Case Report

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

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

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

1. Introduction

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

Approximately 10-20% of individuals treated for Lyme disease with a 2-4-week course of antibiotics will go on to experience chronic, persistent fatigue, musculoskeletal pain and neurocognitive difficulties that persist for more than 6 months, known as Post-Treatment Lyme Disease Syndrome (PTLDS) [10]. The etiology of Chronic Lyme disease/PTLDS is unknown,
although several major hypotheses have been proposed to explain persistent symptoms, including persistence of *Borrelia* and/or borrelial antigens, persistent tick-borne co-infections, immune dysregulation, altered neural networks with central sensitization, and/or overlapping sources of inflammation [11][12][13][14]. *Borrelia’s* ability to persist in the body has been hypothesized to take place through multiple mechanisms. These include immune evasion with borrelia changing its surface antigenic expression in response to host immune responses [15] [16], persistence in the intracellular compartment [17] [18], and changing morphological forms in various environments [19] [20] [21] [22], resulting in atypical cystic forms [23], pleomorphic round bodies (cell wall deficient, L-forms) [20], as well as ‘persister’ and ‘biofilm forms’ [24] [25] [26] [27] [28]. The stationary, persister, and biofilm forms of *Borrelia burgdorferi* (Bb) have been found to be resistant to standard antibiotic treatments and a cause of persistent inflammation [29][30][31]. This phenotypic plasticity of *Borrelia* and its survival in biofilms may help to explain in part clinical conundrums and persistent symptomatology [32].

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

Success in the prior DDS CT trials was operationally defined as improvement in percent of normal after 6 months on DDS CT, and failure was operationally defined as remaining the same or worsening of the percentage of normal after at least 6 months of DDS CT. “Of 181 participants who gave both pre-DDS and DDS percentage scores, 14 participants reported feeling worse currently than they did before the DDS, 22 participants reported no difference, while all other participants (145) currently reported a higher percentage of normal”[14]. Causes of potential failures of DDS CT highlighted in Part 1 of our Precision Medicine study included evidence of chronic persistent infection with *Borrelia, Bartonella,* and *Mycoplasma* species, as well as *B. microti*. These were all shown to persist despite commonly prescribed courses of antibiotics or antimalarial/Babesia therapy [14].

Persistence of bacteria can be explained in part by bacterial biofilms, in which cells are protected from the immune system by surface exopolymers with polysaccharides [41]. Antibiotics have been shown in this model to kill regular cells, leaving dormant persisters alive, and when the concentration of antibiotic drops, they resuscitate and repopulate the biofilm [42].

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Since the dosage of dapsone in the initial two studies varied between 25 and 100 mg/day, and the effect of ‘persister’ drugs like dapsone may depend on drug dependent concentrations and its effect on biofilms [43], we decided to try a higher dose of dapsone (100 mg BID) for one month in several patients with a history of chronic, persistent relapses. We present here three case studies of individuals who took 7-8 weeks of dapsone combination therapy using hydroxychloroquine, cimetidine, nystatin, a tetracycline, rifampin, and dapsone (DDS CT), where the dose of dapsone was increased the second month to 100 mg BID, i.e., double dose dapsone combination therapy (DDC CT). Nutritional support included N-acetyl cysteine 600 mg PO BID, alpha lipoic acid 600 mg PO BID, gradually increasing doses of glutathione up to 1000-2000 mg BID by the end of the first month, folic acid (Leucovorin 25 mg PO BID month one, 25 mg TID month 2), L-methyl folate 15 mg PO BID, along with three biofilm agents (Stevia, oregano oil, Biocidin) and three probiotics (Theralac, Ultra Flora DF, *Saccharomyces boulardii*). Patients signed informed consent forms that listed the major side effects of dapsone combination therapy, which included Herxheimer reactions, anemia secondary to folic acid inhibition (hemolytic anemia was minimized by ensuring all patients had
normal levels of G-6-P-D, or they were ineligible for the trial), rashes (secondary to sulfa sensitivity) and/or methemoglobinemia (secondary to increased oxidative stress and decreased heme oxygen carrying capacity). Patients were instructed to get regular laboratory testing with a complete blood count (CBC), comprehensive metabolic profile (CMP) and methemoglobin levels once at 100 mg of dapsone, and to repeat laboratory testing weekly during the second month of higher dose therapy. Any major changes in their symptoms were to be reported immediately to the first author via an emergency cell phone number. After stopping all antibiotic therapy once the trial was completed, all three individuals remained on biofilm agents, folic acid replacement and probiotics for the next several months. All three patients remained in remission for time periods ranging between 2 and 3 years with no further Lyme and tickborne symptoms after completing DDD CT.

