • General malaise • New food sensitivities, medication

						sensitivities and/or chemical sensitivities	
		Abnormal nailfold capillaries	-	2	The Illness Persists for at Least Six Months	-	It usually has a distinct onset, although it may be gradual. Preliminary diagnosis may be possible earlier. Three months is appropriate for children.
		Pulmonary arterial hypertension and/or interstitial lung disease (maximum score is 2)	Pulmonary arterial hypertension	2			
			Interstitial lung disease	2			
		Raynaud's phenomenon	-	3			
		SSc-related autoantibodies (anticentromere, anti–topoisomerase I [anti–Scl-70], anti–RNA polymerase III) (maximum score is 3)	Anticentromere Anti–topoisomerase I Anti–RNA polymerase III	3			
Requirement for Disease Classification	Fulfillment of all EASL criteria, or two of the three two of the three American Association for the Study of Liver Diseases criteria are met.	The total score is determined by adding the maximum weight (score) in each category. Patients with a total score of ≥ 9 are classified as having definite SSc.			A patient with ME/CFS will meet the criteria for fatigue, post-exertional malaise and/or fatigue, sleep dysfunction and pain; have two or more neurological/cognitive manifestations and one or more symptoms from two of the categories of (a) autonomic, (b) neuroendocrine and (c) immune manifestations; and adhere to item "The Illness Persists for at Least Six Months. It usually has a distinct onset, (**) although it may be gradual. Preliminary diagnosis may be possible earlier. Three months is appropriate for children".		
Reference	[12,13]	[14]			[2]		

1.1 SSc and PBC Commonly Co-occur

SSc is a rare (1 in every 2500 people in Canada) systemic autoimmune rheumatic disease (SARD) characterized by immune system dysregulation, vasculopathy, and fibrosis [15]. Although it primarily affects females, men with SSc develop more severe complications [16]. Importantly, it is one of the most lethal rheumatic diseases, with the poorest quality of life – mainly because the mechanisms promoting its pathogenesis are unknown, and effective therapies are lacking [17,18]. Importantly, reduced quality of life in SSc largely stems from fatigue [19]. However, similar to ME/CFS, the mechanisms promoting fatigue in SSc are largely unknown and are poorly described.

Primary biliary cholangitis (PBC) is also a rare and lethal autoimmune disease with unclear causes. It is characterized by progressive intrahepatic duct destruction with an associated inflammatory cell infiltrate, eventually leading to liver fibrosis, failure, and death [20]. Although rare in the general population, with an incidence rate of approximately two new cases per 100,000 people and a prevalence rate of approximately 22 cases per 100,000 inhabitants, the frequency of PBC in patients with SSc is widespread with a reported prevalence of up to 50% [21,22]. Intriguingly, patients with the limited cutaneous subtype of SSc (lcSSc), who more commonly develop systemic vasculopathy, have an increased risk of PBC [22]. Furthermore, females are more susceptible to PBC with a similar female-to-male sex ratio as reported in SSc (approximately 8:1). In other words, the co-occurrence of SSc and PBC is most likely not due to chance alone. Common mechanisms to both SSc and PBC may be driving their pathogenesis and the fatigue experienced by patients.

1.2 SSc and PBC Share Common Mechanisms

One of the earliest clinical manifestations in patients with SSc is Raynaud's phenomenon [23]. Raynaud's phenomenon is characterized by a transient and chronic ischemia (or hypoxia) of the digits, and is relatively common in females with a prevalence of approximately 15% [24]. Nearly all patients with SSc develop Raynaud's phenomenon

[25]. Intriguingly, the frequency of Raynaud's phenomenon in patients with PBC without an SSc diagnosis is also higher than expected [26]. In SSc, Raynaud's phenomenon is associated with progressive vascular remodeling which can be detected using nailfold capillaroscopy [27]. Recently, similar capillary changes have been reported in more than half of patients with PBC, and these mimicked the patterns observed in SSc patients [28,29].

Chronic hypoxic stressors in SSc and PBC are not only restricted to the digits but are also present systemically. This is well described in SSc where patients describe symptoms of "brain fog", digital ulcers, and progressive obliterative vascular remodeling which can result in pulmonary arterial hypertension. Recently, it has been suggested that systemic hypoxia is also present, as patients with PBC have an increased frequency of pulmonary hypertension [30]. Importantly, patients with PBC also develop cerebral hypoxia which may explain why they also develop brain fog symptoms [31].

The most prominent feature of immune system dysregulation in both SSc and PBC is the presence of anti-nuclear antibodies (ANAs) and anti-mitochondrial antibodies (AMAs). Patients with SSc universally have detectable ANAs, while at least 50 % of patients with PBC have ANA [32]. Importantly, the specific ANAs in both diseases commonly detect components of the nucleosome complex within the nucleus. Similarly, patients with PBC universally generate AMAs, which are also frequently described in at least 25% of patients with SSc [22]. Thus, the generation of ANAs and AMAs are common indicators of immune system dysregulation in both SSc and PBC.

Similar disease characteristics are shared across both PBC and SSc. Histologically, both diseases are characterized by increased activity of $CD8^+$ T cells, altered natural killer (NK) cell cytotoxicity, and potential activation of macrophages/monocytes [33-43]. Although immunosuppressive therapies are only modestly effective, agents that alter cellular metabolism, such as methotrexate, may help manage some patients' symptoms in both diseases [44,45]. This is directly relevant to the disease as other triggers that alter cellular metabolism (such as chronic hypoxia) may

promote inflammatory responses by activating hypoxia-inducible factors [46-48]. In brief, metabolic dysfunctions, oxygen delivery problems, unusual hypoxia responses, and immune system abnormalities are present in both PBC and SSc patients. In other words, could chronic hypoxia promote metabolic remodeling and immune cell dysfunction in both diseases – particularly in patients exhibiting ME/CFS symptoms? In this thesis, we aim to support further the notion that this is a shared mechanism promoting ME/CFS in SSc and PBC patients.

2. Fatigue in Primary Biliary Cholangitis and Systemic Sclerosis

2.1 Measuring Fatigue in SSc and PBC

Fatigue is a complex state of weakness and a lack of perceived energy that can occur for short periods and is generally short-lived. However, chronic severe fatigue is less common in the general population but is a more common and debilitating symptom in PBC and SSc patients. Given the similarities in symptoms associated with fatigue in SSc, PBC, and ME/CFS, the clinically available tools used to measure the severity and impact of fatigue in ME/CFS patients, such as the Multidimensional Fatigue Inventory (MFI) and the Short Form 36 Health Survey (SF-36) vitality subscale, may capture the effects of chronic fatigue in PBC and SSc individuals [49,50].

While fatigue is a distressing symptom in SSc and PBC patients, different tools are available to measure fatigue severity. Accurately measuring fatigue severity is essential to guide researchers in developing better ways to improve the quality of life of SSc patients. SSc individuals experience greater fatigue severity, as captured by the MFI-20 questionnaire's general fatigue subscale, than those with other rheumatic diseases [51]. Similarly, the SF-36 vitality subscale, a 4-question tool, further supports this notion [18]. An association between fatigue severity and both physical symptoms and health-related quality of life in SSc patients exists when using the 13-item Functional Assessment of Chronic Illness Therapy Fatigue (FACIT-F) score [19]. However, it is important to emphasize that most studies in SSc have measured fatigue without measuring other

clinical symptoms associated with chronic fatigue, such as post-exertional malaise and cognitive impairment.

In contrast to SSc, only two available fatigue questionnaires are validated to measure PBC patients' fatigue. The first is the PBC-40, which includes a fatigue domain comprised of eight questions designed to assess PBC patients' fatigue in a standardized manner [52]. While the PBC-40's design effectively captures PBC-specific fatigue, it limits its utility in measuring fatigue in other diseases such as SSc. The second validated fatigue questionnaire for PBC patients is the Fisk Fatigue Severity Score (FFSS) [53]. The FFSS differs from the PBC-40 in that it can evaluate fatigue severity and its effects on daily activities in multiple diseases.

A selected version of the FFSS has been used in fatigue assessments of SSc patients, suggesting that common self-reported fatigue-related symptoms exist in those with SSc and PBC [54]. Unfortunately, there is no consensus on which fatigue questionnaires are best suited for studying chronic fatigue in PBC, or how they compare in their performance to other tools such as the MFI-20, SF-36, or FACIT-F.

2.2 Therapies Targeting Fatigue Can Provide Mechanistic Insights in SSc, PBC, and ME/CFS Patients

The various tools with discordant results relevant to fatigue in SSc and PBC highlight the lack of a clear understanding of the mechanisms promoting fatigue. Nonetheless, therapeutic strategies that improve fatigue may provide a starting point for identifying shared biological mechanisms promoting chronic fatigue in SSc and PBC. **Tables 2.** and **3.** provide lists of fatigue management studies conducted in SSc and PBC patients, respectively. Exercises are a promising way of treating chronic fatigue in SSc patients [55,56]. Notably, plasmapheresis and setanaxib improve fatigue symptoms in patients with PBC [57,58]. Hence, do circulating humoral factors promoted by metabolic remodeling promote severe fatigue?

Table 2. Fatigue Management and Treatment Studies in Systemic Sclerosis.

Year	Findings	Degree of Change	Fatigue Measurement Tool	Study Design	Study Size	Primary Outcome(s)	Secondary Outcome(s)	Ref.	Ref. (Similar Studies)
2008	Complementary and alternative medicine users compared to non-users, improved fatigue scores.	8.2% improvement in mean fatigue scores.	Short Form 36 Item Vitality Subscale	Prospective Cohort Comparative	• 19 SSc Complementary Medicine Users. • 17 SSc Non-Complementary Medicine Users.	Perceived functioning by eight domains were measured by the Short Form-36 Questionnaire.	• Sociodemographic information. • Limited or diffuse cutaneous involvement. • Total skin scores by modified Rodnan skin scores. • Modified Medsger severity scores. • Yearly measurements of diffusing capacity of carbon monoxide. • Short Form 36-Item Questionnaire mental component summary scores and physical component summary scores.	[59]	[60,61]
2013	Self-directed, print-format, mail-delivered self-management programs did not improve fatigue severity.	5.7% decrease in mean fatigue scores.	Multidimensional Assessment of Fatigue Scale	Before–After (Pre–Post)	49 SSc participants.	Multidimensional Assessment of Fatigue Scale.	• Demographics and baseline disease information. • Health log. • Arthritis Self-Efficacy Scale. • Health Assessment Questionnaire. • Scleroderma Functional Assessmen	[62]	[63,64]

							t Questionnaire. • Health Assessment Questionnaire Pain Scale. • Center for Epidemiologic Studies Depression Scale. • Program Evaluation Interview Responses.		
2014	Aerobic and muscular endurance exercises led to a marginal improvement in fatigue.	29.8% decrease in mean fatigue scores.	Visual Analogue Scale	Single Subject Before–After (Pre–Post) Experiment	4 SSc participants.	Six-minute walk test.	• Demographic data. • Working heart rate, saturation, and central and peripheral exertion during and after submaximal treadmill test. • Muscle endurance as measured by the number of correctly performed repetitions. • Perceived muscle exertion according to the Borg CR-10 scale. • Visual Analogue Scales for patients' perceptions of Raynaud's phenomenon, global fatigue and global disease impact on well-being during the last week.	[65]	[55]

