

1 Case Report

2 Efficacy of Double Dose Dapsone Combination 3 Therapy in the Treatment of Chronic Lyme 4 Disease/Post-Treatment Lyme Disease Syndrome 5 (PTLDS) and Associated Co-infections: A Report of 6 Three Cases

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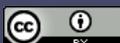
13 **Abstract:** Three patients with multi-year histories of relapsing and remitting Lyme disease and
14 associated co-infections despite extended antibiotic therapy were each given double dose dapsone
15 combination therapy (DDD CT) for a total of 7-8 weeks. At the completion of therapy, all three
16 patients major Lyme symptoms remained in remission for a period of 25-30 months. In conclusion,
17 Double dose dapsone therapy could represent a novel and effective anti-infective strategy in
18 chronic Lyme disease/PTLDS, especially in those individuals who have failed regular dose dapsone
19 combination therapy (DDS CT) or standard antibiotic protocols. A randomized, blinded,
20 placebo-controlled trial is warranted to evaluate the efficacy of DDD CT in those individuals with
21 chronic Lyme disease/PTLDS.

22 **Keywords:** Lyme disease; Post-Treatment Lyme Disease Syndrome (PTLDS); dapsone combination
23 therapy (DDS CT); double dose dapsone combination therapy (DDD CT); babesiosis; persistent
24 infection
25

26 1. Introduction

27 Lyme disease affects over 300,000 Americans per year [1] [2] and at least 2 million individuals in
28 the United States have been reported to be suffering from Post-Treatment Lyme Disease Syndrome
29 (PTLDS) [3]. In Europe, Lyme borreliosis is also the most common tick-borne disease [4] and
30 worldwide estimates suggest an increase in tick-vector-borne disease incidence and distribution [5].
31 Ticks can contain a broad range of bacteria (i.e., *Borrelia* spp., *Rickettsia* spp., tularemia), viruses (i.e.,
32 tick-borne encephalitis virus, Powassan virus) and parasites (babesiosis)[6]. Estimates from the World
33 Health Organization suggest that 17% of human global infectious disease burden are vector-borne,
34 with *Borrelia burgdorferi sensu lato* complex and relapsing fever borreliosis comprising the major
35 *Borrelia* spp. vectored by ticks [7]. Based on the geographical spread and increasing number of
36 individuals suffering from Lyme and associated tick-borne diseases (TBD's), and significant health
37 care costs associated with treatment failures [8][9], the necessity of finding effective treatments for
38 Lyme borreliosis and associated co-infections is vitally important from a public health perspective.

39 Approximately 10-20% of individuals treated for Lyme disease with a 2-4-week course of
40 antibiotics will go on to experience chronic, persistent fatigue, musculoskeletal pain and
41 neurocognitive difficulties that persist for more than 6 months, known as Post-Treatment Lyme
42 Disease Syndrome (PTLDS) [10]. The etiology of Chronic Lyme disease/PTLDS is unknown,



43 although several major hypotheses have been proposed to explain persistent symptoms, including
44 persistence of *Borrelia* and/or borrelial antigens, persistent tick-borne co-infections, immune
45 dysregulation, altered neural networks with central sensitization, and/or overlapping sources of
46 inflammation [11][12][13][14]. *Borrelia*'s ability to persist in the body has been hypothesized to take
47 place through multiple mechanisms. These include immune evasion with borrelia changing its
48 surface antigenic expression in response to host immune responses [15] [16], persistence in the
49 intracellular compartment [17] [18], and changing morphological forms in various environments [19]
50 [20] [21] [22], resulting in atypical cystic forms [23], pleomorphic round bodies (cell wall deficient,
51 L-forms) [20], as well as 'persister' and 'biofilm forms'[24] [25] [26] [27] [28]. The stationary,
52 persister, and biofilm forms of *Borrelia burgdorferi* (Bb) have been found to be resistant to standard
53 antibiotic treatments and a cause of persistent inflammation [29][30][31]. This phenotypic plasticity
54 of *Borrelia* and its survival in biofilms may help to explain in part clinical conundrums and persistent
55 symptomatology [32].

56 There have been several studies to date evaluating persister drugs and biofilm agents in the
57 treatment of Lyme disease. Most of these have been in vitro studies, using essential oils, herbal
58 compounds like Stevia, or drugs found through a search of the NCI compound collection or FDA
59 approved drug library [33][34] [35][29]. Two of these compounds, dapsone and disulfiram, which
60 are both sulfa drugs, have been found to be effective against stationary phase *Borrelia burgdorferi* [36]
61 [37][38] and evaluated in clinical studies. Disulfiram was found in a small case series to have a
62 positive clinical effect in three patients who required intensive open-ended antimicrobial therapy for
63 chronic relapsing neurological Lyme disease and relapsing babesiosis [39]. Dapsone combination
64 therapy (DDS CT) has been published in two, separate retrospective case series, totaling 300
65 patients, to have a positive effect on eight major Lyme symptoms, and improve treatment outcomes
66 among patients with chronic Lyme disease/PTLDS and associated coinfections in those failing
67 traditional antibiotic therapy [40][14].

68 Success in the prior DDS CT trials was operationally defined as improvement in percent of
69 normal after 6 months on DDS CT, and failure was operationally defined as remaining the same or
70 worsening of the percentage of normal after at least 6 months of DDS CT. "Of 181 participants who
71 gave both pre-DDS and DDS percentage scores, 14 participants reported feeling worse currently
72 than they did before the DDS, 22 participants reported no difference, while all other participants
73 (145) currently reported a higher percentage of normal"[14]. Causes of potential failures of DDS CT
74 highlighted in Part 1 of our Precision Medicine study included evidence of chronic persistent
75 infection with *Borrelia*, *Bartonella*, and *Mycoplasma* species, as well as *B. microti*. These were all shown
76 to persist despite commonly prescribed courses of antibiotics or antimalarial/*Babesia* therapy [14].
77 Persistence of bacteria can be explained in part by bacterial biofilms, in which cells are protected
78 from the immune system by surface exopolymers with polysaccharides [41]. Antibiotics have been
79 shown in this model to kill regular cells, leaving dormant persisters alive, and when the
80 concentration of antibiotic drops, they resuscitate and repopulate the biofilm [42].

81 Since the dosage of dapsone in the initial two studies varied between 25 and 100 mg/day, and
82 the effect of 'persister' drugs like dapsone may depend on drug dependent concentrations and its
83 effect on biofilms [43], we decided to try a higher dose of dapsone (100 mg BID) for one month in
84 several patients with a history of chronic, persistent relapses. We present here three case studies of
85 individuals who took 7-8 weeks of dapsone combination therapy using hydroxychloroquine,
86 cimetidine, nystatin, a tetracycline, rifampin, and dapsone (DDS CT), where the dose of dapsone was
87 increased the second month to 100 mg BID, i.e., double dose dapsone combination therapy (DDD
88 CT). Nutritional support included N-acetyl cysteine 600 mg PO BID, alpha lipoic acid 600 mg PO
89 BID, gradually increasing doses of glutathione up to 1000-2000 mg BID by the end of the first month,
90 folic acid (Leucovorin 25 mg PO BID month one, 25 mg TID month 2), L-methyl folate 15 mg PO
91 BID, along with three biofilm agents (Stevia, oregano oil, Biocidin) and three probiotics (Theralac,
92 Ultra Flora DF, *saccharomyces boulardii*). Patients signed informed consent forms that listed the major
93 side effects of dapsone combination therapy, which included Herxheimer reactions, anemia
94 secondary to folic acid inhibition (hemolytic anemia was minimized by ensuring all patients had

95 normal levels of G-6-P-D, or they were ineligible for the trial), rashes (secondary to sulfa sensitivity)
96 and/or methemoglobinemia (secondary to increased oxidative stress and decreased heme oxygen
97 carrying capacity). Patients were instructed to get regular laboratory testing with a complete blood
98 count (CBC), comprehensive metabolic profile (CMP) and methemoglobin levels once at 100 mg of
99 dapsone, and to repeat laboratory testing weekly during the second month of higher dose therapy.
100 Any major changes in their symptoms were to be reported immediately to the first author via an
101 emergency cell phone number. After stopping all antibiotic therapy once the trial was completed, all
102 three individuals remained on biofilm agents, folic acid replacement and probiotics for the next
103 several months. All three patients remained in remission for time periods ranging between 2 and 3
104 years with no further Lyme and tickborne symptoms after completing DDD CT.