2. Case 1

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

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

We performed a complete blood count (CBC), comprehensive metabolic profile (CMP), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), hormone levels (thyroid functions, DHEA/cortisol, pregnenolone), antigliadin antibody/tissue transglutaminase (TTG), IgE food allergy panel, immunoglobulin levels and subclasses, mineral levels (magnesium, copper, zinc), streptococcal titers (anti-streptomyycin O [ASO], anti-DNase), viral titers (Herpesvirus 6 [HHV-6], Epstein-Barr virus [EBV], cytomegalovirus [CMV]), Stachybotrys titer, urinalysis, EKG, as well as a comprehensive tickborne panel. This included a C6 Elisa peptide, IgM/IgG Lyme Western Blot,
Babesia microti immunofluorescent assay (IFA), Babesia duncani antibody, Babesia Fluorescent In Situ Hybridization (FISH) test, Bartonella titer and PCR, Vascular Endothelial Growth Factor (VEGF), Rocky mountain spotted fever (RMSF, rickettsia rickettsii) and tularemia titer (Francisella tularensis), Q fever titer (Coxiella burnetii), and Mycoplasma, chlamydia pneumonia and Brucella titer. Significant results included evidence of multiple food allergies, consistent with leaky gut; low plasma copper (0.64 ug/mL, normal range between 0.80-1.75 ug/ml); prior exposure to HHV6 (antibody titers 1:1280, normal less than 1:80), and an elevated VEGF at 134 picograms/milliliter (normal range 0-115 pg/ml) consistent with possible Bartonellosis, with evidence of prior exposure to Lyme disease (positive 31 kDa, 39 kDa and 41 kDa bands on an IgG Western blot). Urinalysis revealed mucus threads and calcium oxalate crystals, without any history of kidney stones.

