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Systemic Intracellular Bacterial Infections (*Mycoplasma, Chlamydia, Borrelia* species) in Neurodegenerative (MS, ALS) and Behavioral Disorders (ASD)

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Abstract

Patients with neurodegenerative and behavioral disorders often have systemic bacterial, viral and/or fungal infections that may play important roles in their pathogenesis. We and others have examined patients with various neurodegenerative and behavioral neurological conditions, such as Amyotrophic Lateral Sclerosis (ALS), Multiple Sclerosis (MS) and Autistic Spectrum Disorders (Autism, Attention Deficit Disorder, Asperger Syndrome), and found evidence for systemic intracellular bacterial and viral infections in a majority of patients. For example, examination of blood leukocytes for evidence of *Mycoplasma* spp., *Chlamydia pneumoniae*, *Borrelia burgdorferi* and other infections by polymerase chain reaction revealed high incidences of systemic co-infections that were not found in control subjects ($P<0.001$). The results were compared to other chronic illnesses where neurological manifestations are often found, such as Chronic Fatigue Syndrome/Myalgic Encephlomyopathy (CFS/ME), Fibromyalgia Syndrome (FMS), Lyme Disease and Gulf War Illnesses. Most of these chronic illness patients also had multiple intracellular bacterial infections compared to control subjects ($P<0.001$), and the most common co-infection found was *Mycoplasma* species in all of the conditions examined. In contrast, in the few control subjects that tested positive, only single infections were found. The results suggest chronic intracellular bacterial infections are common features of neurodegenerative and behavioral disorders, and treatment regimens should address the multiple infections present in these conditions.

Keywords: Bacterial infections, Multiple Sclerosis, Amyotrophic Lateral Sclerosis, Autistic Spectrum Disorder, Fatiguing Illnesses

1. Introduction

Neurodegenerative diseases are chronic degenerative diseases of the Central Nervous System (CNS) that often cause dementia. Although for the most part the causes and mechanisms of this collection of brain diseases are not well known, they are increasing in incidence in the developed as well as the underdeveloped world and are often found in the aging population. These diseases are characterized by molecular changes in nerve cells that result in nerve cell degeneration and ultimately nerve dysfunction and cell death, resulting in neurological signs and symptoms and in extreme cases dementia [1,2]. There appears to be a genetic link to neurodegenerative diseases, but the genetic changes that occur and the changes in gene expression that are found in these diseases are complex [2]. One of the types of change found in essentially all neurological degenerative diseases is the over-expression of oxidative free radical compounds (oxidative stress) that cause lipid, protein and genetic structural changes [3,4]. In addition to genetics and changes in gene expression, it is thought that nutritional deficiencies, head trauma, environmental toxins, chronic bacterial and viral infections, autoimmune immunological responses, vascular diseases, accumulation of fluid in the brain, changes in neurotransmitter concentrations and other causes are involved in various neurodegenerative diseases [1-5].

An attractive model for neurodegeneration and resulting neurological disease involves the toxic products produced as a result of chronic bacterial and/or viral infections

[6,7]. Infectious agents may enter the CNS in infected migratory macrophages, or they may gain access by transcytosis across the blood-brain-barrier or by intraneuronal transfer from peripheral nerves [6]. Cell wall-deficient bacteria, principally species of *Mycoplasma, Chlamydia, Coxiella, Brucella, Borrelia*, among others, are candidate infectious agents that may play an important role in neurodegenerative diseases [8]. Such infections may also cause disease progression, and since they are usually systemic, they could affect the immune system, CNS and other organ systems.

2. Methods

Blood Collection

Blood was collected in EDTA-containing tubes, immediately brought to ice bath temperature and shipped with wet ice by air courier to the Institute for Molecular Medicine for analysis. All blood samples were blinded. Whole blood was used for preparation of DNA using Chelex as previously described [9, 10]. Multiple tests were performed on all patients and control subjects [9, 10].

Amplification of Gene Sequences by PCR

Amplification of the target gene sequences by Polymerase Chain Reaction (PCR) was accomplished as previously described [9, 10]. Negative and positive controls were present in each experimental run. The amplified samples were separated by agarose gel electrophoresis. After denaturing and neutralization, Southern blotting was performed to confirm the PCR product [9, 10]. Multiple PCR primer sets were used for each species tested to minimize the chance that cross-reacting microorganisms were detected.