105 2. Case 1

106 This 20-year-old Black male, with a past medical history significant for Lyme disease and
107 babesiosis, as well as obsessive-compulsive disorder (OCD), became our patient in June 2017. His
108 chief complaints included unexplained fevers, sweats, chills and/or flushing, significant fatigue,
109 upset stomach, neck stiffness and cracking, headaches, hair loss (secondary to trichotillomania),
110 blurry vision, and disturbed sleep with problems both falling asleep and early awakening. He had a
111 tick bite at age 4 years old, where his ELISA and Western Blot were positive, and he was treated by
112 an infectious disease specialist for the next 6 years. During that timeframe, he was continuously on
113 rotations of hydroxychloroquine (Plaquenil) and clarithromycin for 6 months, followed by rotations
114 of hydroxychloroquine and a tetracycline for 6 months. Each time he tried coming off the antibiotics
115 he had a relapse of his underlying symptoms and was therefore left on continuous antibiotic therapy
116 during that 6-year time frame. At age 12, due to his lack of progress, his parents took him to see a
117 pediatric Lyme disease specialist, who diagnosed him both Lyme disease and babesiosis (*Babesia*
118 titers were positive), since he still had ongoing fevers, sweats and chills, air hunger, fatigue,
119 headaches and insomnia. He was rotated to atovaquone, azithromycin and doxycycline, and
120 remained on this protocol for the next several years, until he plateaued without any further
121 improvement in his symptoms. He then saw a third Lyme specialist, who placed him on
122 doxycycline, sulfamethoxazole/trimethoprim (Bactrim DS) with fluconazole, as well as
123 atovaquone/proguanil (Malarone) for a short period of time, due to his ongoing fevers, sweats, and
124 chills consistent with ongoing babesiosis. These treatments helped his symptoms, but as per prior
125 regimens, was insufficient to maintain his health and remain in school.

126 During his first visit, he had been off antibiotics for several months, and all of his underlying
127 tickborne disease symptoms were relapsing. His prior physician had put him on cefpodoxime
128 proxetil, an oral third-generation cephalosporin before seeing us, which was ineffective. He
129 described moderate fevers, sweats, chills, severe fatigue (which increased postprandially), hair loss,
130 upset stomach, joint pain, headaches, neck stiffness, blurry vision, and insomnia. He denied any
131 other significant past medical history, social history, family history, and his review of systems was
132 otherwise unremarkable, except for occasional nocturia 3 × per night and frequent unexplained skin
133 rashes. Physical examination revealed a well-developed, well-nourished black male in no apparent
134 distress. Sitting blood pressure was 129/80 with a pulse of 73 bpm, and standing 9 minutes, his blood
135 pressure dropped to 119/83 with a pulse rate of 108 bpm. The 10-point drop in systolic blood
136 pressure and 35-point increase in pulse rate was consistent with severe Postural Orthostatic
137 Tachycardia Syndrome (POTS). The rest of his physical examination was unremarkable, except for
138 some evidence of trichotillomania, due to his underlying OCD.

139 We performed a complete blood count (CBC), comprehensive metabolic profile (CMP),
140 C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), hormone levels (thyroid functions,
141 DHEA/cortisol, pregnenolone), anti-gliadin antibody/tissue transglutaminase (TTG), IgE food allergy
142 panel, immunoglobulin levels and subclasses, mineral levels (magnesium, copper, zinc),
143 streptococcal titers (anti-streptomycin O [ASO], anti-DNase), viral titers (*Herpesvirus 6* [HHV-6],
144 *Epstein-Barr virus* [EBV], *cytomegalovirus* [CMV]), *Stachybotrys* titer, urinalysis, EKG, as well as a
145 comprehensive tickborne panel. This included a C6 Elisa peptide, IgM/IgG Lyme Western Blot,

146 *Babesia microti* immunofluorescent assay (IFA), *Babesia duncani* antibody, *Babesia* Fluorescent In Situ
147 Hybridization (FISH) test, *Bartonella* titer and PCR, Vascular Endothelial Growth Factor (VEGF),
148 Rocky mountain spotted fever (RMSF, *rickettsia rickettsii*) and tularemia titers (*Francisella tularensis*),
149 Q fever titers (*Coxiella burnetti*), and *Mycoplasma*, *chlamydia pneumonia* and *Brucella* titers. Significant
150 results included evidence of multiple food allergies, consistent with leaky gut; low plasma copper
151 (0.64 ug/mL, normal range between 0.80-1.75 ug/ml); prior exposure to HHV6 (antibody titers
152 1:1280, normal less than 1:80), and an elevated VEGF at 134 picograms/milliliter (normal range 0-115
153 pg/ml) consistent with possible Bartonellosis, with evidence of prior exposure to Lyme disease
154 (positive 31 kDa, 39 kDa and 41 kDa bands on an IgG Western blot). Urinalysis revealed mucus
155 threads and calcium oxalate crystals, without any history of kidney stones.

156 We discussed the results of the physical examination and blood tests with the patient and his
157 family. He was placed on a high salt diet with 2 liters of fluid per day for evidence of POTS, a strict
158 hypoglycemic diet avoiding food allergens for his postprandial energy swings and placed on a
159 different rotation of *Babesia* medication for his constant night sweats. He was given clindamycin 300
160 mg, 2 PO BID, Bactrim DS one BID, Malarone 2 PO BID, artemisinin one PO TID, grapefruit seed
161 extract 2 PO BID, nystatin 500,000 units tablets, 2 PO BID, along with Stevia extract 15 drops twice a
162 day and triple probiotics, including *Saccharomyces boulardii* to prevent antibiotic associated diarrhea.

163 Follow-up examination 2 months later, revealed mild progress. He still complained of severe
164 fatigue, irritable bladder, neck pain, stiffness of the neck and back, muscle pain, pain in the bottom of
165 his feet which would come and go with shin pain (increased with sports), occasional
166 lightheadedness, and mood swings with disturbed sleep, although his fevers and night sweats had
167 improved on this regimen. On physical examination, his blood pressure dropped 20 points systolic
168 (116/72 dropped to 96/70) with a 10-point increase in pulse rate at 10 minutes standing, consistent
169 with ongoing POTS. Due to lack of adequate improvement, azithromycin 250 mg PO BID was added
170 to his protocol, 4 days in a row per week, to extend the coverage against Bb, *Babesia* and possible
171 *Bartonella*, along with rifampin, 300 mg, 2 capsules PO BID one day per week. Mineral replacement
172 was administered for the history of low copper and gastrointestinal support (glutamine, short chain
173 fatty acids, probiotics) was added for his history of leaky gut and food allergies.

174 Follow-up consultation on November 2017, revealed less joint pain and an improvement in
175 *Babesia* symptoms (sweats and 'air hunger' were better) on clindamycin, azithromycin, and Bactrim
176 along with Cryptolepis, but fatigue was increased on the weekend while doing pulsed rifampin,
177 implying active intracellular infections, and/or biofilm forms of *Borrelia*. Clindamycin was therefore
178 stopped and changed to doxycycline, 150 mg PO BID, with rifampin, azithromycin, and Bactrim,
179 and follow-ups between January and March 2018 showed slow improvement. Severe fatigue was
180 primarily related to use of rifampin, and/or being off his hypoglycemic diet, but *Babesia* symptoms
181 continued to improve with decreased sweats.