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

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

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

As of May 2018, all symptoms were slowly improving with multiple intracellular antibiotics, but he still complained of resistant fatigue, insomnia, neck pain, headaches, mild night sweats and hair loss secondary to trichotillomania and ongoing OCD. The patient and his family did not want him to go on a selective serotonin reuptake inhibitor (SSRI) like paroxetine, so we discussed other options to treat his resistant tickborne symptoms. Since the patient had been on almost continuous antibiotics since age 4 (14 years) and had never taken a ‘persister’ drug regimen like dapsone, we discussed a trial of double dose dapsone combination therapy (DDD CT) for 2 months based on prior published research for DDS CT. The patient and parents signed a consent form, which explained the protocol in detail. His glucose 6 phosphate dehydrogenase level [G6PD] was within normal limits, minimizing the risk of hemolytic anemia; sulfa induced rashes would be unlikely, as he had been on Bactrim DS [sulframethoxazole/trimethoprim] without side effects. The DDD CT protocol consisted of the following: 2 months of doxycycline 150-200 mg PO BID, rifampin 300 mg PO BID, hydroxychloroquine 200 mg PO BID, nystatin tablets 500,000 units, 2 PO BID, cimetidine 400 mg PO BID, and gradually increasing doses of dapsone: 25 mg week one; 50 mg week 2; 100 mg weeks 3 and 4; and 100 mg of dapsone PO BID for 4 weeks, taken with Leucovorin 25 mg PO BID month one, 25 mg TID month 2, along with L-methyl folate 15-20 mg PO BID to help prevent
significant anemia. Methylene blue, 50 mg PO BID, would be used during month 2 if there was any evidence of methemoglobinemia (a signed consent form had been given, explaining potential side effects and contraindications) and folic acid dosing would be increased for any significant increase in anemia. Nutritional support included 3 biofilm agents at the following doses: Stevia [NutraMedix] 15 drops PO BID; oregano oil capsules 60 mg PO BID and Biocinid, 2 sprays in a glass of water. N-acetylcysteine (NAC) 600 mg PO BID, alpha lipoic acid 600 mg PO BID, and liposomal glutathione 1000 mg PO BID were used to reduce the risk of methemoglobinemia and severe Herxheimer reactions, with triple probiotics PO BID taken at least one hour away from antibiotics. A typed flare protocol for severe, persistent Herxheimer reactions was also given, using PRN sodium bicarbonate, up to 2 grams twice a day, or drinking fresh squeezed lemons/limes twice a day through a straw (to alkalize the body), along with 2000 mg of glutathione 2-3 × per day as needed. Laboratory values with a CBC, CMP and methemoglobin levels were checked twice during month one, and weekly during month 2 to rule out significant anemia and/or methemoglobinemia or detect any changes in liver or kidney function. Once the 2-month protocol was finished, the patient was to stop all antibiotics, and only continue on folic acid supplementation, biofilm agents and probiotics.

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

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

By December 2019, the patient was 1 ½ years off of antibiotics and in remission post DDD CT, and only complained of fatigue his 1st year of college if he was up late studying without adequate sleep. According to his mother, he was frequently sick during his childhood, and now he was never ill. Repeat adrenal testing showed phase 3 adrenal dysfunction with low morning and evening cortisol levels, which were contributing to his resistant fatigue. He was therefore placed on adaptogenic herbs and an adrenal glandular supplement which reversed any problems with energy/stamina. He was able to play recreational sports and walk a minimum of 2 miles per day.
He denied any Lyme, Babesia or Bartonella symptoms, his OCD and trichotillomania completely resolved, and POTS/dysautonomia improved with mild tachycardia standing with no further drops in blood pressure. During his last follow-up on August 2020, he was over 2 years without a relapse of tickborne symptoms (25 months) and was able to stay off antibiotics for the 1st time in 14 years.

3. Case 2

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

Her chief complaints consisted of severe fatigue, day sweats, night sweats and chills, neck stiffness and headaches with intermittent migraines, migratory joint and muscle pains in the neck, fingers and toes, hair loss, rare tingling of the extremities, intermittent gas, nausea, vomiting and constipation, blurry vision, balance problems with intermittent dizziness, moderate memory and concentration problems with poor organizational skills, poor sleep secondary to sleep apnea, and periods of sadness with depression and anxiety. Medication regimens used during a time frame of over two decades to address her Bb, Babesia, Bartonella and Mycoplasma exposure according to chart
review included rotations of doxycycline, atovaquone/proguanil and mefloquine; atovaquone and azithromycin; amoxicillin/clavulanic acid; cefuroxime axetil and azithromycin; amoxicillin and azithromycin; cefuroxime axetil, hydroxychloroquine and azithromycin; cefdinir, probenecid, telithromycin and hydroxychloroquine; rotations of double intracellular antibiotic regimens with hydroxychloroquine (minocycline +azithromycin, minocycline +ciprofloxacin, minocycline +rifampin, doxycycline + moxifloxacin), each for several months at a time, along with a course of IV ceftriaxone 2 grams/day for one month, with intermittent use of IV glutathione, which was effective for underlying symptoms and Herxheimer reactions. As of November 2017, post rotations of antibiotics for her tickborne diseases (TBD’s), she remained on Armour thyroid 60 mg/day, methyl folate 15 mg/day, along with multiple cell wall/cystic/double intracellular antibiotic regimens.