							• Stanford Health Assessment Questionnaire – HAQ. • SF-36 domain scores.		
	Internet self-directed self-management programs led to a decrease in fatigue severity.	A 7.2% decrease in mean fatigue scores following the intervention.	Visual Analogue Scale	Before–After (Pre–Post) Pilot	16 SSc participants.	Visual Analogue Scale for fatigue.	• Demographic information. • Chronic Disease Self-Efficacy Scale. • Health Education Impact Questionnaire. • Patient Activation Measure. • Center for Epidemiologic Studies Depression Scale. • Health Assessment Questionnaire. • Visual Analogue Scale for pain.	[63]	[62,64]
2015	Acupuncture treatment led to improvement in fatigue perceptions.	Two patients self-reported improvements in fatigue following acupuncture treatment.	Marked as Present or Absent	Case Series	2 Female SSc participants.	• Physician Assessment of Patients' Symptoms. • Patients' Self-Reported Symptom Improvement.	-	[60]	[59,61]
2016	Glucocorticoid discontinuation did not lead to an increase in fatigue.	A decrease of 6.7% in mean fatigue scores.	Visual Analogue Scale	Prospective Cohort Comparative	• 33 SSc participants who discontinued glucocorticoids. • 15 SSc participants who could not discontin	Visual Analogue Scale for fatigue.	• Demographic information. • Clinical measures. • Laboratory test results. • Visual Analogue Scale for global	[66]	None

					ue glucocorticoids.		health status. • Visual Analogue Scale for pain. • SF-36 mental component summary scores and physical component summary scores. • European Scleroderma Study Group activity index. • Medsger scale.		
2017	Diffuse cutaneous SSc individuals with clinical or subclinical hypothyroidism had their fatigue improve following L-thyroxine treatment.	An improvement of 38.9% in mean general fatigue scores.	Multidimensional Fatigue Inventory 20 Item	Before–After (Pre–Post)	• 10 clinical hypothyroid female SSc patients. • 23 Subclinical hypothyroid female SSc patients.	• Multidimensional Fatigue Inventory 20 Item General Fatigue Scores.	• Age. • Thyroid Stimulating Hormone Levels. • Free Triiodothyronine. • Free Thyroxine.	[67]	None
2018	Tocilizumab, an antibody therapy that targets the IL-6 receptor, did not improve fatigue relative to the placebo group.	A 14.4% decrease in least square mean fatigue scores in patients treated with tocilizumab.	Functional Assessment of Chronic Illness Therapy Fatigue Score	Double-Blinded, Placebo-Randomized Controlled Phase 2 Trial	• 35 SSc participants completed treatment with tocilizumab for 24 weeks. • 30 SSc participants treated with tocilizumab for 48 weeks. • 36 SSc participants completed treatment with placebo	• Difference in mean change from baseline in mRSS.to week 24.	• Health Assessment Questionnaire–Disability Index score. • Patient Global Visual Analogue Scale. • Physician Global Visual Analogue Scale. • Functional Assessment of Chronic Illness Therapy Fatigue Score.	[68]	None

		for 24 weeks. • 33 SSc participants completed treatment with placebo for 48 Weeks.	• Pruritus 5-D Itch). • Change from baseline in mRSS to week 48. • Proportion of patients with change from baseline in the mRSS at Week 48 ≥ the change from baseline in the mRSS at Week 24. • Change in Visual Analogue Scale Scores from baseline (intestinal, breathing, Raynaud's, finger ulcers, overall disease VAS scores from Scleroderma HAQ–DI). • Forced Vital Capacity. • Percent Predicted Forced Vital Capacity. • Percent Predicted Diffusing Capacity for Carbon Monoxide. • Tender Joint Counts. • Adverse Events. • Serious Adverse Events. • Laboratory monitoring. Was performed.

							• Gene Expression Analysis of Skin Biopsy specimens collected at baseline and at week 24. • Immunoassays for serum concentrations of COMP, POSTN, and CCL18.		
2020	Tai Chi improved fatigue compared to pre-intervention in diffuse cutaneous SSc patients.	• 43.9% decrease in mean Fatigue Impact Scale scores. • 33.3% decrease in mean Fatigue Severity Scale scores.	• Fatigue Severity Scale • Fatigue Impact Scale	Randomized Control Trial Parallel Groups	• 14 SSc participants in the Tai Chi Group. • 14 SSc participants in the Home Exercise Group.	• Fatigue Impact Scale. • Fatigue Severity Scale.	• Demographic data and disease information. • Trunk Lateral Endurance Test. • Berg Balance Scale. • Pittsburgh Sleep Quality Index. • Hospital Anxiety and Depression	[56]	None
	There were no significant differences between the internet self-management and control groups in a randomized controlled trial.	Approximately similar mean fatigue scores.	PROMIS-29 Profile v2.0	Randomized Control Trial Parallel Groups	• 123 SSc participants in the Internet Self-Management Program. • 124 SSc participants in the Book Self-Management Program.	• PROMIS-29 Profile v2.0.	• Demographic data and disease information. • PROMIS Self-Efficacy for Managing Chronic Conditions measure. • Patient Health Questionnaire-8. • Patient Activation Measure. • European Quality of Life-5 Dimensions. • European Quality of Life-5 Dimensions	[64]	[62,63]

							Quality Adjusted Life Years. • Brief Satisfaction with Appearance Scale.		
2021	Aerobic exercise improved fatigue severity.	• A 64.3% decrease in mean fatigue scores when supervised. • A 5.2% decrease in mean fatigue scores when done at home.	Fatigue Impact Scale	Randomized Control Trial Parallel Groups	• 18 SSc participants in the Supervised Exercise Group. • 19 SSc participants in the Home Exercise Group.	• Six-minute walk test.	• Demographic data, disease information, smoking history. • Heart Rate. • Oxygen Saturation. • Modified Borg Scale. • Forced Vital Capacity. • Forced Expiratory Volume in 1 Second. • Forced Expiratory Volume in 1 second / Forced Vital Capacity Ratio. • Peak Expiratory Flow. • Diffusion Capacity of the Lungs for Carbon Monoxide. • Maximal Inspiratory Pressure after a Maximal Expiratory Maneuver. • Maximal Expiratory Pressure after a Maximal Inspiratory Maneuver. • Modified Medical Research Council Dyspnea Scale.	[55]	[65]

							• Isometric knee extension strength. • Isometric handgrip strength. • Health Assessment Questionnaire Disability Index. • Scleroderma Health Assessment Questionnaire. • Short Form 36-Item Questionnaire mental component summary scores and physical component summary scores. • Fatigue Impact Scale.		
	Two patients self-reported improvements in fatigue following yoga treatment at discharge and follow-up.	• Compared to baseline, there was a 250% improvement in mean fatigue scores at 5-week discharge. • A 367% improvement in mean fatigue scores at a 4-week follow-up after discharge.	Short Form 36 Item Vitality Subscale	Case Series	2 Female SSc participants.	• Short Form 36-Item Questionnaire	• Occurrence of first symptoms. • Diagnosis. • Commencement of medication. • Erythrocyte Sedimentation Rate. • C-Reactive Protein levels. • Visual Analogue Scale for overall pain and stiffness. • Symptom score checklist.	[61]	[59,60]
2022	There is no improvement in fatigue severity when	• A 49.4% decrease in median Multidimensional	• Multidimensional Assessment of Fatigue Scale	Before–After (Pre–Post)	4 SSc participants.	• First three questions on the Multi-Dimensiona	• Demographic questionnaire.	[69]	None

	participating in the Fatigue and Activity Management Education in Systemic Sclerosis (FAME-iSS) when comparing baseline to post-intervention or between post-intervention and a 3-month follow-up.	Assessment of Fatigue Scale total scores post-intervention relative to baseline. • A 20.3% decrease in median Multidimensional Assessment of Fatigue Scale total scores in the 3-month follow-up relative to post-intervention. • A 37.9% decrease in median Modified Fatigue Impact Scale scores post-intervention relative to baseline. • A 23.4% increase in median Modified Fatigue Impact Scale scores post-intervention relative to post-intervention.	• Modified Fatigue Impact Scale	l Assessment of Fatigue: degree of fatigue, severity of fatigue, level of distress from fatigue.	• Patient-reported outcomes. • FAME-iSS handbook data. • Participant feedback semi-structured interviews. • Individual participant follow-up telephone interviews • Modified Fatigue Impact Scale. • Hospital Anxiety and Depression Scale. • PROMIS Self-Efficacy for Managing Symptoms. • Self-Efficacy for Performing Energy Conservation Strategies Assessment. • Acceptability of FAME-iSS assessed through a 8-item short course evaluation. • Energy Conservation Strategies Survey

Table 3. Fatigue Management and Treatment Studies in Primary Biliary Cholangitis.

Year	Findings	Degree of Change	Fatigue Measurement Tool	Study Design	Study Size	Primary Outcome(s)	Secondary Outcome(s)	Ref.	Ref. (Similar Studies)
1985	Five patients had their fatigue symptoms improve following plasmapheresis.	A decrease of at least 55.6% in mean fatigue scores.	A questionnaire, but the identity of the questionnaire was not reported.	Case Series	5 PBC participants.	• Questionnaire assessing pruritis, fatigability, dryness of eyes or mouth, Raynaud's phenomenon, nausea and vomiting.	• Liver size. • Spleen size. • Xanthomata. • Painful neuropathy. • Duration of therapy. • Aspartate aminotransferase levels. • Alkaline phosphatase levels. • Total bilirubin levels. • Anti-mitochondrial antibody titre. • Cholesterol levels. • Immune complexes.	[58]	[57]
1994	The combination treatment of prednisone and ursodeoxycholic acid led to the disappearance of fatigue.	66.7% of patients had improvement in fatigue.	Marked as Present or Absent	Case Series	7 PBC participants.	Fatigue.	• Pruritis. • Arthralgia. • Total Symptoms. • Aspartate aminotransferase levels. • Alkaline phosphatase levels. • IgM levels.	[70]	None

1999	Bezafibrate improved five patients' fatigue symptoms.	All five patients with fatigue had their symptoms improve.	Marked as Present or Absent	Case Series	11 SSc participants.	Fatigue.	• Age. • Gender. • Pruritis. • Anti-mitochondrial antibody status. • Histological staging. • Duration of prior ursodeoxycholic acid therapy. • Alanine aminotransferase levels. • Alkaline phosphatase levels. • IgM levels. • γGTP levels.	[71]	[72]
2001	The treatment with both the compound antioxidant vitamin Bio-Antox containing the antioxidants selenium, methionine, beta-carotene, vitamin C, and vitamin E, and Bio-Quinone Q10 and Coenzyme Q10 with alpha-tocopherol only improved physical and social, but not cognitive,	• 33.3% improvement in mean Fisk Fatigue Severity physical scores. • 19.2% improvement in mean Fisk Fatigue Severity social scores.	Fisk Fatigue Severity Score	Case Series	• 13 PBC participants in the Bio-Antox and Bioquinone Q10 group. • 11 PBC participants in the Bio-Antox alone group.	Fisk Fatigue Severity Scores: cognitive, physical, social scores.	• Visual Analogue Scale for Itch. • Night itch scores. • Dry eyes. • Dry mouth. • Abdominal pain. • Bone and joint pain.	[73]	[74]

	fatigue and there was a trend towards improvement in total Fisk Fatigue Severity scores.								
2002	Liver transplants did not improve fatigue even when controlling for the geographical location of PBC patients.	~2.6% decrease in median fatigue scores following transplant relative to non-Scheuer stage III-IV patients.	Fatigue Impact Scale	Case-Control	136 PBC participants.	Fatigue Impact Scale	• Demographic and clinical data. • Visual Analogue Scale for pruritis. • Rand MOS Depression Screener • Thyroid stimulating hormone levels. • Albumin levels. • Bilirubin levels. • Alkaline phosphatase levels. • Prothrombin time. • Anti-mitochondrial antibody titre. • Histological stage.	[75]	[76-78]
2003	A randomized placebo-controlled trial showed no improvement in fatigue severity by using only a compound antioxidant	1.5% improvement in median fatigue scores following treatment.	Fisk Fatigue Severity Score	Multicentre, Randomized, Double-Blind, Placebo-Controlled, Cross-Over Trial	44 PBC participants.	Fisk Fatigue Severity Scores	• Visual Analogue Scale for pruritis. • Visual Analogue Scale for degree of eye dryness and mouth. • Hypochondrial pain using a	[74]	[73]

	containing vitamins A, C, and E, selenium, methionine, and ubiquinone.						four-point Likert scale. • Bone pain using a four-point Likert scale. • Hamilton Depression Rating Scale.		
2004	Treatment with the serotonin reuptake inhibitor fluvoxamine did not lead to a significant improvement in fatigue severity.	• 4.1% improvement in the median scores in the social domain of the Fisk Fatigue Severity Score on Day 70 relative to baseline. • 4.7% decrease in median fatigue scores on Day 70 using the Visual Analogue Scale relative to baseline. • 5.6% improvement in the median general fatigue scores on Day 70 using the Multidimensional Fatigue Inventory 20	• Visual Analogue Scale • Fisk Fatigue Severity Score • Multidimensional Fatigue Inventory 20 Item	Randomized, Double-Blind, Placebo-Controlled Trial	• 17 PBC participants treated with fluvoxamine for intention-to-treat analysis. • 12 PBC participants treated with fluvoxamine for per-protocol analysis. • 16 PBC participants treated with placebo for intention-to-treat analysis. • 14 PBC participants treated with placebo for per-protocol analysis.	• Visual Analogue Scale for fatigue. • Fisk Fatigue Severity Score. • Multidimensional Fatigue Inventory 20 Item.	• Short Form 36-Item Questionnaire. • Visual Analogue Scale for pruritis. • Total serum bilirubin • Albumin • γ-GT • Alkaline phosphatase. • Alanine aminotransferase. • Aspartate aminotransferase. • Cholesterol. • IgG • IgM • Prothrombin time. • Antithrombin-III. • Hemoglobin. • Leukocyte count. • Platelet count.	[79]	None