Statistics

Subjects' demographic characteristics were assessed using descriptive statistics and students' t-tests (independent samples test, t-test for equality of means, 2-tailed). The 95% confidence interval was chosen. Pearson Chi-Square test was performed to compare prevalence data between patients and control subjects.

3. Amyotrophic Lateral Sclerosis (ALS)

ALS is an adult-onset, idopathic, progressive degenerative disease affecting both central and peripheral motor neurons. Patients with ALS show gradual progressive weakness and paralysis of muscles due to destruction of upper motor neurons in the motor cortex and lower motor neurons in the brain stem and spinal cord, ultimately resulting in death, usually by respiratory failure [11,12]. The overall clinical picture of ALS can vary, depending on the location and progression of pathological changes found in nervous tissue [13].

The role of chronic infections has attracted attention with the finding of enterovirus sequences in 15 of 17 spinal cord samples from ALS patients by Polymerase Chain Reaction (PCR) [14,15]. Although others have failed to detect enterovirus sequences in spinal cord samples from patients with or without ALS [16], infectious agent(s) may play a role in the etiology of ALS.

We studied the presence of systemic microbial infections in a preliminary number of ALS patients [17]. We found that 8/8 Gulf War veterans diagnosed with ALS from three nations had systemic mycoplasmal infections. All but one patient had *M. fermentans* infections, and one patient had a systemic *M. genitalium* infection. In 22/28 nonmilitary ALS patients from the USA, Canada and Great Britain we also found blood mycoplasmal infections. Of the mycoplasma-positive civilian patients who were further tested for *M. penetrans, M. fermentans, M. hominis* and *M. pneumoniae*, most were positive for *M. fermentans* (13/22, 59%), but we did find other *Mycoplasma* species, such as *M. hominis* (7/22, 31%) and *M. pneumoniae* infections (2/22, 9%). Two civilian ALS patients had multiple mycoplasmal infections (*M. fermentans* plus *M. hominis*, 9%). The difference in incidence of mycoplasmal infections between ALS patients and control subjects was highly significant ($P<0.001$) [17].

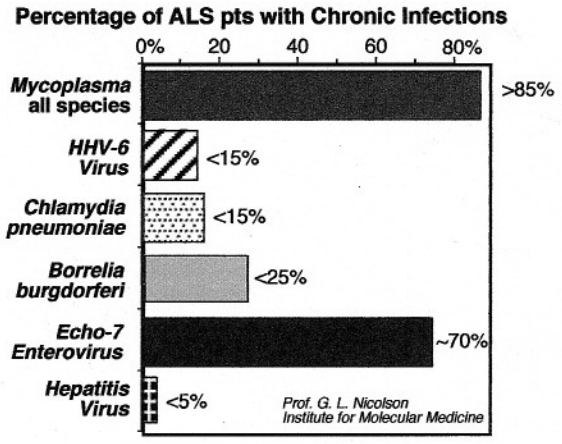

Figure 1. Percent incidence of systemic bacterial and viral infections in 46 patients with Amyotrophic Lateral Sclerosis (ALS).

ALS patients also have other chronic infections, including Human Herpes Virus-6 (HHV-6), *Chlamydia pneumoniae* and *Borrelia burgdorferi* (Figure 1). Similar to the possible role of enteroviruses in the pathogenesis of ALS, the exact role that the other infections play in the pathogene-

sis or progression of ALS is not known. They could be cofactors in the pathogenesis of ALS, or they could simply be opportunistic infections that cause morbidity in ALS patients, such as the respiratory, rheumatic symptoms and other problems often found in ALS patients. They could also be involved in the progression of ALS rather than in its inception.