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

Since she reported symptom improvement on lower dose dapsone (50 mg/day), the patient was placed back on DDS CT with minocycline, rifampin, and a higher dose of dapsone, increasing the dose from 75 mg/day to eventually 100 mg/day. The patient noticed an immediate improvement with the higher dapsone dose, with improvement in fatigue, pain, and cognitive functioning. As the H/H dropped to 9.7/28.6 after one month at 100 mg of dapsone while on Leucovorin 25 mg BID and L-methyl folate 15 mg, with a mild increase in liver functions (LFT’s: ALT 39, normal range < 32 IU/L), folic acid dosing was increased to 25 mg of L-methyl folate, and the patient was given extra liver support (milk thistle, N-acetyl cysteine). The subsequent H/H improved to 10.7/31.4 and LFT’s returned to normal. Blood methemoglobin levels rose to 3.7 % on 4/2016 while on 100 mg of dapsone (normal range < 1.5%), but the patient denied any symptoms of increased fatigue, headaches, shortness of breath or cyanotic extremities, which would have been suggestive of symptomatic methemoglobinemia. Glutathione dosing was therefore increased to 500 mg PO BID for the elevated levels of methemoglobin, and several months later, while also on higher doses of folic acid, blood methemoglobin levels dropped to 2.5%, subsequently returning to normal range (0.6%) with her hypoglycemia/Candida/mast cell diet. This would invariably lead to significant fatigue, gastrointestinal distress, and migraines. Her Babesia symptoms did however eventually resolve after several courses of atovaquone and a macrolide, or tetracycline, mefloquine and atovaquone/proguanil, and her percentage of normal functioning kept increasing over the years, as she was rotated through multiple cell wall/cystic double intracellular antibiotic regimens.

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

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

Since she reported symptom improvement on lower dose dapsone (50 mg/day), the patient was placed back on DDS CT with minocycline, rifampin, and a higher dose of dapsone, increasing the dose from 75 mg/day to eventually 100 mg/day. The patient noticed an immediate improvement with the higher dapsone dose, with improvement in fatigue, pain, and cognitive functioning. As the H/H dropped to 9.7/28.6 after one month at 100 mg of dapsone while on Leucovorin 25 mg BID and L-methyl folate 15 mg, with a mild increase in liver functions (LFT’s: ALT 39, normal range < 32 IU/L), folic acid dosing was increased to 25 mg of L-methyl folate, and the patient was given extra liver support (milk thistle, N-acetyl cysteine). The subsequent H/H improved to 10.7/31.4 and LFT’s returned to normal. Blood methemoglobin levels rose to 3.7 % on 4/2016 while on 100 mg of dapsone (normal range < 1.5%), but the patient denied any symptoms of increased fatigue, headaches, shortness of breath or cyanotic extremities, which would have been suggestive of symptomatic methemoglobinemia. Glutathione dosing was therefore increased to 500 mg PO BID for the elevated levels of methemoglobin, and several months later, while also on higher doses of folic acid, blood methemoglobin levels dropped to 2.5%, subsequently returning to normal range (0.6%) with her hypoglycemia/Candida/mast cell diet. This would invariably lead to significant fatigue, gastrointestinal distress, and migraines. Her Babesia symptoms did however eventually resolve after several courses of atovaquone and a macrolide, or tetracycline, mefloquine and atovaquone/proguanil, and her percentage of normal functioning kept increasing over the years, as she was rotated through multiple cell wall/cystic double intracellular antibiotic regimens.
H/H improving to 11.1/32.9. The patient stayed on this DDS CT protocol for 6 months and stopped it at the end of 2016 when she was feeling close to 100% of normal functioning.