182 As of May 2018, all symptoms were slowly improving with multiple intracellular antibiotics,
183 but he still complained of resistant fatigue, insomnia, neck pain, headaches, mild night sweats and
184 hair loss secondary to trichotillomania and ongoing OCD. The patient and his family did not want
185 him to go on a selective serotonin reuptake inhibitor (SSRI) like paroxetine, so we discussed other
186 options to treat his resistant tickborne symptoms. Since the patient had been on almost continuous
187 antibiotics since age 4 (14 years) and had never taken a 'persister' drug regimen like dapsone, we
188 discussed a trial of double dose dapsone combination therapy (DDD CT) for 2 months based on
189 prior published research for DDS CT. The patient and parents signed a consent form, which
190 explained the protocol in detail. His glucose 6 phosphate dehydrogenase level [G6PD] was within
191 normal limits, minimizing the risk of hemolytic anemia; sulfa induced rashes would be unlikely, as
192 he had been on Bactrim DS [sulfamethoxazole/trimethoprim] without side effects. The DDD CT
193 protocol consisted of the following: 2 months of doxycycline 150-200 mg PO BID, rifampin 300 mg
194 PO BID, hydroxychloroquine 200 mg PO BID, nystatin tablets 500,000 units, 2 PO BID, cimetidine
195 400 mg PO BID, and gradually increasing doses of dapsone: 25 mg week one; 50 mg week 2; 100 mg
196 weeks 3 and 4; and 100 mg of dapsone PO BID for 4 weeks, taken with Leucovorin 25 mg PO BID
197 month one, 25 mg TID month 2, along with L-methyl folate 15- 20 mg PO BID to help prevent

198 significant anemia. Methylene blue, 50 mg PO BID, would be used during month 2 if there was any
199 evidence of methemoglobinemia (a signed consent form had been given, explaining potential side
200 effects and contraindications) and folic acid dosing would be increased for any significant increase in
201 anemia. Nutritional support included 3 biofilm agents at the following doses: Stevia [NutraMedix]
202 15 drops PO BID; oregano oil capsules 60 mg PO BID and Biocidin, 2 sprays in a glass of water.
203 N-acetylcysteine (NAC) 600 mg PO BID, alpha lipoic acid 600 mg PO BID, and liposomal glutathione
204 1000 mg PO BID were used to reduce the risk of methemoglobinemia and severe Herxheimer
205 reactions, with triple probiotics PO BID taken at least one hour away from antibiotics. A typed flare
206 protocol for severe, persistent Herxheimer reactions was also given, using PRN sodium bicarbonate,
207 up to 2 grams twice a day, or drinking fresh squeezed lemons/limes twice a day through a straw (to
208 alkalize the body), along with 2000 mg of glutathione 2-3 × per day as needed. Laboratory values
209 with a CBC, CMP and methemoglobin levels were checked twice during month one, and weekly
210 during month 2 to rule out significant anemia and/or methemoglobinemia or detect any changes in
211 liver or kidney function. Once the 2-month protocol was finished, the patient was to stop all
212 antibiotics, and only continue on folic acid supplementation, biofilm agents and probiotics.

213 The patient and his family returned for an in-office consultation at the end of July 2018. He had
214 finished the double dose dapsone protocol approximately one month prior. He denied any
215 significant Herxheimer reactions during treatment and had significant improvement in all of his
216 baseline symptoms. Energy levels had significantly improved, neck pain and headaches decreased,
217 there were no noticeable night sweats, insomnia improved, and cognition was within normal limits.
218 He had some baseline mild right hip and foot pain, which was primarily increased playing soccer.
219 He stated that post DDD CT, “it was the best he had felt in years” and his overall percentage of
220 normal improved from 75% to 90% functioning. Baseline white cell counts (WBC) of 3.9-4.3 × 10
221 E3/uL remained stable during treatment and hemoglobin/hematocrit levels (H/H) of 14.2/43.5
222 dropped to 12.2/37.9 during month two (a 2 gram drop in hemoglobin) with methemoglobin levels
223 rising from 2.6% month one, to a maximum of 4.4% month two, which subsequently decreased to
224 3.9%, and 1.2% by the end of therapy (normal levels of methemoglobin range between 0 and 2.9%).
225 He denied any symptoms of methemoglobinemia during treatment, using glutathione and
226 methylene blue (no increased fatigue, headaches, shortness of breath and/or blue hands/feet), and by
227 the end of July 2018, his anemia had completely resolved, with an H/H of 15.9/48, increased from
228 baseline values.

229 We repeated *Borrelia burgdorferi*, *Babesia* and *Bartonella* testing posttreatment. The Lyme ELISA
230 was negative, his 31 kDa (Osp A) turned negative on the IgG Western blot, *Babesia microti* and *Babesia*
231 *duncani* titers were negative, *Bartonella henselae* and *Bartonella Quintana* titers were negative, VEGF
232 levels decreased to normal, but he had a positive *Babesia* FISH, consistent with active babesiosis,
233 despite denying clinical symptoms of babesiosis. He therefore remained off all antibiotics and was
234 only placed on Malarone (atovaquone/proguanil 100/250 mg) 2 PO BID with liposomal Artemisia
235 one PO TID for several months with traditional Chinese herbs (Coptis, HH, circulation P). Six
236 months post DDD CT, during a repeat consultation, he was 100% of normal functioning. He denied
237 fatigue, neck pain or headaches, joint pain, fevers, sweats, chills or air hunger, cognitive difficulties
238 or insomnia and his OCD symptoms resolved with a decrease in trichotillomania. He was therefore
239 tapered off Malarone, and remained on mineral supplementation for his low copper, along with a
240 hypoglycemic diet, avoiding food allergens. Repeat VEGF on December 2018 increased from less
241 than 31 pg/mL to 250 pg/mL (normal range 31-86 pg/mL), but follow-up studies 6 months later
242 showed that his VEGF returned to normal range with a negative *Babesia* FISH.

243 By December 2019, the patient was 1 ½ years off of antibiotics and in remission post DDD CT,
244 and only complained of fatigue his 1st year of college if he was up late studying without adequate
245 sleep. According to his mother, he was frequently sick during his childhood, and now he was never
246 ill. Repeat adrenal testing showed phase 3 adrenal dysfunction with low morning and evening
247 cortisol levels, which were contributing to his resistant fatigue. He was therefore placed on
248 adaptogenic herbs and an adrenal glandular supplement which reversed any problems with
249 energy/stamina. He was able to play recreational sports and walk a minimum of 2 miles per day.

250 He denied any Lyme, *Babesia* or *Bartonella* symptoms, his OCD and trichotillomania completely
251 resolved, and POTS/dysautonomia improved with mild tachycardia standing with no further drops
252 in blood pressure. During his last follow-up on August 2020, he was over 2 years without a relapse
253 of tickborne symptoms (25 months) and was able to stay off antibiotics for the 1st time in 14 years.

254 3. Case 2

255 A 39-year-old white female presented to our medical office for the first time in July 1995 with a
256 long and complex medical history significant for Lyme disease and MSIDS (Multisystemic Infectious
257 Disease Syndrome), with more than 16 different overlapping etiologies discovered during a period
258 of two decades which were contributing to her illness. These included Lyme disease (ELISA + [1.20
259 IgG/IgM serology, normal < 1.0], CDC positive IgM Western Blot, serum *Borrelia burgdorferi* PCR +),
260 Babesiosis (positive *Babesia microti* titer 1:160), Ehrlichiosis (*Ehrlichia chaffeensis*, Human Monocytic
261 Ehrlichia [HME] titers 1:160 +), Anaplasmosis (*Anaplasma phagocytophilum*, Human Granulocytic
262 Anaplasma [HGA] titers 1:160 +), Rocky Mountain Spotted Fever (RMSF) IgG, Enzyme
263 Immunoassay (EIA) + (reference range negative/positive), exposure to *Bartonella henselae* (titers 1:64
264 +), positive *Mycoplasma pneumonia* titers with a serum *Mycoplasma fermentans* PCR + (despite years of
265 intracellular antibiotic rotations, including tetracyclines, rifampin, azithromycin and/or quinolones);
266 prior viral exposure to EBV and HHV-6; hormonal dysfunction with phase 2 adrenal dysfunction on
267 a DHEA/cortisol test, hypothyroidism (low T3, free T3, elevated TSH), hypoglycemia/metabolic
268 syndrome (HbA1c 5.9%, hyperlipidemia with an LDL 221), low Vitamin D (18.0 ng/mL, normal
269 range 31-100 ng/mL) and menopause with low estrogen, progesterone and testosterone levels. She
270 also had a history of gluten sensitivity (anti-gliadin antibody + [39 Units, negative values less than 20
271 U], tissue transglutaminase [TTG] negative), multiple food allergies (milk, eggs, nuts, wheat, soy,
272 corn, shrimp, beef and pork) with a history of Candidiasis and leaky gut; inflammation (antinuclear
273 antibody [ANA] +, C4a [1416.7, normal range < 650]) with evidence of Mast Cell Activation Disorder
274 (MCAD), proven by elevated levels of histamine (38 nmol/L, normal < 8) and chromogranin A (415
275 ng/mL, normal < 95) in the blood, which would result in migraines and gastrointestinal distress with
276 dietary indiscretions; black mold exposure (+ *Stachybotrys* titers, black mold found growing on
277 beams in the basement under her bedroom and bathroom, requiring remediation) and heavy metal
278 exposure (elevated levels of mercury [7.7 mcg/day, normal < 5], cadmium [2.6 mcg/d, normal < 2],
279 and aluminum [87 mcg/d, normal < 50] on a 6-hour urine dimercaptosuccinic acid [DMSA]
280 challenge) with elevated levels of mercury on hair analysis (7.8, normal < 1.2), along with
281 detoxification problems compounded by low serum glutathione levels and deficiencies in serum
282 mineral levels (red blood cell [RBC] magnesium, iodine, zinc). She also had a history of
283 Post-Traumatic Stress Disorder (PTSD) secondary to a prolonged history of family trauma, Postural
284 Orthostatic Tachycardia Syndrome (POTS)/dysautonomia (baseline BP 94/54 with a pulse rate of 80
285 beats per minute (BPM), which would decrease to 80/54 standing with a 20 point increase in pulse
286 rate) with episodes of dizziness and pre-syncopal episodes; sleep apnea; abnormal
287 immunoglobulins (IgG 3 subclass deficiency [29 mg/dL, normal range 41-129] and elevated levels of
288 IgM [303 mg/dL, normal range 40-230]. These various medical problems were addressed during a
289 time period of over 20 years. Each time an abnormality on the 16-point MSIDS map was addressed
290 (i.e., multiple infections, environmental toxins, detoxification issues with mineral deficiencies,
291 hormonal dysfunction, POTS/dysautonomia, hypoglycemia, Candida, histamine intolerance with
292 MCAD, sleep apnea, PTSD) clinical improvement was noted, but relapses were common, within
293 days to weeks, especially when treatment for her tickborne diseases were discontinued.