4. Multiple Sclerosis (MS)

Multiple Sclerosis is a disease of the nerves of the central nervous system, and it can occur in young as well as older people. The nerves in various parts of the brain are covered by a protective insulation containing the protein myelin and other proteins imbedded in a lipid sheath so that the electrical impulses that cause nerve conduction are protected. In MS, inflammation and the presence of autoimmune antibodies against myelin and other antigens causes the protective sheath to break down (demyelination), resulting in decrease or loss of electrical impulses along the nerve. In progressive MS the nerve cells are damaged by demyelination and deposition of plaques on the nerve cells to the point where nerve cell death occurs. There is also breakdown of the blood-brain barrier associated with local inflammation caused by glial cells [18,19].

The clinical results of demyelination and blood-brain barrier lesions are variable but usually include impaired vision, alterations in motor, sensory and coordination systems and cognitive dysfunction. Often these are cyclic (relapsing-remitting) over some time, but a subgroup of patients' progress more rapidly [19].

For several years a possible infectious cause for MS has been under investigation [20,21]. Epidemiological and twin studies suggest that MS is acquired not inherited. Since more than 90% of MS patients show immunological and cytokine characteristics of an infection, patients have been examined for various viral and bacterial infections. One of the most common findings is the presence of *Chlamydia pneumoniae* in MS brains [22-24], although this has not been found by all researchers [25,26].

Recent research at the Institute for Molecular Medicine and elsewhere has shown that some of the autoimmune response to nerve cell proteins may be caused by intracellular infections. As many as 80% of MS patients may have intracellular bacterial infections caused by *Mycoplasma, Chlamydia* and other cell wall-deficient bacteria species that were found only at low incidence in age-matched subjects ($P<0.001$). Additional bacterial infections, such as *Borrelia burgdorfii* (Lyme Disease), and other intracellular bacterial infections may be another class of bacteria involved in some MS cases (Figure 2). Viruses may also be involved in MS, and certain viruses have been found at high incidence in MS patients, such as human herpes virus-6 (HHV-6) [27]. We have also found this virus in the systemic circulation of MS patients (Figure 2).

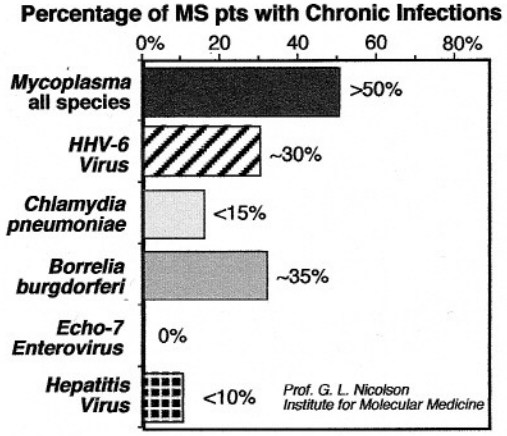

Figure 2. Percent incidence of systemic bacterial and viral infections in 65 patients with Multiple Sclerosis (MS).

Infections can stimulate immunological responses, and the presence, in particular, of intracellular bacterial infections in nerve cells can stimulate autoimmune responses when these intracellular bacteria are released from nerve cells carrying nerve cell antigens. In the case of MS some 20 different bacterial and viral infections have been found, but the link between these infections and the pathogenesis of MS is still being debated [27].

Upon autopsy intracellular bacteria, such as *C. pneumoniae* and *Mycoplasma* species, have been found inside nerve cells in the CNS [28,29], and the presence of such bacteria has been linked to neurological diseases [23]. In addition, infection of non-human primates with bacteria, such as *Mycoplasma fermentans*, results in a fatal disease with neurological complications [30].

5. Autistic Spectrum Disorders (ASD)

Children with Autistic Spectrum Disorders, such as Autism, Attention Deficit Disorder, Asperger Syndrome, etc., generally suffer from an inability to properly communicate, form relationships with others and respond appropriately to their environment. Such patients do not all share the same signs and symptoms but tend to share certain social, communication, motor and sensory problems that affect their behavior in predictable ways. These children often display repetitive actions and develop troublesome fixations with specific objects, and they are often painfully sensitive to certain sounds, tastes and smells

[31,32]. These signs and symptoms are thought to be due to abnormalities in brain function or structure. In some ASD patients there are also a number of other less specific chronic signs and symptoms. Among these are fatigue, headaches, gastrointestinal and vision problems and occasional intermittent low-grade fevers and other signs and symptoms that are generally excluded in the diagnosis of ASD.