		Item relative to baseline. • 6.3% worsening of median physical functioning scores on Day 70 using the Multidimensional Fatigue Inventory 20 Item relative to baseline. • 6.7% reduction in activity on Day 70 using the Multidimensional Fatigue Inventory 20 Item relative to baseline. • 7.7% reduction in motivation on Day 70 using the Multidimensional Fatigue Inventory 20 Item relative to baseline.							
2005	Treatment with ondansetron, a 5-HT_3 receptor	A 13.4% decrease in fatigue scores	• Fatigue Impact Scale • Fisk Fatigue Severity Score	Multicentre, Randomized, Double-Blind, Placebo-Controlled,	• 24 PBC participants received placebo in period 1 and	• Fisk Fatigue Severity Score.	• Demographic data and disease information.	[80]	None

	antagonist, decreased fatigue compared to placebo only in the second experimental period. However, subjects were unblinded as ondansetron treatment led to constipation in 63% of treated patients in a randomized controlled crossover trial.	from baseline using the Fatigue Severity Scale.	• Walter-Reed Performance Assessment Battery	Cross-Over Trial	ondansetron in period 2. • 30 PBC participants received ondansetron in period 1 and placebo in period 2.		• Fatigue Impact Scale. • Walter-Reed Performance Assessment Battery. • The Stanford Sleepiness Scale. • Hamilton Rating Scale for Depression. • Pittsburgh Sleep Quality Index. • Anti-mitochondrial antibody status. • Alkaline phosphatase. • Bilirubin. • International normalised ratio. • Side effects.		
	Five patients' fatigue severity improved following being prescribed modafinil, and improvements were maintained in two to fourteen months of follow-up.	Not Reported	Marked as Present or Absent	Case Series	5 PBC participants.	Fatigue.	• Age. • Duration of PBC. • Disease Stage. • Drugs. • Bilirubin levels. • Albumin levels. • Alkaline phosphatase levels.	[81]	[82-84]
2007	Modafinil improved fatigue severity in an open-label study	• 87% improvement in mean fatigue scores using a	PBC-40 Fatigue Domain	Open Label	21 PBC participants.	PBC-40 Domains.	• Epworth Sleepiness Scale. • Age. • Female Sex	[84]	[81-83]

	relative to baseline.	per-protocol analysis relative to baseline. • 58% improvement in mean fatigue scores using an intention-to-treat analysis.					• Ursodeoxycholic acid dose. • Disease stage at last biopsy. • Bilirubin levels. • Albumin levels. • Alkaline phosphatase levels.		
2009	Modafinil was found to improve fatigue severity, and the improvement was considered a complete response.	74% of patients had a complete response to treatment.	Fatigued was defined as the patient being able to do many activities but not most of them and needing more sleep than usual. Alternatively, they might be unable to carry out their normal activities or hold a regular job.	Open Label	42 PBC participants.	Fatigue.	• Age. • Female Gender. • Disease Stage. • Known duration of disease. • Bilirubin level. • Albumin levels. • Alkaline phosphatase levels. • Alanine Transferase levels. • Mayo risk score.	[83]	[81,82,84]
2010	Methotrexate does not improve fatigue, according to a systematic review.	The reported relative risks for fatigue with methotrexate treatment were 0.92 (95% Confidence Interval 0.06 - 13.18) and	N/A	Systematic Review and Meta-Analysis	455 PBC participants.	• Mortality. • Mortality or liver transplantation combined.	• Liver complications. • Pruritus, fatigue, and jaundice. • Liver biochemistry. • Liver biopsy findings. • Quality of life and	[45]	None

		6.50 (95% Confidence Interval 0.37 - 114.12).					cost-effectiveness. • Adverse events. • Methotrexate versus colchicine. • Subgroup analyses. • Sensitivity analyses.		
2012	Ursodeoxycholic acid does not improve fatigue, according to a systematic review.	The difference between the number of patients experiencing fatigue after treatment with ursodeoxycholic acid and those who did not receive the treatment is 6.4%, and the relative risk is 0.90 (with a 95% confidence interval of 0.81 - 1.00).	N/A	Systematic Review and Meta Analysis	1447 PBC participants.	• All-cause mortality. • All-cause mortality or liver transplantation. • Adverse events. • Quality of life.	• Liver transplantation. • Pruritus and fatigue. • Liver-related morbidity. • Biochemical markers (including serum bilirubin). • Liver histology (including worsening of histological stage).	[85]	[86]
2013	Fatigue worsened after liver transplants only in males, while no difference was observed in females. More than 50% of PBC patients had	26.1% increase in median fatigue scores in males following liver transplants.	PBC-40 Fatigue Domain	Case-Matched Cross-Sectional	• 351 liver transplanted female PBC patients. • 351 non-liver transplanted female PBC patients. • 29 liver transplanted male	• PBC-40 Domains.	• Age at PBC diagnosis. • Total follow-up time. • Age at study. • Age at transplantation.	[76]	[75,77,78]

	severe fatigue after liver transplant.				PBC patients. • 29 non-liver transplanted male PBC patients.		• Diagnosis to transplanted disease duration. • Proportion of transplant failure. • Patients not in treatment. • Post-transplantation follow-up time. • Epworth Sleepiness Scale (ESS). • Orthostatic Grading Scale. • Hospital Anxiety and Depression Scale.		
	Except for the "Emotional" domain, all the PBC-40 domains improved, including the fatigue domain, and fatigue scores decreased 6, 12, and 24 months after their liver transplants. 44% of patients still had moderate to severe fatigue after liver transplant. No correlation was found between	35.6% decrease in mean fatigue scores 24 months after liver transplant.	PBC-40 Fatigue Domain	Case-Matched Cross-Sectional	• 31 PBC participants pre-liver transplant. • 30 PBC participants 6 months post-liver transplant. • 29 PBC participants 12 months post-liver transplant. • 29 PBC participants 24 months post-liver transplant.	PBC-40 Domains.	Model for End-Stage Liver Disease.	[77]	[75,76,78]

	MELD scores and fatigue scores before or after liver transplantation.								
2014	Liver transplants led to a trend in improving total fatigue scores of PBC patients when using the Fisk Fatigue Severity Score.	46.7% improvement in median fatigue scores 12 months after liver transplant.	Fisk Fatigue Severity Score	Case-Matched Cross-Sectional	• 12 PBC participants pre-liver transplant. • 96 non-PBC participants pre-liver transplant. • 10 PBC participants 1-year post-liver transplant. • 50 non-PBC participants 1-year post-liver transplant.	Fisk Fatigue Severity Score Domains	-	[78]	[75-77]
2017	Modafinil, a central nervous system stimulant, did not improve fatigue severity as measured by the Fisk Fatigue Severity Score in a placebo-controlled clinical trial.	• 23.2% decrease in median total Fisk Fatigue Severity Scores relative to baseline. • 3.1% decrease in median Fatigue Severity Scale scores relative to baseline. • 17.1% decrease in median Fatigue Severity Scale scores relative to baseline.	• Fisk Fatigue Impact Scale • Fatigue Severity Scale • PBC-40 Fatigue Domain	Randomized, Double-Blind, Placebo-Controlled Trial	• 17 PBC participants were treated with modafinil. • 19 PBC participants were treated with placebo.	≥ 50% or greater improvement in fatigue severity (quantified by the FFIS) after 12 weeks of treatment, compared with baseline values.	• Age. • Patients with varices at study entry. • Patients with ascites at study entry. • Patients with peripheral edema at study entry. • Ludwig histological stage of PBC at study entry. • Sjogren syndrome. • Thyroid disease. • The frequency of adverse events in each	[82]	[81,83,84]

		• 17.6% of patients treated with modafinil have a ≥50% reduction in FFIS scores relative to baseline.					treatment arm. • Change in fatigue severity as quantified by the Fisk Fatigue Impact Scale compared with baseline. • Change in fatigue severity as quantified by the FSS compared with baseline. • Change in fatigue severity as quantified by the of the PBC-40 fatigue domain compared with baseline. • Change in alkaline phosphatase, aspartate aminotransferase, total bilirubin and albumin levels after 12 weeks of therapy compared with baseline values.		
2018	The pan-peroxisome proliferator-activated receptor-activating drug bezafibrate had an inconsiste	Fatigue improved in 30% of treated patients. However, fatigue	3-stage (absent, intermittent, continuous) fatigue scale	Randomized, Double-Blind, Placebo-Controlled Phase 3 Trial	• 50 PBC participants treated with bezafibrate for an intent-to-treat analysis. • 50 PBC participants treated with	Percentage of patients with a complete biochemical response, which was defined as	• Demographic and disease information. • Percentage of patients with a complete biochemical	[72]	[71]

	nt effect on fatigue in a placebo-controlled clinical trial.	also improved in 19% of placebo-treated patients.		placebo for an intent-to-treat analysis.	normal serum levels of alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total bilirubin, and albumin, as well as a normal prothrombin index (the patient's prothrombin time expressed as a percentage of the normal value) at 24 months.	response at various time points during the trial. • Percentage of patients with a normal alkaline phosphatase level at 24 months. • Changes in serum levels of alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl-transferase, total bilirubin, albumin, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol, and changes in the prothrombin index and platelet count. • Percentage of patients with an adequate biochemical response at 24 months as defined by the Barcelona, Paris

		1, Paris 2, Rotterdam, and Toronto criteria, as well as by the GLOBE score. • Changes in pruritus intensity on a visual-analogue scale. • Changes with respect to fatigue (absent, intermittent, or continuous). • Changes in quality of life (as assessed with the use of the Nottingham Health Profile. • Changes in liver stiffness. • Changes in the Enhanced Liver Fibrosis score. • Development of portal hypertension. • Survival without liver transplantation or liver complications. • Changes in serum levels of total and endogenous bile acids,

							ursodeoxycholic acid, 7α-hydroxy-4-cholesten-3-one, IgM, IgG, high-sensitivity C-reactive protein, tumor necrosis factor α, and interleukin-12. • Projected survival estimated according to the GLOBE score • UK-PBC risk score. • Predictive factors of inadequate response.		
2019	Ursodeoxycholic acid does not improve fatigue, according to a systematic review.	After treatment with ursodeoxycholic acid, there was no decrease in the relative risk of patients with fatigue (0.86, 95% Confidence Interval 0.69-1.08).	N/A	Systematic Review and Meta-Analysis	1706 PBC participants.	Proportion of fatigued patients and change in degree of fatigue as quantified by the fatigue domain of the PBC-40 scale, Fisk Fatigue Impact Scale, Visual Analogue scale Multidimensional Fatigu	Serious Adverse Events	[86]	[85]

						e Inventory, or Fatigue Severity Scale.			
	Fatigue severity did not improve following treatment with rituximab, a therapy that depletes B-cells, at any measured time points.	4.1% decrease in mean fatigue scores after 12 months of rituximab treatment.	PBC-40 Fatigue Domain	Randomized, Double-Blind, Placebo-Controlled Trial Phase 2	• PBC participants treated with rituximab: 29 at baseline, 27 with a 3-month follow-up complete, 27 with a 6-month follow-up complete, 26 with a 9-month follow-up complete, 27 with a 24-month follow-up complete. • PBC participants treated with rituximab: 28 at baseline, 28 with a 3-month follow-up complete, 28 with a 6-month follow-up complete, 28 with a 9-month follow-up complete, 26 with a 24-month follow-up complete.	Fatigue domain score from the PBC-40 questionnaire at 3 months.	• Demographic and disease information. • Other domains of the PBC-40. • Hospital Anxiety and Depression Scale. • Epworth Sleeping Scale. • Orthostatic Grading Scale. • Patient-Reported Outcomes Measurement Information SystemHealth Assessment Questionnaire. • Cognitive Failure Questionnaire. • Fatigue diaries. • Physical activity monitoring using wrist worn tri-axial GENEActiv accelerometers. • Anaerobic threshold (AT)	[87]	None

							assessed using conventional Cardiopulmonary Exercising (CPX). • Muscle bioenergetic function was assessed using Magnetic Resonance Spectroscopy. • 6) Quantification and phenotyping of total B-cell populations and B-cell subsets. . • Anti-PDC antibody total and individual isotype levels. • Change in liver serum biochemistry.		
2021	Plasmapheresis improves chronic fatigue.	15.8% improvement in median fatigue scores following treatment relative to baseline.	PBC-40 Fatigue Domain	Before–After (Pre–Post)	• 13 clinically stable patients with PBC and intractable pruritus not responding to medical therapy. • 103 PBC patients for serum levels of both resistin and tissue growth factor β.	PBC-40 fatigue domain.	• Laboratory biochemical parameters. • Other PBC-40 domains. • Patient Health Questionnaire-9. • Hospital Anxiety and Depression Scale. • Numerical Rating Scale for pruritis.	[57]	[58]

2023	In a randomized placebo-controlled clinical trial, treatment with setanaxib improved fatigue severity in PBC patients, whether given once or twice a day.	• 10.8% reduction in mean fatigue scores when dosed twice a day relative to baseline. • 6.7% reduction in mean fatigue scores when dosed once a day relative to baseline.	PBC-40 Fatigue Domain	Randomized, Double-Blind, Placebo-Controlled Trial Phase 2	• PBC participants given setanaxib 400 mg twice daily: 36 for baseline intention-to-treat analysis, 30 for change from baseline to week 24. • PBC participants given setanaxib 400 mg once daily: 38 for baseline intention-to-treat analysis, 37 for change from baseline to week 24. • PBC participants given oral placebo: 38=7 for baseline intention-to-treat analysis, 36 for change from baseline to week 24.	PBC-40 fatigue domain.	• Other PBC-40 domains. • Change from baseline to week 24 in mean patient-reported score for each PBC-40 domain. • Number and percentage of patients who achieved a meaningful response in each domain at week 24. • Liver stiffness as assessed through transient elastography. • Venous blood serum levels of markers of liver injury and inflammation (alkaline phosphatase, high-sensitivity C-reactive protein and IL-6).	[88]	None

3. Metabolic Remodeling is Common in SSc, PBC, and ME/CFS

Patients with PBC, SSc, and ME/CFS have unique metabolic signatures distinguishing them from individuals with other diseases (**Figure 2, Table 4**). Understanding these alterations can provide clues to the specific metabolic pathways common to these diseases. Adaptations to chronic hypoxia may be a common mechanism linking these metabolic disturbances. Some of these adaptations include those described in increased glycolysis [89,90], with increased production and release of hydrogen ions associated with increased lactate release [90-94], and an altered redox state [95-101]. These metabolic changes may promote some of the symptoms described in PBC, SSc, and ME/CFS, such as diffuse body pain and possibly post-exercise malaise [92,93].