294 Her chief complaints consisted of severe fatigue, day sweats, night sweats and chills, neck
295 stiffness and headaches with intermittent migraines, migratory joint and muscle pains in the neck,
296 fingers and toes, hair loss, rare tingling of the extremities, intermittent gas, nausea, vomiting and
297 constipation, blurry vision, balance problems with intermittent dizziness, moderate memory and
298 concentration problems with poor organizational skills, poor sleep secondary to sleep apnea, and
299 periods of sadness with depression and anxiety. Medication regimens used during a time frame of
300 over two decades to address her Bb, *Babesia*, *Bartonella* and *Mycoplasma* exposure according to chart

301 review included rotations of doxycycline, atovaquone/proguanil and mefloquine; atovaquone and
302 azithromycin; amoxicillin/clavulanic acid; cefuroxime axetil and azithromycin; amoxicillin and
303 azithromycin; cefuroxime axetil, hydroxychloroquine and azithromycin; cefdinir, probenecid,
304 telithromycin and hydroxychloroquine; rotations of double intracellular antibiotic regimens with
305 hydroxychloroquine (minocycline +azithromycin, minocycline +ciprofloxacin, minocycline
306 +rifampin, doxycycline + moxifloxacin), each for several months at a time, along with a course of IV
307 ceftriaxone 2 grams/day for one month, with intermittent use of IV glutathione, which was effective
308 for underlying symptoms and Herxheimer reactions. As of November 2017, post rotations of
309 antibiotics for her tickborne diseases (TBD's), she remained on Armour thyroid 60 mg/day,
310 liothyronine 10 mcg BID, fludrocortisone 0,1 mg/day, midodrine 10 mg TID, Bioidentical hormone
311 therapy (Bi-Est) with estradiol (0.25 mg), progesterone (40 mg), DHEA (5mg), testosterone (2.5 mg),
312 pregnenolone (10 mg), cetirizine (10 mg), famotidine (20 mg), occasional courses of fluconazole for
313 Candida flare-ups, and low dose naltrexone 4.5 mg HS.

314 During the above 20 + year medical history, when each of the above abnormalities were treated,
315 she would often feel better with improved levels of functioning, but never maintain her health if
316 anti-infective therapies against *Borrelia* and co-infections were stopped. Relapses of underlying
317 tickborne symptoms would often happen within days of stopping her various antibiotic protocols,
318 requiring anti-infective herbal protocols to help stay in longer periods of remission (Zhang,
319 Traditional Chinese Medicine [TCM, Coptis/circulation P/HH]; or the Cowden protocol, with
320 Samento/Banderol/Parsley/Burbur). There was also a significant increase in underlying symptoms of
321 fatigue, headaches, myalgias and cognitive difficulties if there were dietary indiscretions off of a
322 strict hypoglycemic/Candida/mast cell diet. This would invariably lead to significant fatigue,
323 gastrointestinal distress, and migraines. Her Babesia symptoms did however eventually resolve
324 after several courses of atovaquone and a macrolide, or tetracycline, mefloquine and
325 atovaquone/proguanil, and her percentage of normal functioning kept increasing over the years, as
326 she was rotated through multiple cell wall/cystic/double intracellular antibiotic regimens.

327 Due to frequent relapses within weeks off antibiotics, dapsone combination therapy (DDS CT)
328 was instituted for the first time in 2015 after signing informed consent on potential side effects, using
329 minocycline 100 mg PO BID, rifampin 300 mg PO BID, hydroxychloroquine 200 mg BID, dapsone 50
330 mg/day, nystatin 500,000 units BID, Leucovorin 25 mg BID, L-methyl folate 15 mg/day, along with
331 triple probiotics, and Stevia and Biocidin for biofilms. There was a significant improvement in
332 baseline symptoms, with improvement in fatigue, pain, headaches, and cognitive difficulties. Only
333 mild anemia (H/H 11/33.3) without any evidence of methemoglobinemia was noted. The regimen
334 was subsequently stopped after the patient felt better, stabilizing after 6 months on the protocol, and
335 within several months off medications, symptoms relapsed again, with significant fatigue and brain
336 fog. A *Borrelia burgdorferi* PCR was sent out January 2016 to IgeneX laboratories, Palo Alto, California
337 and returned positive, confirming ongoing active infection.

338 Since she reported symptom improvement on lower dose dapsone (50 mg/day), the patient was
339 placed back on DDS CT with minocycline, rifampin, and a higher dose of dapsone, increasing the
340 dose from 75 mg/day to eventually 100 mg/day. The patient noticed an immediate improvement
341 with the higher dapsone dose, with improvement in fatigue, pain, and cognitive functioning. As the
342 H/H dropped to 9.7/28.6 after one month at 100 mg of dapsone while on Leucovorin 25 mg BID and
343 L-methyl folate 15 mg, with a mild increase in liver functions (LFT's: ALT 39, normal range < 32
344 IU/L), folic acid dosing was increased to 25 mg of L-methyl folate, and the patient was given extra
345 liver support (milk thistle, N-acetyl cysteine). The subsequent H/H improved to 10.7/31.4 and LFT's
346 returned to normal. Blood methemoglobin levels rose to 3.7 % on 4/2016 while on 100 mg of dapsone
347 (normal range < 1.5%), but the patient denied any symptoms of increased fatigue, headaches,
348 shortness of breath or cyanotic extremities, which would have been suggestive of symptomatic
349 methemoglobinemia. Glutathione dosing was therefore increased to 500 mg PO BID for the elevated
350 levels of methemoglobin, and several months later, while also on higher doses of folic acid, blood
351 methemoglobin levels dropped to 2.5%, subsequently returning to normal range (0.6%) with her

352 H/H improving to 11.1/32.9. The patient stayed on this DDS CT protocol for 6 months and stopped it
353 at the end of 2016 when she was feeling close to 100 % of normal functioning.

354 May 2017, having remained off antibiotics for 6 months, the patient again began to relapse with
355 typical Lyme symptoms, although not as severely as with prior episodes. She complained of
356 increased fatigue, headaches, neck pain/stiffness, paresthesia's and moderate to severe cognitive
357 difficulties, despite strict compliance with diet and addressing all other abnormalities on the
358 16-point MSIDS map. A Lyme Western Blot showed an increase in the 31kDa band (Osp A). *Babesia*
359 symptoms however had not returned post rotation of prior anti-malarial medications (no day
360 sweats, night sweats, flushing, unexplained cough, or air hunger) and *Babesia* titers returned to
361 normal with negative *Babesia* FISH testing. *Bartonella henselae* titers also came down to normal over
362 time, with negative PCR's and normal levels of vascular endothelial growth factors (VEGF). It was
363 therefore decided to try a double dose dapsone protocol (DDD CT) for two months, after signing an
364 informed consent. She gradually increased the doses of dapsone month one (up to 100 mg/day) and
365 used dapsone 100 mg BID month 2, with the same antibiotic combinations as before (minocycline,
366 rifampin, hydroxychloroquine, leucovorin, L-methyl folate, biofilm agents and probiotics). Doses
367 of glutathione were increased month 2 to 1000 mg BID to help reduce methemoglobin levels, with an
368 increase in folic acid dosing to leucovorin 75 mg/day with 45 mg of L-methyl folate once baseline
369 levels of H/H decreased.