The causes of ASD are unknown and may include genetic defects, heavy metal, chemical and biological exposures, among others, and are probably different in each patient. However, among ASD patients there may be similarities in genetic defects and environmental exposures [31, 32] that are important in patient morbidity (sickness) or in illness progression. Other chronic illnesses have some of the same chronic signs and symptoms, suggesting that there may be some overlap in the underlying causes of these conditions or at least in the factors that cause illness or morbidity or illness progression.

The signs and symptoms in many, perhaps even a majority, of chronic illness patients may be due, in part, to systemic chronic infections (bacteria, viruses, fungi) that can penetrate into the CNS. Such infections often follow acute or chronic heavy metal, chemical, biological (viral, bacterial, fungal infections) exposures or environmental insults or even multiple vaccines that have the potential to suppress the immune system and leave children susceptible to opportunistic infections [33-35]. These illnesses generally evolve slowly over time in a multi-step process that may require genetic susceptibility along with multiple toxic exposures.

Chronic infections may be an important element in the development of ASD. Such infections are usually held in check by immune surveillance, but they can take hold and become a problem if they can avoid host immunity and penetrate and hide in various tissues and organs, including cells of the CNS and peripheral nervous system. When such infections occur, they may cause many of the complex signs and symptoms seen in various chronic illnesses [34, 35]. Changes in environmental responses and increased titers to various endogenous viruses as well as bacterial and fungal infections have been commonly seen in chronic illnesses [34, 35].

ASD patients often show their first signs and symptoms after multiple childhood immunizations [2]. Rimland [2] noted that the sharp rise in Autism rates only occurred after the multiple vaccine MMR came into widespread use. In the U.S. children typically receive as many as 33 vaccines, a dramatic increase in the use of childhood vaccines over the last few decades. Such vaccines often contain mercury and other preservatives [36]. Commercial vaccines have also been examined for contaminating microorganisms, and one study found that approximately 6% of commercial vaccines were contaminated with Mycoplasmas [36]. Thus we examined the extent of intracellular bacterial infections in patients with ASD. We were aided in this examination by data that we collected on families of Gulf War veterans where there was a high incidence of Autism in their children [37].

As found previously [38,39], veterans of the Gulf War with chronic fatiguing illness (GWI) exhibited multiple signs and symptoms. Upon examination, the signs and symptoms of GWI were indistinguishable from civilian patients diagnosed with Chronic Fatigue Syndrome/ Myalgic Encephalomyopathy (CFS/ME) [35], except for symptomatic children aged 3-12 who were also diagnosed with Autism or Attention Deficit Hyperactivity Disorder (ADHD), two disorders that fall under ASD [40]. Here 45 of 110 GWI patients or ~42% had mycoplasmal infections (Figure 1), and almost all of these (37 out of 45 or ~82%) were single infections (one species of mycoplasma) [37]. *M. fermentans* was found in ~85% of these single infection cases (Figure 3). When the few multiple infection cases were examined, most were found to have combinations of *M. fermentans* plus either *M. pneumoniae*, *M. hominis* or *M. genitalium* (Figure 2). In contrast, in healthy control subjects only 6 of 70 subjects (8.5%) were positive for any mycoplasmal infection, and all of these were single species infections of various types [37]. Comparing GWI patients and non-symptomatic control subjects, there was a significant difference in the incidence of mycoplasmal infections ($P<0.001$). Differences in infection incidence or species of mycoplasmal infection between male and female GWI patients or control subjects were not seen [37].

In family members of Gulf War veterans with GWI there was evidence of illness transmission. We found that 57/107 (53.2%) of these family members from families with one or more Gulf War veteran diagnosed with GWI and with a positive test for a mycoplasmal infection showed symptoms of CFS/ME. Among the CFS-symptomatic family members, most (40/57 or 70.2%) had mycoplasmal infections compared to the few non-symptomatic family members who had similar mycoplasmal infections (6/50 or 12%) (Figure 3). When the incidence of mycoplasmal infection was compared within families, the CFS/ME family members were more likely to have mycoplasmal infections compared to non-symptomatic family members ($P<0.001$). Symptomatic

children (mostly diagnosed with Autism and ADD) were also infected with mycoplasmas at high incidence (Figure 1), but this was not seen in aged-matched control subjects (data not shown). Although some non-symptomatic family members did have mycoplasmal infections (5/50 or 10%), this was not significantly different from the incidence of mycoplasmal infections in healthy control subjects (6/70 or 8.5%) (Figure 3).