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

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

3. Case 3

A 50-year-old white female with a past medical history significant for Lyme disease, babesiosis, Graves’ disease with hyperthyroidism, hypertension, hyperlipidemia, obesity with insulin resistance and non-alcoholic fatty liver (NASH), chemical sensitivity, supraventricular tachycardia requiring cardiac ablation, left knee arthroscopic surgery for a torn meniscus, status post MVA with neck torsion, ADD and bilateral cataract surgery presented to our office for a Lyme consultation in July 2010. She had a history of at least 10 tick bites and spent much of her life in heavily Lyme endemic areas such as Martha’s Vineyard. Approximately 10 years ago, she began to develop multiple symptoms including significant fatigue, night sweats, joint pain, swollen glands, neck pain and stiffness, headaches, palpitations, shortness of breath, anxiety, depression, and insomnia. She went to see a local endocrinologist and was diagnosed with hyperthyroidism. She was placed on methimazole, which controlled her abnormal thyroid functions, but her health never returned to normal. One year later, her local physician diagnosed her with Lyme disease based on her symptoms and a CDC positive IgM and IgG Lyme Western blot. She was placed on doxycycline 100 mg PO PID and felt much better. She then went to see a local infectious disease specialist, who placed her on tetracycline HCL and hydroxychloroquine, but did not feel well on this regimen, and returned to her local physician to go back on doxycycline. She did 4 months of antibiotic therapy and got to between 70 and 80% of normal functioning. She then was rotated to azithromycin and...
rifampin for ongoing resistant symptoms, but did not feel as well with this protocol, and went to see
a physician specializing in Lyme disease for a 2nd opinion. Her testing returned positive for Babesia
and Borrelia burgdorferi, and she was rotated to atovaquone and azithromycin. On this protocol her
symptoms improved during the 1st 2 weeks, she then had a steroid injection in her neck for her chronic pain and severely
worsened her symptomatology. At the time of our initial consultation, she felt much worse before
starting her Lyme disease and Babesia therapy and was functioning at 10% of her normal
functioning.

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

Physical examination was unremarkable except for an enlarged thyroid gland, consistent with
Graves’ disease, and some left elbow tenderness on palpation. Laboratory testing done during the
initial consultation included thyroid functions, DHEA/cortisol testing, vitamin and mineral levels,
evaluating for heavy metal exposure (Mercury, lead, arsenic, cadmium, aluminum), checking for
food allergies and expanding her tickborne panel for exposure to Q-fever, RMSF and tularemia, as
well as checking for exposure to Bartonella, chlamydia pneumonia and Mycoplasma pneumonia. Heavy
metal testing returned showing elevated levels of lead (25 µg/g creatinine, normal range <2) and
Mercury (17 µg/g creatinine, normal range <4), and adrenal testing revealed phase 2 adrenal
dysfunction with low cortisol in the morning (0.20 ng/ml, normal range 1-8.0 ng/ml) and at 2 pm
(0.46 ng/ml, normal range 1-8.0 ng/ml). She was therefore placed on dietary restrictions, and told to
decrease intake of larger fish, along with alpha lipoic acid 600 mg PO BID, and low dose
hydrocortisone in the morning and at noon (5 mg PO BID) with adaptogenic herbs (B vitamins,
ashwagandha, rhodiola). These measures improved her energy/stamina. Since she had a significant
improvement during the 1st 2 weeks on amoxicillin therapy before her steroid injection, she was
placed on benzathine penicillin (Bicillin LA) 1.2 million units twice a week, hydroxychloroquine 200
mg PO BID, clarithromycin XL 500 mg PO BID and atovaquone/proguanil 1 PO QD, along with
probiotic support. Low-dose naltrexone (LDN) 2 mg HS was added for anti-inflammatory effects,
and she was also started on bupropion (Wellbutrin XL) 150 mg PO BID for depression. She
responded well to the Bicillin injections and oral antibiotics and remained on this therapy for the
next several months. She also was eventually started on valsartan/hydrochlorothiazide for
hypertension, rosuvastatin for hyperlipidemia (total cholesterol 317, triglycerides 253, HDL 47, LDL
219), and metoprolol XL 25 mg one QD for palpitations (a 24-hour Holter monitor revealed frequent
PVCs without SVT). A stress echocardiogram was done for her chest pain and cardiac risk factors,
which showed no ischemic changes, and a Doppler/ultrasound of the carotids showed no evidence
of plaque or stenosis. A low carbohydrate, Paleo style diet was prescribed for significant weight gain
(5ft 7 in, 206 lbs.) with hyperinsulinemia (70.7 uIU, normal < 24.9 uIU), hyperuricemia (uric acid 8.2.
normal range < 6.0 mg/dL), elevated hs-CRP (3.7 mg/L, normal < 1) and a CT abdomen suggestive of a fatty liver.