370 The patient immediately felt better going back on DDS CT November 2017, with all symptoms
371 improving at the end of month one. H/H dropped from 13.1/38.9 at the beginning of therapy to
372 10.1/29.2 after one month, with methemoglobin levels remaining within normal limits (WNL) at
373 0.9%. Beginning the second month of higher dose dapsone therapy (DDD CT), she experienced a
374 Herxheimer reaction for 3-4 days, with an increase in fatigue and cognitive difficulties, after which
375 she felt 100 % back to normal functioning, remaining at that level for the duration of treatment.
376 Laboratory values towards the end of month 2 on dapsone 100 mg BID, showed that methemoglobin
377 levels remained WNL (1.2%) but her H/H gradually dropped to 8.8/26.9. As there was no significant
378 dyspnea except walking up steep hills, the regimen was continued until completion of the 8-week
379 course, increasing folic acid doses as noted above during month 2. One month later, off dapsone, the
380 H/H improved to 10.1/30.4 and month 2 post DDD CT, her H/H was back into normal range at
381 12.4/37.8. Folic acid dosing was subsequently discontinued, and she remained on probiotic and
382 biofilm support for the next several months. As of August 2020, 30 months post DDD CT, the patient
383 has remained well without any relapse of Lyme and tick-borne symptoms, for the first time in over
384 25 years.

385 3. Case 3

386 A 50-year-old white female with a past medical history significant for Lyme disease, babesiosis,
387 Graves' disease with hyperthyroidism, hypertension, hyperlipidemia, obesity with insulin
388 resistance and non-alcoholic fatty liver (NASH), chemical sensitivity, supraventricular tachycardia
389 requiring cardiac ablation, left knee arthroscopic surgery for a torn meniscus, status post MVA with
390 neck torsion, ADD and bilateral cataract surgery presented to our office for a Lyme consultation in
391 July 2010. She had a history of at least 10 tick bites and spent much of her life in heavily Lyme
392 disease and *Babesia* endemic areas such as Martha's Vineyard. Approximately 10 years ago, she
393 began to develop multiple symptoms including significant fatigue, night sweats, joint pain, swollen
394 glands, neck pain and stiffness, headaches, palpitations, shortness of breath, anxiety, depression,
395 and insomnia. She went to see a local endocrinologist and was diagnosed with hyperthyroidism. She
396 was placed on methimazole, which controlled her abnormal thyroid functions, but her health never
397 returned to normal. One year later, her local physician diagnosed her with Lyme disease based on
398 her symptoms and a CDC positive IgM and IgG Lyme Western blot. She was placed on doxycycline
399 100 mg PO BID and felt much better. She then went to see a local infectious disease specialist, who
400 placed her on tetracycline HCL and hydroxychloroquine, but did not feel well on this regimen, and
401 returned to her local physician to go back on doxycycline. She did 4 months of antibiotic therapy and
402 got to between 70 and 80% of normal functioning. She then was rotated to azithromycin and

403 rifampin for ongoing resistant symptoms, but did not feel as well with this protocol, and went to see
404 a physician specializing in Lyme disease for a 2nd opinion. Her testing returned positive for *Babesia*
405 and *Borrelia burgdorferi*, and she was rotated to atovaquone and azithromycin. On this protocol her
406 sweats got better, but otherwise there was a steady regression of her baseline underlying symptoms.
407 She was therefore switched to amoxicillin 5 to 6 weeks before our initial consultation, titrating the
408 dose upwards. Although she initially responded well, with a significant decrease in symptoms for
409 the 1st 2 weeks, she then had a steroid injection in her neck for her chronic pain and severely
410 relapsed, followed by a chemical exposure with hair straightening products, which also significantly
411 worsened her symptomatology. At the time of our initial consultation, she felt much worse before
412 starting her Lyme disease and *Babesia* therapy and was functioning at 10% of her normal
413 functioning.

414 Chief complaints during the initial history and physical were fevers, sweats, chills and flushing;
415 weight gain; fatigue; sore throat and swollen glands; unexplained menstrual irregularity; loss of
416 libido; upset stomach with constipation; chest pain/rib soreness with shortness of breath and an
417 unexplained cough; palpitations; migratory joint pain in the knees, neck, hips and ankles with neck
418 and back stiffness; myalgias; headaches with intermittent migraines; tingling, numbness, burning or
419 stabbing sensations of the extremities; blurry vision; tinnitus and increased motion sickness;
420 dizziness with poor balance; tremors; confusion with difficulty thinking; difficulty with
421 concentration or reading; forgetfulness with poor short term memory; disorientation and getting
422 lost, going to the wrong places; difficulty with speech and writing; mood swings with irritability,
423 anxiety and depression; insomnia with early awakening, and exaggerated symptoms from alcohol
424 use. Review of systems also revealed problems with a chronic postnasal drip, occasional wheezing,
425 early satiety, and stress incontinence. Medication included methimazole 5 mg BID, amoxicillin 875
426 mg, 3 PO QD, Imitrex PRN, Nystatin 500,000 units 3 PO QD, an estradiol patch, progesterone 100 mg
427 HS, as well as various vitamin and mineral supplements.

428 Physical examination was unremarkable except for an enlarged thyroid gland, consistent with
429 Graves' disease, and some left elbow tenderness on palpation. Laboratory testing done during the
430 initial consultation included thyroid functions, DHEA/cortisol testing, vitamin and mineral levels,
431 evaluating for heavy metal exposure (Mercury, lead, arsenic, cadmium, aluminum), checking for
432 food allergies and expanding her tickborne panel for exposure to Q- fever, RMSF and tularemia, as
433 well as checking for exposure to *Bartonella*, *chlamydia pneumonia* and *Mycoplasma pneumonia*. Heavy
434 metal testing returned showing elevated levels of lead (25 µg/g creatinine, normal range <2) and
435 Mercury (17 µg/g creatinine, normal range <4), and adrenal testing revealed phase 2 adrenal
436 dysfunction with low cortisol in the morning (0.20 ng/ml, normal range 1-8.0 ng/ml) and at 2 pm
437 (0.46 ng/ml, normal range 1-8.0 ng/ml). She was therefore placed on dietary restrictions, and told to
438 decrease intake of larger fish, along with alpha lipoic acid 600 mg PO BID, and low dose
439 hydrocortisone in the morning and at noon (5 mg PO BID) with adaptogenic herbs (B vitamins,
440 ashwagandha, rhodiola). These measures improved her energy/stamina. Since she had a significant
441 improvement during the 1st 2 weeks on amoxicillin therapy before her steroid injection, she was
442 placed on benzathine penicillin (Bicillin LA) 1.2 million units twice a week, hydroxychloroquine 200
443 mg PO BID, clarithromycin XL 500 mg PO BID and atovaquone/proguanil 1 PO QD, along with
444 probiotic support. Low-dose naltrexone (LDN) 2 mg HS was added for anti-inflammatory effects,
445 and she was also started on bupropion (Wellbutrin XL) 150 mg PO BID for depression. She
446 responded well to the Bicillin injections and oral antibiotics and remained on this therapy for the
447 next several months. She also was eventually started on valsartan/hydrochlorothiazide for
448 hypertension, rosuvastatin for hyperlipidemia (total cholesterol 317, triglycerides 253, HDL 47, LDL
449 219), and metoprolol XL 25 mg one QD for palpitations (a 24-hour Holter monitor revealed frequent
450 PVCs without SVT). A stress echocardiogram was done for her chest pain and cardiac risk factors,
451 which showed no ischemic changes, and a Doppler/ultrasound of the carotids showed no evidence
452 of plaque or stenosis. A low carbohydrate, Paleo style diet was prescribed for significant weight gain
453 (5ft 7 in, 206 lbs.) with hyperinsulinemia (70.7 uIU, normal < 24.9 uIU), hyperuricemia (uric acid 8.2.