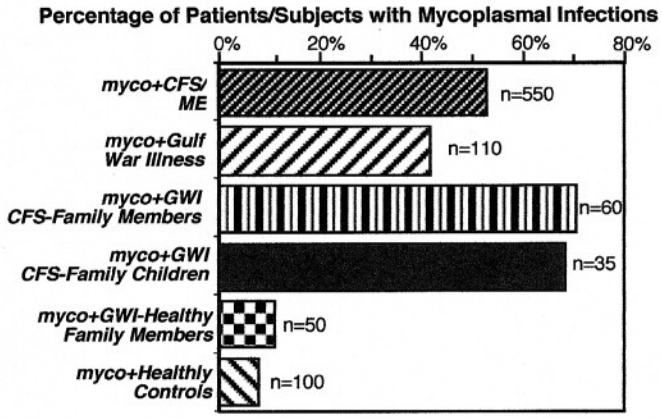

Figure 3. Percent incidence of mycoplasmal infections in family members of veterans with Gulf War Illnesses [37].

The mycoplasma species were also similar between GWI patients and their CFS/ME-symptomatic family members. In 45 mycoplasma-positive CFS/ME-symptomatic family members, most (31 out of 40 or 77.5%) had single species infections (*M. fermentans*), similar to the mycoplasma-positive Gulf War veterans (37 out of 45 or 82%). These results were highly significant ($P<0.001$). We did not find differences in the incidence of infection or type of infections between males and females, children versus adults or spouses versus other family members (data not shown). However, similar to previous reports, the time of onset of CFS/ME illness after the Gulf War tended to be shorter in spouses than other family members, but these differences did not achieve significance [40].

We next examined a small cohort of ASD patients in Central California [40]. This comprised 28 patients aged 3-12 who were diagnosed with ASD. Most of these children had at least one parent with a chronic illness, and the most common diagnosis of adults or adolescents in the same family was CFS/ME or Fibromyalgia Syndrome. When the Autism patients were examined for mycoplasmal infections, 15 children tested positive (54%) for mycoplasmal infections. However, in contrast to the children of GWI patients who for the most part had only one type of mycoplasmal infection, *M. fermentans*, the Central California group that tested positive for mycoplasmal infections had a variety of different species of mycoplasmas [40]. We also tested a few siblings without apparent signs and symptoms, and for the most part few had these infections (5/41 subjects or 12%). Similar results were found in the Gulf War veterans' families where 12% of non-symptomatic family members had mycoplasmal infections [39].

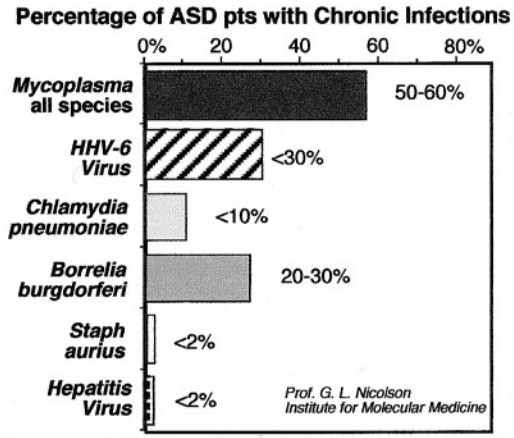

Figure 4. Percent incidence of bacterial and viral infections in 48 patients with Autistic Spectrum Disorders (ASD). The range indicates results from different laboratories.