These adaptations are well described in both PBC and SSc. For instance, fatigued PBC patients have more extended periods of decreased muscle pH following exercise than non-fatigued PBC patients [94]. Similarly, SSc patients display a reduced oxygen content gradient, leading to decreased peak oxygen consumption and compromised skeletal muscle microcirculation [102,103]. Hypoxia in SSc can also promote inflammatory signals by increasing ATP levels and IL-6 production [104]. These signals, in turn, promote immune cell activation and other adaptations that result in disease specific-changes (such as organ-limited and/or systemic fibrosis and vasculopathy) and perhaps ME/CFS symptom manifestation. This is why it is unsurprising that many patients with SSc and PBC develop severe fatigue without severe visceral complications such as interstitial lung disease [105]; or cirrhosis [75-78], respectively.

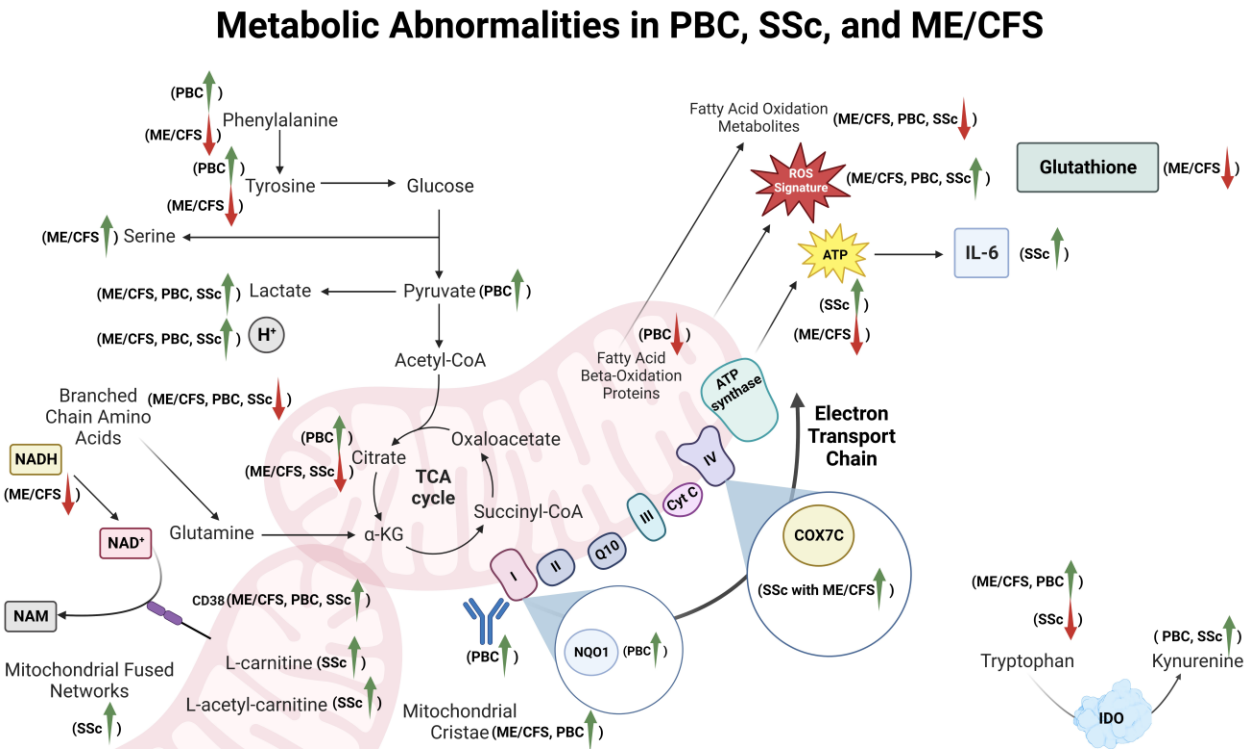

Figure 2. The metabolic abnormalities observed in primary biliary cholangitis, systemic sclerosis, and myalgic encephalomyelitis/chronic fatigue syndrome are similar and involve the same metabolites and metabolic pathways. The down red arrows represent decreased levels, while the green up arrows indicate increased levels. α-KG, alpha-ketoglutarate; ATP, adenosine triphosphate; CoA, coenzyme A; COX7C, cytochrome c oxidase subunit 7C; Cyt C, cytochrome C; H^+, hydrogen ions; IL-6, interleukin 6; NAD^+, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide with a hydrogen; NAM, nicotinamide adenine mononucleotide; NQO1, NAD(P)H dehydrogenase quinone 1; ME/CFS, myalgic encephalomyelitis/chronic fatigue syndrome; PBC, primary biliary cholangitis; Q10, Coenzyme Q10; ROS, reactive oxygen species; SSc, systemic sclerosis.

Table 4. Metabolic Changes in Primary Biliary Cholangitis (PBC), Systemic Sclerosis (SSc), and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS).

Diseases(s)	Findings	Biological Specimen(s) Analyzed	Ref.
PBC, SSc, ME/CFS	• All three diseases have increased signatures of oxidative stress species. A source of this in SSc patients are fibroblasts' mitochondria.	• Blood Serum (PBC & SSc) • Blood Plasma (SSc & ME/CFS) • Fibroblasts (SSc) • Skin (Diffuse SSc) • Liver (PBC) • Bile Ducts (PBC)	[95-101]
	• All three diseases have decreased extracellular pH levels and increased lactate release.	• Magnetic Resonance Spectroscopy Following Plantar Flexion (PBC & ME/CFS) • Human Dermal Fibroblasts (SSc) • Ventricular Cerebrospinal Fluid (ME/CFS)	[90-94]
	• All three diseases have decreased levels of both fatty acid oxidation metabolites and branched-chain amino acids. However, fatigued PBC patients do not have decreased branched-chain amino acid levels.	• Blood Plasma • Blood Serum (PBC & ME/CFS)	[95,106-111]
	• All three diseases have increased CD38 expression in B-cells.	• Peripheral Blood Mononuclear Cells • Peripheral Blood (SSc)	[112-115]
PBC & SSc	• Increased kynurenine levels.	• Blood Serum	[116,117]
PBC & ME/CFS	• Both diseases have increased muscular acidosis following exercise.	• Magnetic Resonance Spectroscopy Following Plantar Flexion	[92-94]
	• Reports of increased tryptophan in PBC and ME/CFS.	• Blood Serum (PBC) • Blood Plasma (ME/CFS)	[111,118]
	• There are reports of increased numbers of mitochondrial cristae in both diseases.	• Intrahepatic Bile Ducts (PBC) • Peripheral Blood Mononuclear Cells (ME/CFS)	[119,120]
SSc & ME/CFS	• Reduced citrate levels.	• Blood Serum (SSc) • Blood Plasma (ME/CFS)	[121,122]
	• There are increased mRNA levels of *COX7C* in ME/CFS patients and in SSc patients with ME/CFS.	• Whole Blood	[105,123]
SSc	• Increased glycolysis, and perhaps glutamine metabolism, are present.	• Human Dermal Fibroblasts	[90,124]
	• Hypoxia stimulates increased ATP levels which in turn stimulates the increased release of IL-6 by fibroblasts.	• Human Dermal Fibroblasts	[104]
	• SSc individuals' fibroblasts have increased glycolytic metabolism and hydrogen ion release.	• Human Dermal Fibroblasts	[90,124]
	• Decreased numbers of mitochondrial cristae.	• Human Dermal Fibroblasts	[125]
	• There is an increase in hyper-fused mitochondrial networks.	• Human Dermal Fibroblasts	[126]
	• There are decreased mRNA levels of *ND4* and *CyB* in SSc patients with ME/CFS.	• Whole Blood	[105]
	• Increased upregulation of genetic signatures associated with glycolysis and oxidative phosphorylation.	• Monocyte-Derived Macrophages	[127]
	• Higher levels of L-carnitine and L-acetyl-carnitine.	• Monocyte-Derived Dendritic Cells	[107]
	• Decreased tryptophan levels.	• Blood Plasma	[110]
	• Increased CD38 expression.	• Human Skin Biopsies • Human Dermal Fibroblasts	[128]

PBC	• There are anti-mitochondrial antibodies targeting mitochondrial Complex 1.	• Blood Serum	[129,130]
	• PBC patients have increased NQO1 protein levels.	• Liver	[131]
	• PBC individuals have increased citrate levels.	• Blood Serum	[132]
	• PBC patients have increased phenylalanine and tyrosine levels, the latter of which correlates with fatigue.	• Blood Plasma	[111]
ME/CFS	• ME/CFS patients have decreased phenylalanine levels, and tyrosine levels decreased in blood plasma but increased in urine.	• Blood Plasma • Urine	[133,134]
	• Up to 45% of ME/CFS individuals have a Coenzyme Q10 deficiency.	• Blood Plasma	[135]
	• There are decreased mRNA levels of *NQO1*.	• Vastus Lateralis Muscle	[136]
	• Decreased mRNA levels of *PDK1*, *PDK2*, *PDK4*, and *PPARD*.	• Peripheral Blood Mononuclear Cells	[137]
	• B-cells have higher expressions of CD24 and CD38, which was associated with increased use of glucose and lactate.	• Peripheral Blood Mononuclear Cells	[114]
	• Cytotoxic $CD8^+$ T-cells have reduced glycolysis at rest and after activation, reduced mitochondrial membrane potential, and decreased mitochondrial ATP production.	• Peripheral Blood Mononuclear Cells	[138]
	• $CD56^+$ natural killer cells have increased CD38 expression.	• Peripheral Blood Mononuclear Cells	[139]
	• Decreased occipital lobe glutathione levels and increased ventricular lactate.	• Ventricular Cerebrospinal Fluid	[91]

3.1 Abnormal Hypoxic Responses and Oxygen Delivery Problems are Common in SSc, PBC, and ME/CFS

3.11 Peripheral and Central Blood Oxygenation Problems are Common in ME/CFS, PBC, and SSc

A chronic hypoxic state may be the central mechanism that promotes SSc, PBC, and associated ME/CFS. Blood oxygenation problems occur in ME/CFS patients' bodies, especially in their muscles and the brain. A correlation between reduced muscle oxygen delivery and oxidative metabolism peripherally characterizes those with ME/CFS [140]. This reduced oxygen delivery could explain the previous finding of increased lactate levels in the blood of individuals with ME/CFS, even at rest [92]. Problems in delivering oxygen to brain regions may also lead to adverse neurological effects in ME/CFS

patients. Individuals with ME/CFS exhibit reduced cerebral blood flow in the carotid and vertebral arteries, despite accounting for the effects of orthostatic hypotension and tachycardia, indicating impaired cerebral vascular autoregulation [141]. ME/CFS patients' brains also have increased ventricular lactate and decreased occipital lobe glutathione [91], the former of which could result from decreased cerebral perfusion and oxygen supply to ME/CFS patients' brains, while reduced glutathione may be indicative of increased oxidative stress.

Decreased cerebral blood flow may cause other clinical effects in those with ME/CFS. A strong correlation exists between brain grey matter volume and cerebral vascular autoregulation in ME/CFS patients [142]. These grey matter perfusion issues may account for the autonomic dysfunctions reported in ME/CFS and PBC patients [143,144]. Cerebral perfusion issues may impact the brainstem and overall brain functions which could explain the cognitive impairments reported in ME/CFS patients, such as brain fog, impaired memory, and concentration difficulties [141]. Although not yet definitively established, the decreased cerebral blood flow and reduced oxygen utilization in brain tissue may contribute to reduced cognitive functioning in ME/CFS individuals.