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

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

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

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

The patient had a telemedicine consult April 2018, since she continued to slowly relapse 9 months off antibiotics. She had gone from 90% of normal functioning, down to 60% normal functioning, with increasing fatigue, neck pain with neuralgia on the right side of the neck, head pressure and increased brain fog. Since her G6PD levels were WNL (239, normal range between...
46-376 U) with a normal CBC (H/H 14.5/42.2) and no baseline elevations in methemoglobin (0.3% normal range < 1.9%), we discussed a trial of DDD CT, starting at the beginning of May 2018. After signing an informed consent, she began doxycycline 200 mg PO BID, rifampin 300 mg PO BID, gradually increasing doses of dapsone until at 100 mg BID, hydroxychloroquine 200 mg PO BID, nystatin 500,000 units PO BID, cimetidine 400 mg PO BID, leucovorin 15 mg BID with 15 mg of L-methyl folate during month one, increasing L-methyl folate to 25 mg PO BID month two. This was along with triple biofilm agents and triple probiotics with NAC, ALA and GSH. A CBC, CMP, and methemoglobin levels were drawn every 2 weeks during the 1st month of treatment, and weekly during the 2nd month of DDD CT, at which point, she would stop the therapy and remain on biofilm agents, folic acid and probiotics.

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

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

4. Discussion

There has been a longstanding medical debate regarding the etiology of chronic Lyme disease/PTLDS with two published standards of care for the diagnosis and treatment [44] [45]. Current IDSA treatment guidelines (2006) have relied on 4 randomized, controlled trials (RCT’s) for persistent disease and advised against retreatment [44], although earlier National Institutes of Health (NIH) placebo controlled, double blind, RCT’s of PTLDS showed some benefit on primary or secondary outcome measures in two out of the four trials using IV ceftriaxone [46]. A further biostatistical review of antibiotic retreatment in all 4 Lyme disease RCT’s was therefore performed to understand the discrepancies [47]. DeLong et al. concluded that the design assumptions in the two Klempner trials were unrealistic and underpowered to detect meaningful treatment effects; that the Krupp trial was well designed, finding statistically significant and meaningful improvement in fatigue; and that the Fallon trial corroborated this finding, while also demonstrating improvement in cognitive functioning at week 12, which declined by week 24 [47]. Although improvement in fatigue and cognitive functioning was noted in two of the four trials, sustained improvement was lacking, requiring new treatment strategies.
Other authors have similarly noted relapses in Lyme symptoms post discontinuation of antibiotics and long term impairment of functional status [48]. Although case reports and uncontrolled trials have reported the efficacy of prolonged antibiotic therapy [49] [50] [51], symptoms have often been shown to relapse after discontinuation of therapy [52]. Relapse in underlying Lyme symptoms with persistent fatigue, musculoskeletal pain, neuropathy, and neurocognitive/neuropsychiatric difficulties can potentially be explained by multiple etiologies. These include persistence of spirochetal antigens/peptidoglycans in the joints post treatment leading to Lyme arthritis [12]; persistent infection with Borrelia burgdorferi [14] [53] [54] [55], due to invasion of the spirochete into the joints [56] [57], ligaments [58], eyes [59] [60], central nervous system [61] [62], protected niches including the intracellular compartment [17] [18] and fibroblasts [63] [64]; persistent tickborne co-infections [14], including Babesia [65] [66] [67] [68] [69] and associated co-infections i.e., Bartonella [70] [71], mycoplasma spp. [72] [73], and viral infections such as Human Herpes Virus 6 (HHV-6) [74] [14], as well as other medical problems causing overlapping sources of inflammation with downstream effects [13]. These include up to 16 factors identified on the MSIDS model [75] [76], including immune dysfunction/immune deficiency [15] [77] [13], environmental toxins with detoxification problems [78], GI problems, food allergies [79] [80], nutritional deficiencies [81] [82], hormone and autonomic nervous system dysregulation [83] [84], mitochondrial dysfunction [85] [86], neuropsychiatric problems, and/or sleep disorders [13] [88]. All 3 patients reported here had evidence of multiple overlapping etiologies accounting in part for resistant symptoms, including evidence of associated co-infections (Babesia, Bartonella, Mycoplasma fermentans), hormonal dysfunction, food allergies and leaky gut, environmental toxin exposure with mineral deficiencies, POTS/dysautonomia, neuropsychiatric problems and sleep disorders. Patient 2 also had evidence of persistent Borrelia and mycoplasma species by serum polymerase chain reaction (PCR) despite years of targeted antibiotic therapy. Although addressing these multiple etiologies led to clinical improvements, it was not however until DDD CT therapy was instituted, using a higher dose ‘persister’ drug regimen with dapsone 100 mg PO BID along with a tetracycline, rifampin and biofilm agents, that all 3 patients were finally able to stay in remission for prolonged periods of time (24-30 months).