In a larger study we examined the blood of 48 ASD patients from Central and Southern California and found that a large subset (28/48 or 58.3%) of patients showed evidence of *Mycoplasma* spp. infections compared to two of 45 (4.7%) age-matched control subjects (Odds Ratio=13.8, $P<0.001$) [41]. Since ASD patients had a high prevalence of one or more *Mycoplasma* species and some also show evidence of infections with *Chlamydia pneumoniae*, we examined ASD patients for other infections (Figure 4). In addition, the presence of one or more systemic infections may predispose ASD patients to other infections, thus we examined the prevalence of *C. pneumoniae* (4/48 or 8.3% positive, Odds Ratio=5.6, $P<0.01$) and Human Herpes Virus-6 (HHV-6, 14/48 or 29.2%, Odds Ratio=4.5, $P<0.01$) co-infections in ASD patients. We found that *Mycoplasma*-positive and –negative ASD patients had similar percentages of *C. pneumoniae* and HHV-6 infections, suggesting that such infections occur independently in ASD patients. Control subjects also had low rates of *C. pneumoniae* (1/48 or 2.1%) and HHV-6 (4/48 or 8.3%) infections, and there were no co-infections in control subjects. The results indicated that a large subset of ASD patients show evidence of bacterial and/or viral infections (Odds Ratio=16.5, $P<0.001$) [41].

6. Chronic Fatigue Syndrome (CFS/ME)

Chronic fatigue is reported by 20% of all patients seeking medical care [42]. It is associated with many well-known

medical conditions and may be an important secondary condition in several chronic illnesses. Although chronic fatigue is associated with many illnesses, CFS/ME and Fibromyalgia Syndrome (FMS) are distinguishable as separate syndromes based on established clinical criteria [43]. However, their clinical signs and symptoms strongly overlap. CFS/ME is characterized by unexplained, persistent long-term disabling fatigue plus additional signs and symptoms, whereas patients with FMS suffer primarily from muscle pain, tenderness and soreness [44]. In patients with either diagnosis other conditions that can explain their signs and symptoms are absent; thus in many patients with overlapping signs and symptoms it is difficult to make a clear distinction between a diagnosis of CFS/ME and FMS.

CFS/ME and FMS have been associated with immunological abnormalities and infectious illnesses [34,45]. CFS/ME patients can be subdivided into clinically relevant subcategories that may represent different disease states or co-morbid conditions or illnesses [46]. An important subset of CFS/ME patients is characterized by the presence of chronic bacterial and viral infections [9,10,33-35]. Identifying systemic infections in CFS/ME patients, such as those produced by *Mycoplasma* species, *Chlamydia pneumoniae*, *Brucella* species, *Borrelia burgdorferi* and HHV-6 infections (Figure 5), is likely to be important in determining the treatment strategies for many CFS/ME patients.

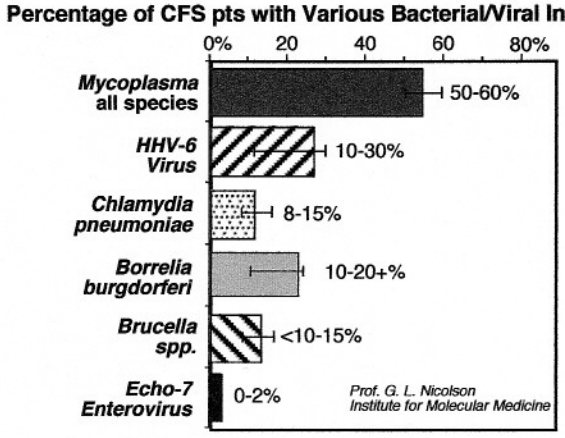

Figure 4. The incidence of various bacterial and vial co-infections in 100 patients with CFS/ME. The bars indicate the range of values found in different independent studies.

Although no single underlying cause has been established for CFS/ME, there is growing awareness that CFS/ME can have an infectious nature that is either causative for the illness, a cofactor for the illness or appears as an opportunistic infection(s) that aggravate patient morbidity [34]. There are several reasons for this [39], including the nonrandom or clustered appearance of CFS/ME, sometimes in immediate family members, the presence of certain signs and symptoms associated with infection, the often cyclic course of the illness and its response to antimicrobial therapies [47].