Significant reductions in oxygen levels and blood flow issues are present in SSc and PBC patients' brains. More than half of neurologically asymptomatic SSc patients have cerebral hypoperfusion [145]. This cerebral hypoperfusion could lead to chronic brain microvascular disease. Such a hypothesis is supported by increased white matter hyperintense foci in those with SSc, which correlates with the severity of peripheral vascular disease and cognitive symptoms [146,147]. Patients with SSc and white matter hyperintense lesions exhibit increased common carotid artery intima-media thickness, left and right carotid artery atheromas [147]. Furthermore, SSc patients commonly report neurological symptoms, and the brain issues observed in these patients may explain these symptoms [148]. When studying these brain problems it is crucial that the specific type of magnetic resonance imaging scan used to study brain abnormalities must be accounted for, as it has been demonstrated at least in SSc patients that certain magnetic resonance imaging types may not detect all white matter hyperintense lesions [149].

Individuals with PBC also experience cerebral vascular issues before cirrhosis onset [150]. Reduced cerebral vascular autoregulation and increased cerebral vascular resistance in PBC patients could explain the higher levels of cerebral hypoxia and altered cerebrovascular activity reported in their brains [31,151]. PBC patients also have increased cerebral deoxygenated hemoglobin, which compounds these individuals' cerebral oxygenation problems [31]. These findings may explain why PBC patients' worsened fatigue severity and cognitive performance are linked to altered deep gray matter, white matter lesions, and reduced bilateral brain white matter observed in PBC individuals [9,10,152], as oxygen levels can affect the structure and function of gray matter. The impaired central nervous activation and abnormal intra-cortical inhibition seen in PBC patients, independent of exercise and liver transplant status, could also be explained by reduced brain oxygenation [8]. Further research is needed to fully characterize how brain hypoxia contributes to cognitive difficulties in SSc and PBC patients.

3.12 HIF-1α Activation May Be an Effect of Decreased Oxygenation in SSc and PBC

Chronic hypoxia is a known driver of the metabolic adaptations that are described in SSc, PBC, and ME/CFS [153]. Patients with ME/CFS have impaired cellular mitochondrial respiratory activity, as suggested by lower peak oxygen extraction in muscle cells after cardiopulmonary exercise compared to controls [154]. Even when blood oxygen levels are normal, a decrease in oxygen utilization may trigger a cellular hypoxia response to activate the transcription factor HIF-1α. Hence, exploring the link between hypoxic responses and downstream mitochondrial adaptations may provide added insights that could be valuable in devising better treatments for chronic fatigue in PBC and SSc patients.

SSc-associated vasculopathy can lead to a chronic hypoxic state and downstream activation of HIF-1α [155], as suggested by increased HIF-1α/VEGF signaling [156].

Although a chronic hypoxic state is not traditionally thought to be a characteristic of PBC, such hypoxia may ensue from oxygen delivery problems irrespective of blood vessel pathology (e.g., cerebral vascular autoregulation problems [151]). This hypoxic state in SSc and PBC patients may lead to an increased cognitive dysfunction and chronic fatigue (**Figure 3**). Furthermore, this hypoxic state may lead to an altered expression and function of HIF1-α, as HIF1-α regulates muscle endurance, vascularization, and vascular remodeling [157,158]. HIF1-α activation may also promote disease progression in SSc and PBC patients through other mechanisms. This may include fibrosis as suggested by skin fibroblasts with higher levels of profibrotic mediators such as connective tissue growth factor expression via HIF1-α [159]. Similarly, *HIF1A* SNPs may promote the development of pulmonary arterial hypertension, potentially through modifications in HIF-1α/VEGF-A signaling in vascular endothelial cells [156,160].

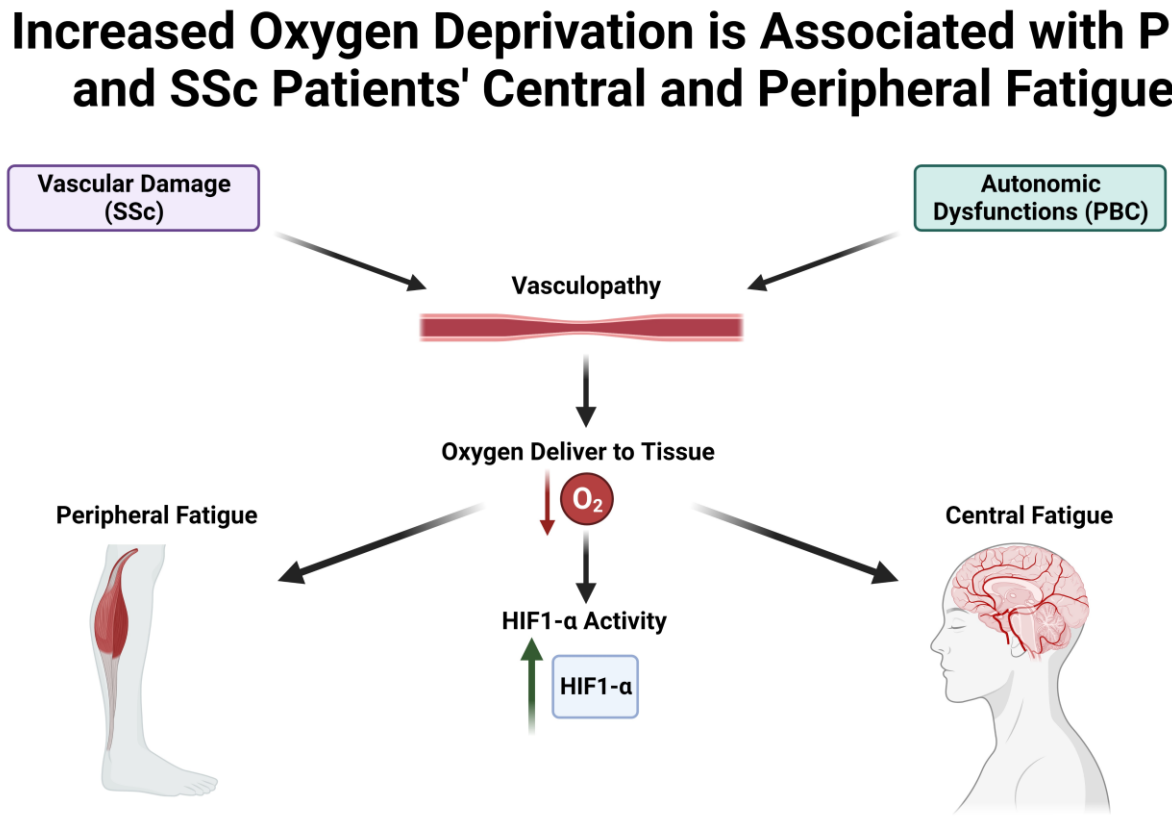

Figure 3. A possible way in which vasculopathy is linked to central and peripheral fatigue in systemic sclerosis (SSc) and primary biliary cholangitis (PBC) patients. The low oxygen (O_2) levels may lead to the development of central and peripheral fatigue, and the further activation of hypoxia-inducible factor 1 alpha (HIF1-α) leading to downstream hypoxia responses. The red down arrows show decreased levels, while the green up arrows indicate increased levels.

3.13 Issues with Red Blood Cells are Present in ME/CFS, SSc, and PBC Patients

Oxidative species causing reductions in red blood cell deformability could be another reason for the oxygen delivery problems in ME/CFS, SSc, and PBC patients. Reduced blood cell deformability decreases oxygen delivery to tissues by physically blocking capillaries thereby causing an increase in microvascular resistance and viscosity [161,162]. ME/CFS patients exhibit a significant reduction in red blood cell deformability, possibly due to oxidative damage to the red blood cells [163]. This hypothesis is supported by reports of elevated levels of reactive oxygen species scavengers, increased methemoglobin levels, and increased numbers of stomatocytes in some ME/CFS patients' blood [164,165]. These findings illustrate a link between changes in red blood cells and chronic fatigue, and such a relationship may also hold in SSc and PBC.

The link between chronic fatigue and oxidative species-induced red blood cell deformability reduction is also supported by studies in SSc and PBC patients. SSc patients' red blood cell membranes have decreased fluidity and increased lipid peroxidation products [166,167], while decreased red blood cell membrane fluidity and altered lipid composition problems have also been reported in individuals with liver diseases, including those with cholestatic viral hepatitis [168]. Furthermore, PBC patients with a higher red blood cell distribution width to platelet ratio, a measure of inflammation, have worse fibrotic histological severity [169].

Interestingly, a higher red blood cell distribution width to platelet ratio has been linked with poorer outcomes in stroke patients [170,171]. These findings suggest that higher red blood cell distribution width to platelet ratio values might be an indicative clinical marker of chronically PBC and SSc patients suffering from oxygen delivery problems to their brains. It is also worth mentioning that increased oxidative stress is a known inducer of inflammatory mediators that are elevated in both SSc and PBC (e.g., type I interferon signals). Hence, chronic hypoxia associated with associated oxidative stress may potentiate inflammation, particularly in genetically susceptible individuals.

4. Shared Immune System Abnormalities in SSc, PBC, and ME/CFS

Numerous studies have investigated the link between immune system abnormalities and fatigue severity. Many of these ideas propose that central fatigue mechanisms are associated with changes in peripheral immune system signaling molecules, especially through pro-inflammatory cytokines that act on brain targets [172]. PBC, SSc, and ME/CFS share autoimmune characteristics and immune system abnormalities, namely in decreased immune system homeostasis mechanisms (e.g., altered NK cell cytotoxicity, macrophage activation, and regulatory T-cell suppressive abilities) and in increased production of inflammatory cytokines that promote fatigue such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and interleukin-12 (IL-12) (**Figure 4**).

Particularly of interest are lines of evidence that the immune systems of these patients exhibit signs of senescence, inflammaging, and hypoxic responses. The former two could amplify fatigue by promoting an inflammatory reaction that leads to worse fatigue symptoms, while hypoxic responses could lead to an energy shortage in the immune cells. The following sections put forth evidence supporting the idea that increased effects of fatigue-inducing immune system factors may stem from genetics, hypoxic responses, and inflammaging, a form of chronic inflammation associated with aging.

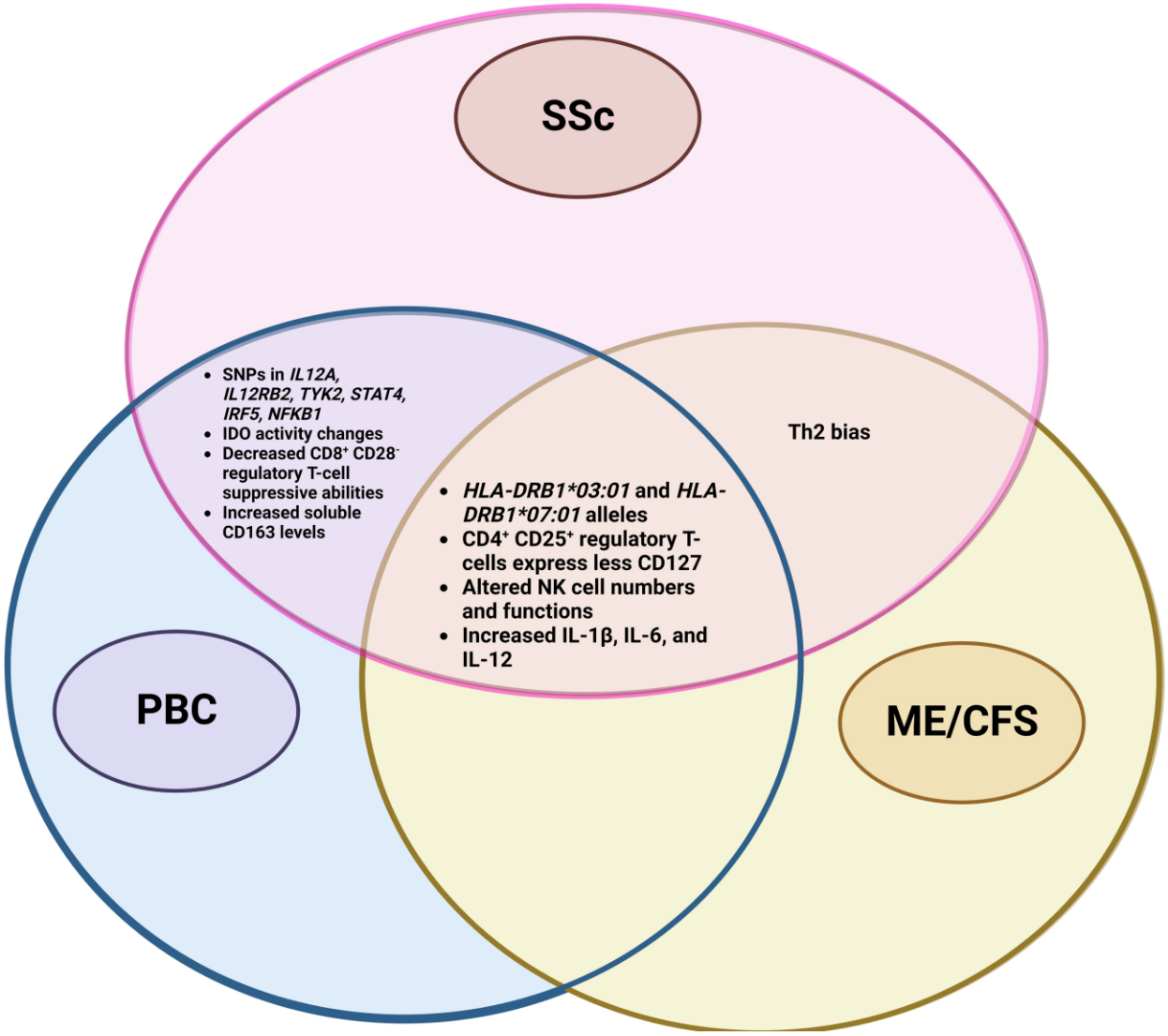

Figure 4. A visual representation of the most notable immune system abnormalities commonly found in primary biliary cholangitis, systemic sclerosis, and myalgic encephalomyelitis/chronic fatigue syndrome. These include genetic variants, T-cell abnormalities, NK cells, and cytokine levels. IL, interleukin; NK, natural killer cell; ME/CFS, Myalgic Encephalomyelitis/Chronic Fatigue Syndrome; PBC, primary biliary cholangitis; SNPs, single nucleotide polymorphisms; SSc, systemic sclerosis.