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

Innovative approaches for treating persister and biofilm forms of Borrelia have emerged during the past decade. New compounds with high activity against stationary form Borrelia burgdorferi persisters have been identified from the NCI compound collection [35], screening an FDA approved drug library [29], as well as identifying new drug candidates using high throughput screening [37]. Potential candidates with efficacy against Bb persisters in culture included daptomycin, cefoperazone and doxycycline [102], sulfa drugs [36] including dapsone [40] and disulfiram [37] [38],...
bee venom/mellitin [103], essential oils (oregano oil, cinnamon bark, clove) [33], as well as Stevia extract [34]. For in vitro biofilm forms of Bb [27], effective killing of Bb has been noted with novel herbal compounds such as Biocidin [104], Stevia [34], baicalein and monolaurin [105] [106] as well as oregano oil [33]. Only dapsone and disulfiram have been published in retrospective clinical trials to date [39] [40] [14]. In one study, three patients who had required open-ended antimicrobial therapy for symptoms of chronic, relapsing Lyme disease and babesiosis had a positive response to a finite course of disulfiram, and remained clinically well without a relapse for a period of 6-23 months [39]. One patient relapsed on disulfiram and required retreatment. Dapsone combination therapy (DDS CT) has been published to be effective in TBD’s in both a case study of a patient with Lyme disease, co-infections and autoimmune disease [107] and in two retrospective clinical trials, involving a total of 300 patients [40] [14]. Eight major Lyme symptoms showed statistical improvement (p < 0.05) with DDS CT, including fatigue, sweat, fatigue, musculoskeletal pain, neuropathy, headaches, cognitive difficulties, and sleep disorders. However, the dose and length of time on DDS CT varied in the two trials (from 25 mg to 100 mg/day), and relapses off DDS CT were not uncommon. The primary difference in this study of 3 patients with chronic Lyme disease/PTLDS was the 100 mg BID dose of dapsone the second month, as compared to doses ranging up to 100 mg QD in our previous studies. The higher dose of dapsone was effective in keeping our patients in symptomatic remission for the first time in decades, with only 7-8 weeks of DDD CT leading to remission times between 25-30 months. This was significantly different than our experience of using lower doses of dapsone, which was effective, but led to relapses upon discontinuation of therapy.