454 normal range < 6.0 mg/dL), elevated hs-CRP (3.7 mg/L, normal < 1) and a CT abdomen suggestive of
455 a fatty liver.

456 Over the following several years, due to ongoing resistant symptoms, the patient was rotated
457 through multiple antibiotic regimens including various oral cephalosporins (cefuroxime axetil,
458 cefdinir), metronidazole, doxycycline, minocycline, azithromycin, rifampin, and quinolones
459 (ciprofloxacin, levofloxacin, moxifloxacin). Although there were temporary improvements in
460 symptomatology with these protocols, as well as improvements using rotations of anti-malarial
461 therapy for babesiosis for ongoing sweats (lumefantrine/artemether [Coartem], Clindamycin), she
462 would relapse with an increase in her baseline symptoms each time she was taken off therapy. In
463 May 2017, her primary care physician decided to institute a trial of IV ceftriaxone, 2 grams QD, along
464 with DDS CT, for increased *Borrelia* specific banding on her Western blot (23 kDa, Osp C)
465 accompanied by resistant symptoms of fatigue, joint pain, and severe ongoing
466 memory/concentration problems off treatment.

467 She was placed back on DDS CT with minocycline 100 mg PO BID, pulse rifampin 300 mg, two
468 PO BID one day/week (for GI tolerance) and dapsone, slowly increasing doses to 100 mg/day with
469 Leucovorin 25 mg BID and L-methyl folate 15 mg/day. N-acetyl-cysteine (NAC), alpha lipoic acid
470 (ALA), and liposomal glutathione (GSH) were given twice a day for inflammation and detoxification
471 support with triple biofilm agents (Serrapeptase, monolaurin, Stevia) and triple probiotics. Rifabutin
472 150 mg PO BID eventually was used instead of rifampin, with better GI tolerance. She improved
473 with this protocol, and by June 2017, one month later, she had significantly less fatigue,
474 musculoskeletal pain, and improved cognition, functioning at 90% of normal. Cognitive problems
475 were still present, but slowly improving, and IV glutathione (GSH) was highly effective for both
476 Herxheimer reactions and resistant brain fog, relieving her symptomatology within 15 minutes of an
477 IV infusion. Her PCP therefore left her on the same protocol until she decided to go to Germany for
478 hyperthermia treatment, in early August 2017.

479 She was taken off her DDS CT by the German physicians and left on IV ceftriaxone and
480 metronidazole, with low dose niacin, while being given IV ozone and stem cells during her visit. She
481 tolerated the treatment well and felt an improvement after the second course of hyperthermia. Upon
482 returning to the United States, despite jet lag, her joint pain and flexibility as well as her cognition
483 had improved, so we removed the PICC line, stopped all antibiotics, and administered two months
484 of mitochondrial support with glycosylated phospholipids (NT Factors), CoQ10 and
485 acetyl-L-carnitine, adding nicotinamide adenine dinucleotide (NADH) if fatigue were to persist.
486 During an in-office follow-up two months later, she was still gradually improving, only complaining
487 of mild fatigue, mild cognitive problems, and neck pain. *Babesia* symptoms were gone, and a repeat
488 *Babesia* titer was negative. Repeat adrenal function with a DHEA/cortisol test also showed that she
489 no longer suffered from adrenal fatigue, even off low dose hydrocortisone.

490 In December 2017, after 4-5 months off IV and oral antibiotics and post hyperthermia treatment,
491 she began to have a relapse of underlying symptoms. These included fatigue, flushing, increased
492 neck pain, blurry vision, and ongoing memory/concentration problems. There were also new,
493 unexplained allergy symptoms that were arising, with an intermittent red face and swollen lips. She
494 went for an allergy evaluation, and we checked an IgE food panel and antibodies against the alpha
495 gal allergen, which were all negative. Magnetic Resonance Imaging (MRI) of the neck did not reveal
496 any structural cause for her neck pain, and an eye examination was performed for her blurry vision,
497 which was WNL. Since the patient's symptoms were not yet severe, she decided to try an alternative
498 treatment regimen with her PCP, using alpha and beta thymosin injections, which gave some relief
499 of symptoms during a three-month trial. The flushing also resolved off Niacin, and she was given
500 fenofibrate (Tricor) 145 mg instead for her hypertriglyceridemia, but this was stopped 3 days later
501 secondary to a possible allergic reaction with ongoing facial erythema.

502 The patient had a telemedicine consult April 2018, since she continued to slowly relapse 9
503 months off antibiotics. She had gone from 90% of normal functioning, down to 60% normal
504 functioning, with increasing fatigue, neck pain with neuralgia on the right side of the neck, head
505 pressure and increased brain fog. Since her G6PD levels were WNL (239, normal range between

506 46-376 U) with a normal CBC (H/H 14.5/42.2) and no baseline elevations in methemoglobin (0.3%
507 normal range < 1.9%), we discussed a trial of DDD CT, starting at the beginning of May 2018. After
508 signing an informed consent, she began doxycycline 200 mg PO BID, rifampin 300 mg PO BID,
509 gradually increasing doses of dapsons until at 100 mg BID, hydroxychloroquine 200 mg PO BID,
510 nystatin 500,000 units 2 PO BID, cimetidine 400 mg PO BID, leucovorin 25 mg BID with 15 mg of
511 L-methyl folate during month one, increasing L-methyl folate to 25 mg PO BID month two. This was
512 along with triple biofilm agents and triple probiotics with NAC, ALA and GSH. A CBC, CMP, and
513 methemoglobin levels were drawn every 2 weeks during the 1st month of treatment, and weekly
514 during the 2nd month of DDD CT, at which point, she would stop the therapy and remain on biofilm
515 agents, folic acid and probiotics.

516 By the July 2018, the patient had completed DDD CT, having missed the last week of treatment
517 secondary to gastrointestinal upset. She was functioning at 90% of normal and reported feeling the
518 best she had felt in the past 20 years. Her CBC had slightly decreased on dapsons while remaining
519 on high dose folic acid (H/H 13.6/40.5) and there was no significant methemoglobinemia on
520 glutathione 1000 milligrams twice a day (methemoglobin 0.3%, normal < 1.9%). Fatigue and nerve
521 pain had completely resolved, generalized arthralgias and myalgias were gone, except for some
522 residual neck pain, moods were significantly better with decreased anxiety, and brain fog was mild,
523 but improved. As of November 2018, she remained at a higher level of functioning than before doing
524 DDD CT, although she had slipped from 90% to 85% of normal functioning. Her anxiety was
525 returning along with palpitations, mild fatigue, neck stiffness, bladder dysfunction with urgency
526 and frequency (history of a dropped bladder), with difficulty concentrating and reading. Laboratory
527 values 6 months post DDD CT on 12/2018, were also WNL (H/H 14.4/42.7) with a normal creatinine
528 [0.9, normal < 0.95] and normal liver functions, despite the history of fatty liver (AST 26, normal < 34
529 U/L; ALT 37, normal < 36).

530 As of March 2019, most of her baseline Lyme symptoms had resolved, but she still complained
531 of some fatigue and daily morning headaches, which would promptly resolve with 400 mg of
532 ibuprofen. She was placed on a stricter hypoglycemic diet for her hyperinsulinemia and occasional
533 low blood sugars on her CMP (glucose 62). By August 2019, the patient felt she was in complete
534 remission, at 100% of her normal functioning, with no further Lyme symptoms. Only residual neck
535 stiffness remained, which was felt to be due to her status post MVA with multiple neck traumas
536 falling off horses. Her fatigue and headaches had resolved with a stricter hypoglycemic diet, and her
537 urinary and bowel problems (constipation) also improved post total abdominal hysterectomy, for
538 fibroids that were compressing her bladder and colon. It was the best she had ever felt since falling
539 ill 20 years ago. As of August 2020, she has remained off antibiotics for over 2 years, with no further
540 relapse of symptoms of chronic Lyme disease/PTLDS.