4.1 HLA Receptor Genetic Variants

Similar human leukocyte antigen (HLA) receptor genetic alleles, namely *HLA-DRB1*03:01* and *HLA-DRB1*07:01*, are shared between SSc, PBC, and ME/CFS patients. These alleles are responsible for encoding the major histocompatibility complex class II chain DRB1. Consequently, the alleles have varying impacts on the function of HLA-DRB1. *HLA-DRB1*03:01* increases the preference for arginine over citrulline in the "anchor sequence" within pockets P6 and P9 [173]. *HLA-DRB1*07:01* encodes for a glutamine residue at amino acid 74 in HLA-DRB1 [174]. Thus, it is highly likely that these alleles alter antigen presentation in SSc, PBC, and ME/CFS patients.

In PBC patients, *HLA-DRB1*03:01* is highly prevalent and is associated with the anti-nuclear anti-sp100 autoantibody [175,176]. Furthermore, this HLA allele haplotype is associated with the lcSSc subtype, an SSc subtype that is more at risk for developing PBC [177]. However, the *HLA-DRB1*03:01* allele haplotype may be less frequently present in chronically fatigued SSc and PBC patients as *HLA-DRB1*03:01* has been reported to be less commonly present in ME/CFS patients [178,179] although further studies are needed to confirm this hypothesis.

As for the other HLA-DRB1 allele, although *HLA-DRB1*07:01* has a protective effect on SSc susceptibility [180], it is more commonly present in PBC and ME/CFS patients [181,182]. Notwithstanding, these HLA genetic alleles could potentially contribute to the development, or even simultaneous occurrence, of PBC, SSc, and ME/CFS through alterations in immune system functions. Similarly, an additional important consideration are non-HLA genetic variants that are contributors to the co-occurrence of SSc, PBC, and/or ME/CFS. A summary of HLA and non-HLA genetic variants is provided in **Table 5**.

4.2 IL-12 Signaling Pathway Genetic Variants are Present in PBC and SSc Patients

Increased IL-12 levels are present in SSc, PBC, and ME/CFS patients, suggesting a possible link between increased IL-12 levels and chronic fatigue [183-185]. The causes of the increased IL-12 levels are unclear, although they may stem from increased production because of immune cell activation; or increased signaling from the IL-12 receptor and its components. For instance, the SNP rs589446 in the *IL12A* gene, which encodes for an IL-12 cytokine subunit, is associated with increased susceptibility in SSc and PBC patients [186,187]. While the biological impact of this SNP on PBC and SSc patients remains uncertain, other SNPs commonly present in the IL-12 signaling pathway could potentially elucidate the dysfunctions associated with both diseases.

PBC and SSc patients' shared SNP rs3790567 in the gene *IL12RB2*, which encodes for an IL-12 receptor subunit, is associated with increased susceptibility in both diseases [188,189]. Further research has indicated that PBC patients carrying this specific SNP have a heightened risk of developing cirrhosis and increased anti-mitochondrial M2 autoantibody serum concentrations, suggesting that this SNP contributes to the PBC disease process [190]. There is evidence to suggest that this SNP has other effects in both diseases. Interestingly, the SNP rs3790567 is associated with a dose-related decrease in IL-1β secretion following smallpox vaccination [191]. However, the elevated IL-1β levels observed in SSc and PBC patients imply that an alternate mechanism could counteract the SNP's effect of reducing IL-1β levels [192-194]. This explanation merits further investigation.

Shared genetic problems also exist in genes that facilitate downstream IL-12 signals. Notably, the *TYK2* SNP rs34536443 (which encodes a known Janus kinase downstream of IL-1, IL-12, and interferon signals and activates STAT4) is associated with increased susceptibility to SSc and PBC [187,195]. This SNP results in a missense mutation that may decreases TYK2's activation state [196]. One might anticipate that a

decrease in TYK2 activation would reduce STAT4 activation via phosphorylation, leading to an overall impairment of the downstream IL-12 signaling pathway. However, this contradicts reports of increased phosphorylated STAT4 in PBC [41]. As suggested by SSc-related findings, this phenomenon could be due to the increased activity of JAK2, an alternative activator STAT4 which stimulates STAT4 through phosphorylation [197,198]. However, further research is necessary to confirm this hypothesis.

Additional genetic data suggests that SSc and PBC may share a similar mechanism leading to increased STAT4 mRNA expression levels and STAT-related signaling. PBC and SSc patients share the *STAT4* SNP rs7574865, an SNP reportedly associated with increased *STAT4* mRNA levels [199-203]. Likewise, an increased predisposition to PBC and SSc has also been linked to another *STAT4* SNP, specifically rs11889341 [199,200,204]. In PBC patients, other effects of the rs11889341 SNP have been noted. Specifically, this SNP has been associated with ANAs, an autoantibody category typically linked with SSc [199]. Furthermore, the SNP rs11889341 has also been associated with increased *STAT1* and *STAT4* mRNA levels which may explain the increased expression of STAT1 in SSc patients [204-206]. While it is not yet clear exactly how the rs11889341 affects *STAT1* mRNA expression levels, it may be that the close physical proximity of the *STAT1* and *STAT4* genes may allow the *STAT4* SNP to influence *STAT1* mRNA expression levels [207,208]. Although these SNPs are associated with increased susceptibility to PBC and SSc, other findings indicate that genetic variations in HLA, IL-12, and downstream signaling mechanisms are not the sole determinants driving these diseases.

4.3 Interferon Signaling Pathway Genetic Variants are Present in PBC and SSc Patients

A hallmark feature of SSc, PBC, and ME/CFS is severe myalgia (or muscle pain), which is clinically linked to the development of fibromyalgia. In patients with fibromyalgia, this diffuse pain may stem from abnormal interferon signatures [209]. In PBC and SSc, patients have increased expression of genes associated with a type I interferon response [210,211]. Increased levels of type I interferons promote the release of IL-12 and immune system activation. One of the most critical downstream transcriptional regulators of interferon-associated genes are the transcription factors nuclear factor kappa B subunit 1 (NFKB1) and interferon regulatory factor 5 (IRF5) [212,213]. While the SNP rs230534 in the *NFKB1* gene has been reportedly linked to an increased risk of developing PBC and SSc, the biological effect of this SNP is currently unknown [187,214,215]. However, more information exists to speculate on the biological effects of the shared SNPs in the *IRF5* gene.

Two SNPs in the IRF5 gene, rs10488631 and rs4728142, have been reported to be associated with increased susceptibility in SSc and PBC (rs10488631 only when associated with *IL12RB2* SNP rs72678531 in PBC patients) [187,216,217]. The effects of these SNPs in both diseases may be through their association with altered *IRF5* mRNA expression. The SNP rs10488631 is associated with increased *IRF5* mRNA expression levels [218]. Conversely, the minor allele of rs4728142 has been linked with reduced *IRF5* mRNA levels in SSc patients, possibly due to the allele's potential to regulate the usage of the *IRF5* gene's alternative promoters [217,219]. The contradictory impacts of these SNPs on *IRF5* mRNA expression complicates predicting the overall effects of these SNPs on PBC and SSc patients' *IRF5* mRNA expression, although the findings suggest that there must be some changes in *IRF5* mRNA expression levels that are present. Even though these *IRF5* and *NFKB1* SNPs have not been identified in patients with ME/CFS, these pathways might still be crucial in promoting ME/CFS as inflammatory signals downstream of their activation are elevated in ME/CFS patients [185].

Table 5. HLA and Non-HLA Genetic Variants for Systemic Sclerosis (SSc), Primary Biliary Cholangitis (PBC), and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS).

Chromosome			SNP	Associated Gene	Potential Biological Functions Affected	Association with Disease	Study Methods Used	Ref.
Number	**Locus**	**Base Pair Position**						
HLA Region Alleles								
6	6p21.32	32,577,902 - 32,589,848	N/A	*HLA-DRB1*03:01*	Affects antigen presentation by increasing the preference for arginine over citrulline in the "anchor sequence" within pockets P6 and P9.	• High allele prevalence and is associated with the anti-sp100 autoantibody in PBC patients. • Associated with the lcSSc subtype in SSc patients. • Lower allele prevalence in ME/CFS patients.	• PCR sequence-based typing in PBC, SSc, and ME/CFS. • Genome-wide association studies in PBC.	[175-179]
				*HLA-DRB1*07:01*	Affects antigen presentation by encoding for a glutamine residue at amino acid 74 in HLA-DRB1.	• Protective effect on SSc susceptibility. • Increased risk of developing PBC. • Higher allele prevalence in ME/CFS patients.	• PCR with sequence-based typing in SSc and PBC. • Next generation sequencing in ME/CFS.	[180-182]
Non-HLA Region SNPs								
1	1p31.2	67356694	rs3790567	*IL12RB2*	An intronic variant associated with decreased IL-1β secretion.	• Increased susceptibility for PBC and SSc. • Increased likelihood of developing cirrhosis and anti-mitochondrial M2 antibodies serum concentrations for PBC.	• GWAS for PBC • PCR for SSc.	[188-190]

2	2q32.2	191079016	rs11889341	*STAT4*	An intronic variant associated with increased *STAT1* and *STAT4* mRNA levels.	• Increased susceptibility for PBC and SSc. • Associated with anti-nuclear autoantibody status in PBC. • Susceptibility to SSc further increases with the *TBX21* SNP rs11650354.	• GWAS for PBC. • PCR for SSc.	[199,200]
		191099907	rs7574865		An intronic variant associated with increased *STAT4* mRNA levels.	• Increased susceptibility for PBC and SSc. • Increased susceptibility for developing SSc and fibrosing alveolitis with the *IRF5* SNP rs2004640 in SSc.	PCR for PBC and SSc.	[200,202,203]
3	3q25.33	160015740	rs589446	*IL12A*	An intronic variant associated with the long non-coding RNA transcript from the *IL12A-AS1* gene.	Increased susceptibility for SSc and PBC.	GWAS	[186,187]
4	4q24	102527884	rs230534	*NFKB1*	An intron variant located within intron 2 of NFKB1.	Increased susceptibility for SSc and PBC.	• GWAS for PBC and SSc • PCR for PBC	[187,214,215]
7	7q32.1	128933913	rs4728142	*IRF5*	A variant associated with altered *IRF5* mRNA expression through regulating alternative promoter usage of the *IRF5* gene.	• Increased susceptibility for PBC and SSc. • Increased susceptibility for developing fibrosing alveolitis in SSc.	• GWAS and immunochip for SSc. • Meta-GWAS for PBC.	[187,216,217]
		128954129	rs10488631		An intergenic variant that is associated with increased *IRF5* mRNA expression levels.	• Increased susceptibility in SSc. • Increased susceptibility in PBC only when associated with the	• Meta-GWAS for PBC. • GWAS for SSc.	[187,216]

						IL12RB2 SNP rs72678531 in PBC patients		
19	19p13.2	10352442	rs34536443	*TYK2*	An intronic variant that is associated with missense mutation in TYK2's protein tyrosine domain leads to TYK2 having a decreased activation state.	Increased susceptibility for SSc and PBC.	ImmunoChip	[187,195]

4.4 Elevated Levels of Inflammaging Cytokines in SSc, PBC, and ME/CFS Patients

IL-1β and IL-6 are the most promising senescence-associated secretory phenotype (SASP) cytokines linked to chronic fatigue in ME/CFS, SSc, and PBC patients. Both IL-1β and IL-6 have increased levels in these patient groups which suggests a relationship between increased cytokine levels and fatigue severity [192-194]. These two cytokines may directly link to chronic fatigue by directly on brain regions to trigger increased fatigue-related behaviours [172,220]. Alternatively, IL-1β and IL-6 may induce fatigue in these patients by affecting mitochondrial activity and metabolism. IL-1β can inhibit mitochondrial oxidative phosphorylation and super-complex formation, while IL-6 can modulate mitochondrial activity in skeletal muscles and $CD4^+$ T-cells [221,222]. It should be noted that chronic hypoxia, a common condition in these patients, could further exacerbate fatigue symptoms by amplifying these cytokines' fatigue-inducing effects [223-226]. Therefore, the effects of IL-1β and IL-6 on both central brain functions and metabolic activity, especially under hypoxic conditions, are perhaps the link connecting immune cell senescence and fatigue manifestation in PBC and SSc patients.