Prior studies done by Dr Eva Sapi and researchers at the University of New Haven, showed that DDS CT (tetracycline, rifampin, dapsone) was highly effective against the biofilm forms of Bb and that higher concentrations of dapsone were more effective in lowering biofilm mass [43]. The major findings were that dapsone, as a single drug and in combination with doxycycline and doxycycline + rifampin had the most significant effect in reducing the mass and viability of B. burgdorferi biofilm. In a repeat in vitro study of DDS CT by Horowitz and researchers at the University of New Haven, showed that disulfiram had the most significant effects on residual GAG amounts of Borrelia biofilm compared to the untreated control (p value <0.01). These culture results support the clinical findings of the superiority of higher dose dapsone therapy for chronic tick-borne symptoms.

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

Based on the above in vitro and our in vivo studies, there is a need for safe and effective drugs that can eliminate all morphological forms of B. burgdorferi including persisters and attached biofilm forms. Both disulfiram and dapsone are known to have potential adverse effects. Disulfiram commonly causes fatigue, sleepiness, headaches, and a metallic taste, although more severe reactions including dermatological, hepatic (hepatitis, hepatotoxicity), cardiac, neurological (peripheral and/or axonal polyneuropathy, sensory-motor polyneuropathy, optic neuritis, seizures), and psychiatric (confusion, psychosis) may result [112]. In patients with Lyme disease and associated co-infections, underlying cardiac, hepatic, neurological and psychiatric conditions can
also co-exist [113] [114] [115] [116] [88] [117], confounding etiologies, requiring a differential diagnosis with monitoring of symptoms, and/or use of lower doses of disulfiram to minimize side effects [39]. Dapsone, also has 4 common side effects, described as ‘Do No H.A.R.M.’, i.e.,

Herxheimer reactions (due to increased inflammatory cytokine production), Anemia (secondary to inhibition of folic acid metabolism, or hemolysis due to G-6-P-D deficiency), Rashes (due to sulfadiazine sensitivity) and Methemoglobinemia (due to increased oxidative stress and diminished oxygen carrying capacity) [14] [118] [119] [120]. Although some of these symptoms were seen in our patients undergoing treatment with DDD CT (Herxheimer reactions, anemia, mild elevations in methemoglobin), adverse side effects were minimized by ruling out G-6-P-D deficiency, using high dose folic acid therapy with folic acid (50-75 mg/day) and L-methyl folate (30-45 mg/day), as well as administering glutathione precursors (NAC 600 mg BID), alpha lipoic acid (ALA, 600 mg BID) and glutathione (GSH, 1000 mg BID) with methylene blue 50 mg BID as needed. Any decrease in red cell counts or significant anemia secondary to dapsone resolved in all patients within 1-2 months of stopping DDD CT while remaining on folic acid supplementation, and none of our patients developed rashes, nor significantly elevated levels of methemoglobin. Use of NAC, ALA and GSH helped to decrease oxidative stress, support detoxification and minimize the risk of methemoglobinemia [121] [122] [123], while doses of glutathione were increased to 2000 mg QD or BID along with alkalinization (using sodium bicarbonate or fresh squeezed citrus) for Herxheimer reactions and/or any increased levels of methemoglobin [124] [76] [125]. Methylene blue can also be given orally to mitigate and rapidly reduce methemoglobinemia [120]. It was only necessary in one of our 3 patients reported here, although in other chronically ill Lyme-MSIDS patients given dapsone at 100 mg or higher [14], oral methylene blue was occasionally needed, and effective in keeping methemoglobin levels below 5%, allowing continuation of therapy. Finally, high dose probiotics (greater than 80 billion CFU’s/day) with multiple strains of Acidophilus, Bifidobacterium, and Saccharomyces boulardii were effective in preventing antibiotic associated diarrhea [126].

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

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

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