541 4. Discussion

542 There has been a longstanding medical debate regarding the etiology of chronic Lyme
543 disease/PTLDS with two published standards of care for the diagnosis and treatment [44] [45].
544 Current IDSA treatment guidelines (2006) have relied on 4 randomized, controlled trials (RCT's) for
545 persistent disease and advised against retreatment [44], although earlier National Institutes of
546 Health (NIH) placebo controlled, double blind, RCT's of PTLDS showed some benefit on primary or
547 secondary outcome measures in two out of the four trials using IV ceftriaxone [46]. A further
548 biostatistical review of antibiotic retreatment in all 4 Lyme disease RCT's was therefore performed to
549 understand the discrepancies [47]. DeLong et al. concluded that the design assumptions in the two
550 Klempner trials were unrealistic and underpowered to detect meaningful treatment effects; that the
551 Krupp trial was well designed, finding statistically significant and meaningful improvement in
552 fatigue; and that the Fallon trial corroborated this finding, while also demonstrating improvement in
553 cognitive functioning at week 12, which declined by week 24 [47]. Although improvement in fatigue
554 and cognitive functioning was noted in two of the four trials, sustained improvement was lacking,
555 requiring new treatment strategies.

556 Other authors have similarly noted relapses in Lyme symptoms post discontinuation of
557 antibiotics and long term impairment of functional status [48]. Although case reports and
558 uncontrolled trials have reported the efficacy of prolonged antibiotic therapy [49] [50] [51],
559 symptoms have often been shown to relapse after discontinuation of therapy [52]. Relapse in
560 underlying Lyme symptoms with persistent fatigue, musculoskeletal pain, neuropathy, and
561 neurocognitive/neuropsychiatric difficulties can potentially be explained by multiple etiologies.
562 These include persistence of spirochetal antigens/peptidoglycans in the joints post treatment leading
563 to Lyme arthritis [12]; persistent infection with *Borrelia burgdorferi* [14] [53] [54] [55], due to
564 invasion of the spirochete into the joints [56] [57], ligaments [58], eyes [59] [60], central nervous
565 system [61] [62], protected niches including the intracellular compartment [17] [18] and fibroblasts
566 [63] [64]; persistent tickborne co-infections [14], including *Babesia* [65] [66] [67] [68] [69] and
567 associated co-infections i.e., *Bartonella* [70] [71], *Mycoplasma* spp. [72] [73], and viral infections such
568 as Human Herpes Virus 6 (HHV-6) [74] [14], as well as other medical problems causing overlapping
569 sources of inflammation with downstream effects [13]. These include up to 16 factors identified on
570 the MSIDS model [75] [76], including immune dysfunction/immune deficiency [15] [77] [13],
571 environmental toxins with detoxification problems [78], GI problems, food allergies [79] [80],
572 nutritional deficiencies [81] [82], hormone and autonomic nervous system dysregulation [83] [84]
573 [85], mitochondrial dysfunction [86] [87], neuropsychiatric problems, and/or sleep disorders [13] [88]
574 [89] [90]. All 3 patients reported here had evidence of multiple overlapping etiologies accounting in
575 part for resistant symptoms, including evidence of associated co-infections (*Babesia*, *Bartonella*,
576 *Mycoplasma fermentans*), hormonal dysfunction, food allergies and leaky gut, environmental toxin
577 exposure with mineral deficiencies, POTS/dysautonomia, neuropsychiatric problems and sleep
578 disorders. Patient 2 also had evidence of persistent *Borrelia* and *Mycoplasma* species by serum
579 polymerase chain reaction (PCR) despite years of targeted antibiotic therapy. Although addressing
580 these multiple etiologies led to clinical improvements, it was not however until DDD CT therapy
581 was instituted, using a higher dose 'persister' drug regimen with dapsone 100 mg PO BID along
582 with a tetracycline, rifampin and biofilm agents, that all 3 patients were finally able to stay in
583 remission for prolonged periods of time (24-30 months).

584 In the past several years, university researchers have determined that may change
585 morphological forms in different environments [20] [21], forming cystic forms (L-forms, S-forms,
586 round bodies, cell wall deficient forms) [91] [92], and metamorphosing into drug tolerant 'persister'
587 and biofilm forms [24] [25] [27] [28]. Persisters are known to occur in a broad range of bacterial
588 infections, leading to dormancy and persistent infection [30] [93] [22] [94]. These persister cells
589 escape the effects of antibiotics without genetic modification, do not grow in the presence of
590 antibiotics, and become a significant fraction of cells in the stationary phase and in biofilms [95].
591 Persister forms and biofilm formation has been published to not only potentially play an important
592 role in antibiotic resistance and reoccurrence of Lyme disease (*Borrelia burgdorferi*) and
593 neuroborreliosis with other *Borrelia* species including *Borrelia afzelii* and *Borrelia garinii* [29] [28]
594 [96], but the importance of biofilms has been identified in other chronic, resistant infections. These
595 include biofilm formation with *Pseudomonas aeruginosa* in patients with cystic fibrosis,
596 *Haemophilus influenzae* and *Streptococcus pneumoniae* in chronic otitis media, and enteropathogenic
597 *E. coli* and *Klebsiella* in recurrent urinary tract infections [97] [98]. Biofilm formation has also been
598 shown to play a role in *Candida* infections in chronic periodontitis [99], in parasitic infections with
599 *Pneumocystis* spp. in lung tissue [100], as well as posing a risk to medical implants, such as central
600 venous catheters, heart valves, ventricular assist devices, coronary stents, neurosurgical ventricular
601 shunts, and breast implants [101].

602 Innovative approaches for treating persister and biofilm forms of *Borrelia* have emerged during
603 the past decade. New compounds with high activity against stationary form *Borrelia burgdorferi*
604 persisters have been identified from the NCI compound collection [35], screening an FDA approved
605 drug library [29], as well as identifying new drug candidates using high throughput screening [37].
606 Potential candidates with efficacy against Bb persisters in culture included daptomycin,
607 cefoperazone and doxycycline [102], sulfa drugs [36] including dapsone [40] and disulfiram [37] [38],

608 bee venom/mellitin [103], essential oils (oregano oil, cinnamon bark, clove) [33], as well as Stevia
609 extract [34]. For in vitro biofilm forms of Bb [27], effective killing of Bb has been noted with novel
610 herbal compounds such as Biocidin [104], Stevia [34], baicalein and monolaurin [105] [106] as well as
611 oregano oil [33]. Only dapsone and disulfiram however have been published in retrospective clinical
612 trials to date [39] [40] [14]. In one study, three patients who had required open-ended antimicrobial
613 therapy for symptoms of chronic, relapsing Lyme disease and babesiosis had a positive response to a
614 finite course of disulfiram, and remained clinically well without a relapse for a period of 6-23
615 months [39]. One patient relapsed on disulfiram and required retreatment. Dapsone combination
616 therapy (DDS CT) has been published to be effective in TBD's in both a case study of a patient with
617 Lyme disease, co-infections and autoimmune disease [107] and in two retrospective clinical trials,
618 involving a total of 300 patients [40] [14]. Eight major Lyme symptoms showed statistical
619 improvement ($p < 0.05$) with DDS CT, including sweats, fatigue, musculoskeletal pain, neuropathy,
620 headaches, cognitive difficulties, and sleep disorders. However, the dose and length of time on DDS
621 CT varied in the two trials (from 25 mg to 100 mg/day), and relapses off DDS CT were not
622 uncommon. The primary difference in this study of 3 patients with chronic Lyme disease/PTLDS
623 was the 100 mg BID dose of dapsone the second month, as compared to doses ranging up to 100 mg
624 QD in our previous studies. The higher dose of dapsone was effective in keeping our patients in
625 symptomatic remission for the first time in decades, with only 7-8 weeks of DDS CT leading to
626 remission times between 25-30 months. This was significantly different than our experience of using
627 lower doses of dapsone, which was effective, but led to relapses upon discontinuation of therapy.

628 Prior studies done by Dr Eva Sapi and researchers at the University of New Haven, showed that
629 DDS CT (tetracycline, rifampin, dapsone) was highly effective against the biofilm forms of Bb and
630 that higher concentrations of dapsone were more effective in lowering biofilm mass [43]. The major
631 findings were that dapsone, as a single drug and in combination with doxycycline and doxycycline +
632 rifampin had the most significant effect in reducing the mass and viability of *B. burgdorferi* biofilm.
633 In a repeat in vitro study of DDS CT by Horowitz and researchers at the University of New Haven
634 [108] (pending publication), in order to further evaluate the effectiveness of the antibiotics on the
635 attached biofilm form of *B. burgdorferi*, crystal violet biofilm, Baclight LIVE/DEAD viability, and
636 Dimethylmethylene blue (DMMB) glycosaminoglycan assays (GAG) were used to evaluate the
637 amount of the protective layers of biofilm polysaccharide matrix before and after antibiotic
638 treatments. The results showed that at 50 μ M doses of dapsone (vs 10 μ M), the higher dapsone dose
639 had the most significant effects on residual GAG amounts of *Borrelia* biofilm compared to the
640 untreated control (p value < 0.01). These culture results support the clinical findings of the
641 superiority of higher dose dapsone therapy for chronic tick-borne symptoms.