Increased levels of other SASP cytokines, including CCL2, IL-8, IL-12, and IL-18, have been reported in SSc, PBC, and ME/CFS [183-185,227-235]. This constellation of findings supports the potential link between the emergence of SASP cytokines and these patients' fatigue symptoms. Furthermore, chronic hypoxic conditions, which are

associated with these diseases, can further stimulate SASP cytokine production and intensity inflammaging, exacerbating the chronic fatigue experienced by individuals with SSc, PBC, and ME/CFS [236]. However, more research is needed to clarify these cytokines' exact relationship with both hypoxia and inflammaging, and their link to chronic fatigue development in these patient groups.

4.5 Evidence of Monocyte/Macrophage Abnormalities in SSc, PBC, and ME/CFS

Evidence suggests that monocyte/macrophage abnormalities are present in PBC, SSc, and ME/CFS. While there is no direct evidence of modified macrophage functions in ME/CFS patients, a preliminary study indicates that there is a dysregulation of classical monocytes, specifically in genes that control their differentiation, tissue migration, and hypoxia responses [237]. This finding suggests that monocyte/macrophage abnormalities, perhaps due to genetic causes, are associated with chronic fatigue symptoms. The association may also hold for chronic fatigue in PBC and SSc. SSc and PBC patients have increased levels of soluble CD163, a marker indicative of non-classical M2 macrophage activation with pro-fibrotic properties [238-241]. These increased levels suggest a significant involvement of these non-classical M2 macrophages in the progression of both PBC and SSc.

Further findings report that PBC patients' monocytes display increased sensitivity to toll-like receptor ligand stimulation and pro-inflammatory cytokine secretion in peripheral blood monocytes [242]. Such cells may be operating in a hypoxic environment as PBC patients' liver $CD68^+$ cells have increased expression and nuclear localization of HIF-1α [243]. In the case of SSc, while no changes in monocyte/macrophage activity have been reported so far, treatments that target macrophages, such as mycophenolate mofetil and tocilizumab, could potentially alleviate symptoms by regulating the abnormal levels of pro-macrophage cytokines (e.g., CCL2) [68,244,245]. Thus, these findings collectively suggest a pivotal role for macrophage abnormalities, possibly through

dysregulation leading to M2 monocyte/macrophage activation, in the onset and progression of chronic fatigue symptoms in these patient groups.

4.6 Shared NK Cell Dysfunctions are Common in SSc, PBC, and ME/CFS

NK cells are important members of the innate immune system that play an essential role in immunoregulation by eliminating IL-12 cytokine family-producing cells such as monocytes and dendritic cells [246]. They also play an essential role in limiting viral infections and cancers [247,248]. Their dysfunction may explain why PBC and SSc patients are more likely to have infections and cancers [26,249-251]. There is supporting evidence of NK cell cytotoxicity impairments, decreased expression of NK cell cytotoxic proteins, and altered NK cell numbers in ME/CFS literature, suggesting a link between chronic fatigue and NK cell cytotoxicity problems [33,34]. These dysfunctions may be due to a chronic hypoxic environment resulting in decreased NK cell cytotoxicity although research must be conducted to confirm the validity of this theory [252,253].

Similarly, SSc individuals' NK cells have decreased cytotoxic ability through decreased NK cell expression of chemokines and NK cell receptor expression (e.g., NKG2D) [39,40]. Evidence of NK cell dysfunctions in SSc patients is further supported by reports of reduced CD69 expression, a marker of immune system activation, on NK cells in the peripheral blood [40,254]. However, when analyzing by SSc clinical subtype, there are increased $CD56^{+}$ NK cell numbers in dcSSc, but not lcSSc, subtype [39]. These findings suggest that differences across clinical disease subtypes must be accounted for when analyzing NK cell numbers and cytotoxicity.

PBC patients' NK cells exhibit increased numbers, an 'activated' phenotype by IL-12, upregulation of genes related to metabolic reprogramming, and increased cytotoxicity [41-43]. Furthermore, PBC patients with reduced transplant-free survival scores have decreased NK cell numbers [255], and perhaps a similar decrease in cytotoxicity may be uncovered in the future. It remains unclear if PBC and SSc patients with chronic fatigue

experience a decrease in NK cell cytotoxicity and whether this is due to hypoxia. Nonetheless, the abnormalities in NK cell populations in PBC, SSc, and ME/CFS patients further suggest a potential link to inflammaging.

4.7 T-Cell Related Dysfunctions are Prominent Features of SSc, PBC, and ME/CFS

The pathophysiology of PBC, SSc, and ME/CFS, suggests that chronic fatigue in these patient groups may be related to altered T-cell senescence and aging. Genetic evidence in the *PTPN22* gene, which encodes for a protein tyrosine phosphatase that decreases T-cell receptor signaling, supports this hypothesis. The SNP rs2476601 in the *PTPN22* gene, associated with both increased SSc and infection-onset chronic fatigue risk, has also been linked to a higher likelihood of frailty in older patients [256-258]. This finding suggests a potential link between genetically influenced changes in T-cell functions and the aging process, which may contribute to worsening physical symptoms.

4.71 Th2 Responses are Possible Features Associated with Chronic Fatigue in SSc, PBC, and ME/CFS

Immune dysregulation characterized by an imbalance between Th1 and Th2 responses is a shared hallmark in PBC, SSc, and ME/CFS. More specifically, a skew towards Th2 responses is evident in ME/CFS and SSc patients [259-261]. While many factors can result in a Th2 bias, the effects of hypoxia and IL-6 are particularly worthy of investigation. Interestingly, exercise in a hypoxic environment tends to promote Th2 responses [262]. This effect may partly explain the post-exertional malaise observed in ME/CFS patients, which may result from further amplifying their already elevated Th2 response. Furthermore, the elevated levels of the cytokine IL-6, known for inducing fatigue, in both patient groups may be promoting Th2 responses while suppressing Th1 responses [263]. These findings suggest that Th2 responses and the influence of IL-6 in

enhancing these responses should be explored further in chronically fatigued SSc patients.

On the other hand, PBC patients typically exhibit a more robust Th1 response promoted by IL-12 and non-classical $CD14^{low}$ $CD16^{+}$ monocytes [264-266]. Despite this Th1 dominance, there may be a role for Th2 responses in PBC. Th2-associated cytokines, such as IL-5, IL-9, and IL-10, are found at increased levels in PBC patients [185,267-269]. This begs the question of whether PBC patients' chronic fatigue symptoms are linked to an increased Th2 response, as such a response has been observed in ME/CFS patients.

4.72 $CD4^{+}$ $CD25^{+}$ Regulatory T-cells

Common abnormalities in $CD4^{+}$ $CD25^{+}$ regulatory T-cells are also present in SSc and PBC patients. It has been consistently shown that PBC patients have fewer $CD4^{+}$ $CD25^{high}$ regulatory T-cells [270,271]. The observation of similar reduced cell frequencies in the PBC patients' immediate female relatives – mothers and sisters – hints at a shared cause, genetic or environmental, driving these changes [271]. However, these cells exhibit reduced immunosuppressive capabilities only when expressing low levels of CD127 [270].

Similarly, SSc patients' decreased numbers of $CD4^{+}$ $CD25^{high}$ regulatory T-cells expressing CD127 have also been reported [272,273]. These patients' $CD4^{+}$ $CD25^{+}$ regulatory T-cells also have increased CD62L and CD69 expression which negatively correlates with their suppressive function, suggesting that these cells have impaired migration and activation in SSc [274]. Interestingly, as $CD4^{+}$ $CD25^{+}$ T-cells undergo more population divisions, there is an increase in these cells' CD95-mediated apoptosis [275]. This apoptosis could explain both diseases' decreased $CD4^{+}$ $CD25^{+}$ regulatory T-cell numbers. However, so far, no studies have reported the levels of CD95 expression in these T-cells in PBC or SSc. It is crucial to note that the observed frequencies and the

effectiveness of these regulatory T-cells can fluctuate based on the progression stage of SSc [272,273], and this must be accounted for when studying this cell type in PBC and SSc.

Similar anomalies in ME/CFS patients' $CD4^+$ $CD25^+$ regulatory T-cells imply a strong association between these cellular abnormalities and the chronic fatigue experienced by patients with SSc and PBC. While it is clear that ME/CFS patients' $CD4^+$ $CD25^+$ regulatory T cells have lower CD127 expression levels [276], decreased or increased numbers of these cells have been reported depending on which ME/CFS classification criteria were used to classify the patients [276,277]. Therefore, careful consideration must be taken when choosing criteria for classifying patients as chronically fatigued. In short, given these findings, it is likely that chronically fatigued PBC and SSc patients' $CD4^+$ $CD25^+$ regulatory T-cells have an even lower expression of CD127 compared to other individuals.

4.73 $CD8^+$ $CD28^-$ Regulatory T-cells

$CD8^+$ $CD28^-$ regulatory T-cells, a class of senescent exhausted regulatory T-cells that play an important role in preventing chronic antigen activation of $CD4^+$ T-cells, are dysfunctional in PBC and SSc. In PBC individuals, these cells have diminished suppressive capacity following IL-10 incubation, reduced CD39 expression, and increased CD127 expression [278]. While there are conflicting reports regarding whether the cytotoxic or suppressor functions of $CD8^+$ $CD28^-$ T-cells are enhanced in individuals with SSc [35], these patients have fewer $CD8^+$ $CD28^-$ regulatory T-cells [273]. The disparities in findings regarding these cells' capabilities in SSc patients may stem from the use of different study settings - *in vivo* versus *ex vivo*. Notably, SSc patients' $CD8^+$ $CD28^-$ cells exhibit enhanced suppressive activity only when studied in an *ex vivo* environment [273]. Thus, research examining the role of dysfunctional $CD8^+$ $CD28^-$ regulatory T-cells in PBC and SSc patients, especially their connection to chronic fatigue

due to their ‘exhausted’ ($CD28^-$) state, should consider how their chosen study setting might influence these cells' suppressive capabilities.

$CD8^+$ $CD28^-$ regulatory T-cells’ ability to skew the balance between Th17 cells and regulatory T-cells towards a Th17 response could play a role in the observed imbalance between Th17 and regulatory T-cells in PBC patients (who also have Sjogren’s syndrome) and in SSc patients [279-281]. The bias towards a pro-Th17 response may functionally contribute to the cerebrovascular complications seen in both diseases. This theory is supported by changes in vascular endothelial cell markers that are associated with elevated Th17 cytokine levels following acute cerebral infarctions [282]. The exact biochemical mechanism for how this occurs requires further study, but it could be through HIF1-α’s ability to promote differentiation of T-cells into Th17 cells [283]. Therefore, understanding the intricate role of $CD8^+$ $CD28^-$ regulatory T-cells in these diseases and their connection to hypoxic responses could provide potential insights into a chronic fatigue mechanism that is associated with these cells’ effects on altered cerebrovascular functioning.

4.8 Immune System Metabolic Abnormalities

Evidence suggests that metabolic changes in immune cells are present in ME/CFS, SSc, and PBC patients, perhaps in response to the hypoxic environment in which these cells operate. Pyruvate dehydrogenase kinases inhibit pyruvate dehydrogenase’s activity, which ultimately lead pyruvate to be converted into lactate instead of being metabolized in the mitochondrial tricarboxylic acid cycle. ME/CFS patients’ peripheral blood mononuclear cells have increased mRNA expressions of pyruvate dehydrogenase kinases 1, in addition to increased mRNA expressions of pyruvate dehydrogenase kinases 2 and 4 [137]. It is noteworthy that pyruvate dehydrogenase kinases 1 and 4 are genetically regulated by HIF1-α, suggesting that this increased expression may be linked to hypoxia in these patients [284,285]. This theory is reinforced by observations in ME/CFS patients, where their $CD8^+$ T-cells have diminished

mitochondrial membrane potential and decreased mitochondrial ATP production [138]. These changes align well with metabolic changes typically seen under hypoxic conditions, suggesting that shifts in immune cell metabolism could be a crucial factor associated with chronic fatigue symptoms.