Using the blood of 100 CFS/ME patients and forensic polymerase chain reaction we found that a majority of patients show evidence of multiple, systemic bacterial and viral infections (Odds Ratio = 18.0, 95% CL 8.5-37.9, $P<0.001$) that could play an important role in CFS/ME morbidity [10,48]. CFS/ME patients had a high prevalence of one of four *Mycoplasma* species (Odds Ratio = 13.8, 95% CL 5.8-32.9, $P<0.001$) and often showed evidence of co-infections with different *Mycoplasma* species, *Chlamydia pneumoniae* (Odds Ratio = 8.6, 95% CL 1.0-71.1, $P<0.01$) and/or active Human Herpes Virus-6 (HHV-6) (Odds Ratio = 4.5, 95% CL 2.0-10.2, $P<0.001$). We found that 8% of the CFS patients showed evidence of *C. pneumoniae* and 31% of active HHV-6 infections. In a separate study we found that a sizable percentage of CFS/ME patients were infected with *Borrelia burgdorferi*, and therefore, they had active Lyme Disease (LD).

Since the presence of one or more chronic systemic infections may predispose patients to other infections, we examined the prevalence of *C. pneumoniae* and active HHV-6 infections in mycoplasma-positive and –negative patients. The incidence of *C. pneumoniae* or HHV-6 was similar in mycoplasma-positive and -negative patients, suggesting that such infections occur independently in CFS patients. Also, the incidence of *C. pneumoniae* in active HHV-6-positive and –negative patients was similar. Control subjects (N=100) had low rates of mycoplasmal (6%), active HHV-6 (9%) or chlamydial (1%) infections, and there were no co-infections in control subjects. Differences in bacterial and/or viral infections in CFS/ME patients compared to control subjects were highly significant. The results indicate that a relatively large subset of CFS/ME patients show evidence of bacterial and viral co-infections.

7. Lyme Disease (LD)

Lyme Disease (LD) is the most common tick-borne disease in North America. First described in Old Lyme, Connecticut in 1975, the infection is caused by a tick bite and the entry of the spiral-shaped spriochete *Borrelia burgdorferi* and other co-infections [49]. *Borrelia b.* and its co-infections has been carried into new habitats by a variety of ticks and their vectors. After incubation for a few days to a month, the *Borrelia* spriochete and co-infections migrate through the subcutaneous tissues into

the lymph and blood where they can travel to near and distant host sites [50]. Transplacental transmission of *Borrelia b.* and co-infections can occur in pregnant animals, including humans, and blood-borne transmission in humans by blood transfusion is likely but unproven. The tick-borne LD co-infections can and usually appear clinically at the same time.

As mentioned above, the signs and symptoms of LD overlap with other chronic conditions; thus LD patients are often diagnosed with other illnesses, such as CFS/ME or Rheumatoid Arthritis. However, many patients with LD have not received an adequate diagnosis for years, and during this period ineffective treatments may have contributed to the refractory nature of the disease.

About one-third of LD cases start with the appearance of a round, red, bulls-eye skin rash (*erythema migrans)* at the site of the tick bite, usually within 3-30 days [50]. Within days to weeks mild flu-like symptoms can occur that include shaking chills, intermittent fevers and local lymph node swelling. After this localized phase, which can last weeks to months, the infection(s) can spread to other sites (disseminated disease), and patients then show malaise, fatigue, fever and chills, headaches, stiff neck, facial nerve palsies (Bell's palsy) and muscle and joint pain and other signs/symptoms.

LD can eventually become persistent or chronic and involve the central and peripheral nervous systems as well as ophthalmic, cardiac, musculoskeletal and internal organ invasion. At this late chronic stage rheumatoid arthritis, neurological impairment with memory and cognitive loss, cardiac problems (mycocarditis, endocarditis causing palpitations, pain, bradycardia, etc.) and severe chronic fatigue are often apparent [51,52].

In the late chronic phase of the disease usually overlap with other chronic conditions, such as CFS/ME, FMS, Rheumatoid Arthritis, among others, causing confusion in the diagnosis and treatment of the chronic phase in LD patients. Some contend that this late phase is not even related to LD, resulting in failure to successfully identify and treat the chronic condition.

The involvement of co-infections in causing chronic signs/symptoms in patients has not been carefully investigated; however, such infections on their own have been shown to produce comparable signs/symptoms. Diagnostic laboratory testing for LD at various clinical stages is, unfortunately, not full-proof, and experts often use a checklist of signs and symptoms and potential exposures, along with multiple laboratory tests to diagnose LD [53].