642 *B. burgdorferi* biofilm, the most antibiotic resistant form of the bacteria, has been shown to be
643 present in borrelial lymphocytomas [28], and was recently found to be a dominant form in a human
644 autopsy study from a Lyme disease patient [109]. The clinical significance of biofilm like
645 microcolonies was further highlighted in a recent animal study by Johns Hopkins researchers where
646 the biofilm like microcolony and stationary phase planktonic forms (free cells) caused more severe
647 Lyme arthritis and inflammation than actively growing log phase spirochetes [31]. Dapsone and
648 disulfiram have both been published to cause significant Herxheimer reactions in patients with
649 chronic Lyme disease/PTLDS [40] [14] [39], consistent with biofilm/persister forms causing
650 significant inflammation. The production of inflammatory chemokines and cytokines by *Borrelia*
651 *burgdorferi*, is known to be a primary factor driving clinical symptomatology [110] [111].

652 Based on the above in vitro and our in vivo studies, there is a need for safe and effective drugs
653 that can eliminate all morphological forms of *B. burgdorferi* including persisters and attached
654 biofilm forms. Both disulfiram and dapsone are known to have potential adverse effects. Disulfiram
655 commonly causes fatigue, sleepiness, headaches, and a metallic taste, although more severe
656 reactions including dermatological, hepatic (hepatitis, hepatotoxicity), cardiac, neurological
657 (peripheral and/or axonal polyneuropathy, sensory-motor polyneuropathy, optic neuritis, seizures),
658 and psychiatric (confusion, psychosis) may result [112]. In patients with Lyme disease and
659 associated co-infections, underlying cardiac, hepatic, neurological and psychiatric conditions can

660 also co-exist [113] [114] [115] [116] [88] [117], confounding etiologies, requiring a differential
661 diagnosis with monitoring of symptoms, and/or use of lower doses of disulfiram to minimize side
662 effects [39]. Dapsone, also has 4 common side effects, described as 'Do No H.A.R.M.', i.e.,
663 Herxheimer reactions (due to increased inflammatory cytokine production), Anemia (secondary to
664 inhibition of folic acid metabolism, or hemolysis due to G-6-P-D deficiency), Rashes (due to sulfa
665 sensitivity) and Methemoglobinemia (due to increased oxidative stress and diminished oxygen
666 carrying capacity) [14] [118] [119] [120]. Although some of these symptoms were seen in our patients
667 undergoing treatment with DDD CT (Herxheimer reactions, anemia, mild elevations in
668 methemoglobin), adverse side effects were minimized by ruling out G-6-P-D deficiency, using high
669 dose folic acid therapy with folinic acid (50-75 mg/day) and L-methyl folate (30-45 mg/day), as well
670 as administering glutathione precursors (NAC 600 mg BID), alpha lipoic acid (ALA, 600 mg BID)
671 and glutathione (GSH, 1000 mg BID) with methylene blue 50 mg BID as needed. Any decrease in red
672 cell counts or significant anemia secondary to dapsone resolved in all patients within 1-2 months of
673 stopping DDD CT while remaining on folic acid supplementation, and none of our patients
674 developed rashes, nor significantly elevated levels of methemoglobin. Use of NAC, ALA and GSH
675 helped to decrease oxidative stress, support detoxification and minimize the risk of
676 methemoglobinemia [121] [122] [123], while doses of glutathione were increased to 2000 mg QD or
677 BID along with alkalization (using sodium bicarbonate or fresh squeezed citrus) for Herxheimer
678 reactions and/or any increased levels of methemoglobin [124] [76] [125]. Methylene blue can also be
679 given orally to mitigate and rapidly reduce methemoglobinemia [120]. It was only necessary in one
680 of our 3 patients reported here, although in other chronically ill Lyme-MSIDS patients given
681 dapsone at 100 mg or higher [14], oral methylene blue was occasionally needed, and effective in
682 keeping methemoglobin levels below 5%, allowing continuation of therapy. Finally, high dose
683 probiotics (greater than 80 billion CFU's/day) with multiple strains of *Acidophilus*, *Bifidobacterium*,
684 and *Saccharomyces boulardii* were effective in preventing antibiotic associated diarrhea [126].

685 A 7-8-week regimen of DDD CT using the above persister and biofilm protocol with higher
686 doses of dapsone was therefore found to be safe and effective in our three patients. It was superior to
687 lower dose dapsone combination therapy (DDS CT), leading to long term remission. Several
688 important questions however remain regarding the safety and efficacy of persister drug regimens.
689 Bacterial cells in biofilms have been shown to have increased antibiotic resistance compared to
690 planktonic forms leading to recalcitrant infections [127] [128] and persister cells have been shown to
691 retain their phenotype for days or weeks after withdrawal from colony-biofilm culture [129].
692 Although 7-8 weeks of treatment with DDD CT was adequate in our case studies, would longer
693 treatments be more effective in addressing persister cells in other resistant patients? Another
694 important question that needs to be addressed is how different *Borrelia* species and/or associated
695 co-infections affect treatment outcomes. Subsequent to our experience here, a review of three select
696 patients who received temporary benefit from DDD CT, but who relapsed upon discontinuation of
697 therapy, were found to have evidence of active *Babesia* and/or *Bartonella* spp. by Fluorescent In Situ
698 Hybridization (FISH) testing [130] [131]. Previously dapsone and disulfiram were both found to be
699 effective in decreasing symptoms of babesiosis, although relapses were noted with both medications
700 [14] [39]. Resistance of *Babesia* and *Bartonella* spp. to standard treatments have both been reported
701 [69] [70] and resistant biofilm and persister forms have also recently been reported for *Bartonella*
702 [132] [133]. Would addressing *Babesia* and/or *Bartonella* with newer medication regimens including
703 tafenoquine for babesia [134] and/or novel combination therapies for *Bartonella* (i.e., macrodantin,
704 rifampin, methylene blue, gentamycin with essential oils) [133] prior to DDD CT improve clinical
705 outcomes in co-infected patients? Similarly, the three biofilm agents (*Stevia*, oregano oil, Biocidin)
706 we used in our study were all published to have efficacy against biofilms and morphological forms
707 of *Borrelia* [34] [33] [104], but would other biofilm agents or combinations against *Borrelia* and/or
708 associated co-infections be more efficacious [33] [135]? Randomized, controlled trials would be
709 necessary to answer these important questions.

710 5. Conclusion

711 None of the 4 prior RCT's for the treatment of persistent symptoms of Lyme disease [136] [137]
712 [138], nor the PLEASE trial which used doxycycline, clarithromycin, hydroxychloroquine and IV
713 ceftriaxone [139], evaluated the use of newer persister medications or biofilm agents. Similarly, the
714 multiple overlapping etiologies on the 16-point MSIDS model causing inflammation with
715 downstream effects found in our patients (including co-infections, environmental toxin exposure,
716 mineral deficiencies, food allergies, Mast Cell Activation, hormonal dysfunction,
717 POTS/dysautonomia, and sleep disorders) [13], were not evaluated and addressed in prior RCT's.
718 These factors may have interfered with the long-term success of antibiotic therapy in both the
719 Klempner, Krupp and Fallon RCT's, where resistant symptoms and relapses were seen after
720 discontinuation of therapy. Based on recent published research implicating the importance of
721 biofilm and persister forms of Bb in chronic infection, the clinical superiority of a 7-8 week protocol
722 of DDD CT leading to long term remission, and the significant number of new patients contracting
723 Lyme disease and PTLDS each year in the United States and worldwide [3], it is vital that an
724 evaluation of these new persister protocols be performed in placebo controlled, blinded, randomized
725 clinical trials.

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727 Conceptualization; Research Methodology; Formal Analysis; Investigation; Data curation; Writing—Original
728 Draft Preparation; Writing—Review & Editing; Project Administration.; R.I.H. provided care for the 3 patients
729 in this study.

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