Another common type of metabolic abnormalities in SSc, PBC, and perhaps ME/CFS are problems with the enzyme indoleamine 2,3-dioxygenase (IDO). SSc and PBC patients' blood serum have similarly increased IDO activity associated with increased kynurenine levels [116,117]. Kynurenine is a metabolic product associated with IDO's metabolization of tryptophan that suppresses effector T-cell functions and promotes regulatory T-cell functions. Although it would typically be anticipated that higher kynurenine levels would coincide with enhanced regulatory T-cells' suppressive abilities, this is not the case in SSc and PBC patients, whose cells have reduced suppressive capabilities.

This paradox could be attributed to specific genetic variants impacting IDO activity. For instance, SSc patients have decreased $CD8^+$ $CD28^-$ regulatory T-cell suppressive abilities when carrying the SNP rs7820268 T allele in their IDO1 gene despite this allele typically being associated with elevated IDO1 expression [286,287]. This finding hints at the increased IDO activity as a compensatory response to this allele's potential impact in reducing IDO activity. A similar mechanism has been proposed for ME/CFS, in which a possible genetic mutation in the IDO gene could lead to reduced kynurenine production [288]. This latter hypothesis suggests that such a metabolic alteration could contribute to chronic fatigue symptoms and inflammaging in SSc and PBC patients.

While the precise cells responsible for modified IDO activity have not yet been fully characterized, one potential immune cell type to consider are IDO-expressing macrophages. Reports suggest that when macrophages are stimulated by interferon gamma, they can increase their IDO expression [289]. This increased IDO can then exert a suppressive effect on T-cell activity [289]. As such, studies focusing on IDO activity

levels in chronically fatigued PBC and SSc patients must account for the effects of this and other IDO-expressing cell types.

Further evidence suggests that SSc patients' monocyte-derived cells may undergo other metabolic changes. For instance, SSc patients' monocyte-derived macrophages have increased upregulation of genes related to glycolysis, oxidative phosphorylation, and hypoxia [127]. These changes may not only be limited to monocyte-derived macrophages but might also affect other monocyte-derived cells. In support of this hypothesis, SSc patients' monocyte-derived dendritic cells have higher levels of L-carnitine and L-acetyl carnitine, both of which are vital players in mitochondrial fatty acid oxidation [107]. While the effects of immune system metabolism changes on the SSc and PBC disease processes needs to be further characterized, existing research in ME/CFS strongly suggests a connection between these changes and chronic fatigue in these patient groups.

5. Conclusions and Future Directions

Chronic fatigue, a commonly overlooked symptom, affects many PBC and SSc patients. It is associated with reduced physical function, increased cognitive dysfunction, and a decreased quality of life. Shared disease characteristics among PBC, SSc, and ME/CFS suggest that chronic fatigue has a physical basis rather than a purely psychological one. Interventions for managing or alleviating fatigue have produced conflicting outcomes, highlighting the need to understand the underlying causes of chronic fatigue in PBC and SSc patients. Thus, developing more effective and targeted treatments is crucial in treating these patients' chronic fatigue.

The intricate nature of autoimmune diseases poses a significant challenge in their classification and study. These diseases, which emerge from a complex interplay of genetic, environmental, and lifestyle factors, are characterized by an extensive range of symptoms that can impact virtually any part of the body. This broad spectrum of

manifestations, coupled with the individual variability in symptom presentation, adds a layer of complexity to their classification, as aberrant autoimmunity may not necessarily be the sole cause of the emergence of autoimmune disease.

The task of diagnosing and treating autoimmune diseases is further complicated by the inconsistent presence of certain antibodies and other immune markers. While these markers are often associated with specific autoimmune diseases, they are not always detected in every case, and the same markers may be present in different autoimmune diseases (or even healthy individuals). Even more perplexing is the evolutionary nature of autoimmune diseases; a person's diagnosis can change over time as new symptoms emerge or existing ones intensify.

The study of autoimmune diseases is no less challenging. Given their multifactorial nature, establishing clear cause-and-effect relationships is a formidable task. Further complicating are the rarity and long latency period of some autoimmune diseases, which poses significant obstacles to large-scale epidemiological prospective studies. Finally, the lack of standardized diagnostic criteria for many autoimmune diseases contributes to inconsistent diagnoses, complicating the comparison of findings reported in different studies.

Similarly, ME/CFS classification criteria are recommended to ensure consistency when classify chronically fatigued patients, especially for PBC and SSc patient groups. This approach establishes a common language and framework for chronic fatigue, a symptom often perceived subjectively and fraught with challenges when it comes to defining the severe chronic form of this symptom. The move away from arbitrary fatigue severity definitions, which tend to differ significantly across studies, and the adoption of ME/CFS classification criteria is expected to pave the way for more consistency in research outcomes. Such standardization allows for a more reliable comparison of results across different studies, bolstering the validity and applicability of the collected data.

More than just a tool for research, the adoption of ME/CFS classification criteria as a standard classification for chronic fatigue can lead to more precise diagnoses, enhanced patient management, and the development of more targeted treatment strategies. Ultimately, this is expected foster a richer and more accurate body of knowledge, guiding future research and potentially improving patient outcomes in the context of chronic fatigue associated with autoimmune diseases like PBC and SSc.

Investigations into immune system abnormalities, metabolic dysfunctions, oxygenation problems, and unusual hypoxia responses offer clues into potential causes of chronic fatigue in PBC and SSc. Though these possible causes are often researched separately, they have considerable overlap in their contribution to both diseases, and hypoxia-inducible factors might be the connecting these different causes. Hypoxia-inducible factors are associated with metabolic shifts and downregulation of the mitochondrial electron transport chain [290,291]. Additionally, metabolic reprogramming induced by hypoxia-inducible factors can alter immune cell functions, contributing to chronic inflammation [46-48]. Therefore, future research should explore the role of hypoxia-inducible factors to understand the underlying causes of chronic fatigue in PBC and SSc patients.

Considering the interrelated abnormalities discussed in this thesis, we suggest a potential mechanism that could be linked to the emergence of chronic fatigue symptoms in SSc and PBC patients (as illustrated in **Figure 5**). SSc and PBC patients have genetic predispositions that result in enhanced HLA sensing, increased IL-12 signalling, and interferon signalling. This predisposition, combined with chronic hypoxia and metabolic changes, results in both reduced red blood cell deformability and increased hypoxia-inducible factor signaling through factors such as HIF-1α which further exacerbates the disease processes. As inflammaging progresses, there is an upsurge in SASP cytokine release, such as the fatigue-inducing cytokines IL-1β and IL-6. This is further accompanied by poor NK cell function, heightened macrophage activation, possible M2 macrophage polarization, and diminished regulatory T-cell (T-reg) function. These

interconnected factors eventually lead to manifestation of ME/CFS-like symptoms, both in central nervous system and in the periphery, in SSc and PBC patients.

Future treatments for chronic fatigue symptoms in both PBC and SSc patients should consider a combination therapy approach targeting multiple pathways given the similarities between their disease processes and the ME/CFS disease process. Such a therapy should include therapeutics that improves mitochondrial function, incorporates immune system-modulating drugs, and enhances oxygen delivery to tissues. Hopefully, the development of this therapy will improve many people's lives by providing relief from the severe impacts of chronic fatigue.

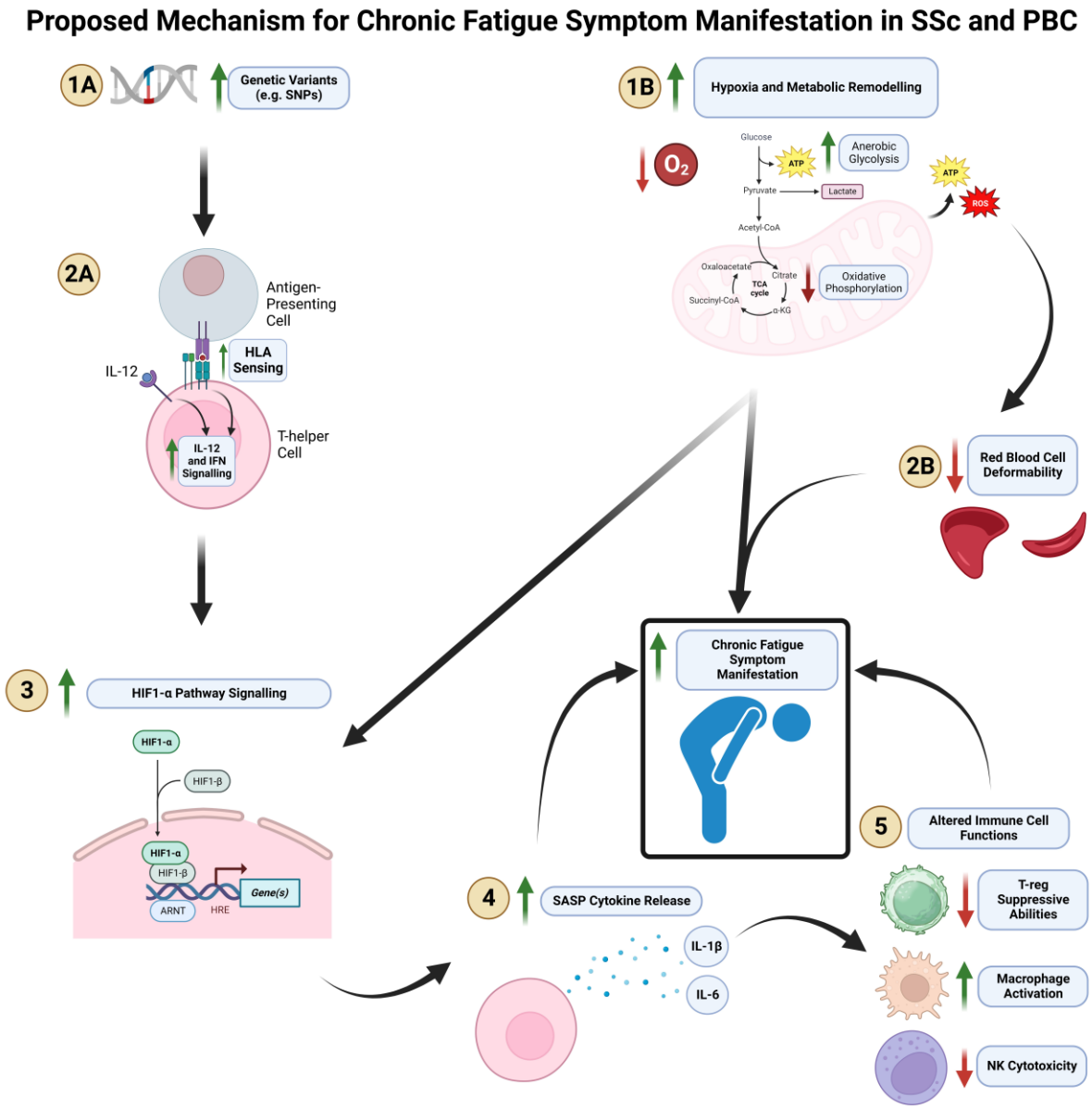

Figure 5. An illustration of the proposed mechanism underlying the emergence of chronic fatigue symptoms in SSc and PBC patients. Genetic predispositions in these patients enhance HLA sensing, and augment IL-12 and interferon signalling. These factors, in conjunction with chronic hypoxia and metabolic changes, intensify the signaling of hypoxia-inducible factors, such as HIF-1α. These chronic hypoxia and metabolic changes result in the increased production of reactive oxygen species which results in a reduced red blood cell deformability. The progression of inflammaging sees an upsurge in SASP cytokine release, notably IL-1β and IL-6. Concurrently, immune cell

dysfunctions manifest, including reduced NK cell function, heightened macrophage activation (with possible M2 polarization), and decreased T-reg function. Collectively, these interconnected factors contribute to the onset of ME/CFS-like symptoms, affecting both the central nervous system and peripheral tissues in SSc and PBC patients. α-KG, alpha-ketoglutarate ATP, ARNT, aryl hydrocarbon receptor nuclear translocator; adenosine triphosphate; HIF-1α, hypoxia-inducible factor-1alpha; HIF-1β, hypoxia-inducible factor-1beta; HLA, human leukocyte antigen; HRE, hypoxia-response element; IFN, interferon; IL-1β, interleukin 1β; IL-6, interleukin 6; IL-12, interleukin 12; NK, natural killer cell; O_2, oxygen; PBC, primary biliary cholangitis; ROS, reactive oxygen species; T-reg, regulatory T-cell; SNP, single nucleotide polymorphisms; SSc, systemic sclerosis.

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