The laboratory tests used for LD diagnosis include: detection of *Borrelia b.* surface antigens by enzyme-linked immunoassay (EIA), immunofluorescent assay (IFA), and Western immunoblot of *Borrelia* proteins. Alternatively, polymerase chain reaction (PCR) for *Borrelia* DNA has been used to detect the DNA of the intact organism in blood. A true-positive test result usually consists of more than one positive test from the above list, usually EIA followed by Western imunoblot [54]. The problem with these tests is that they are blood tests requiring the presence of antibodies or *Borrelia* proteins in the blood, or they are dependent on the spirochete and thus its DNA being present in the blood (PCR).

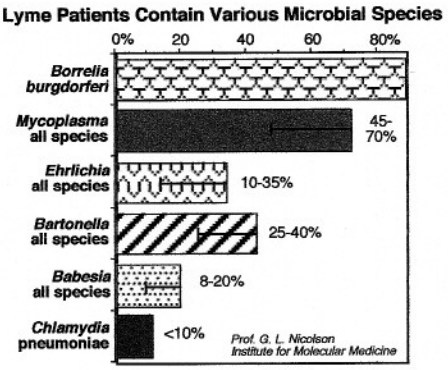

Figure 5. The incidence of various bacterial co-infections in 100 patients with Lyme Disease. The bars indicate the range of values found in various laboratories.

We and others [55] have found that the most common co-infection with *Borrelia b.* are various species of Mycoplasma (Figure 5). Approximately 50-70% of LD patients also have mycoplasmal co-infections (*Mycoplasma fermentans* > *Mycoplasma hominis* > *Mycoplasma pneumoniae, M. genitalium, M. penetrans,* other species). In some cases multiple mycoplasmal infections are present in LD patients. The presence of mycoplasmal infections complicates the diagnosis and treatment of LD, and some of the generalized signs/symptoms found in *Borrelia*-positive patients are also found in mycoplasma-positive patients. Like the *Borrelia b.* spirochete, *Mycoplasma* species are found at intracellular locations in various tissues and are only rarely found free in the blood. This can make detection difficult, and in some patients the appearance of *Borrelia b.* and various Mycoplasmas in their white blood cells can be cyclic.

Another co-infection commonly found in LD is a rickettsial infection caused by *Ehrlichia* species [51]. These small, gram-negative, pleomorphic, obligate intracellular infections are similar to mycoplasmas in their structures, intracellular locations and resulting signs/symptoms. Commonly found species are *E. chaffeensis* and *E.*

phagocytophila, and these microorganisms can cause signs/symptoms within 1-3 weeks of exposure, such as fever, shaking chills, headache and muscle pain and tenderness and less commonly nausea, vomiting, abdominal pain, diarrhea, cough and confusion [51]. Laboratory features include mild to moderate transient hemolytic anemia, decreases in white blood cell count (leucopenia, thrombocytopenia) and elevated erythrocyte sedimentation rate, and sometimes increases in liver enzymes and less often increases in blood urea nitrogen and creatinine. Serology is usually only positive after 1-2 weeks with the limitations discussed above. Since culturing the microorganism is not practical, antibody and PCR testing have been used for confirmation of the infection.

Co-infections complicate the diagnosis and produce different signs/symptoms of LD. These infections can also occur in various combinations. For example, the intracellular protozoan *Babesia* spp. [56]. There are over 100 species of the genus *Babesia*, but most infections in humans in North America are caused by *Babesia microti* and in Europe by *Babesia divergens* and *Babesia bovis.* About 10-40% of cases of LD show *Babesia* co-infections. In addition, LD patients also have *Bartonella* species infections (Figure 5).

When multiple infections are present, the number of signs/symptoms, their severity and duration, can be greater in the early stages of disease [56], including high fever, chills, generalized weakness, gastrointestinal symptoms (anorexia, nausea, abdominal pain, vomiting, diarrhea, among others), anemia, muscle and joint pain, respiratory problems and dark urine. The combination of *Borrelia*, *Mycoplasma* and *Babesia* infections can be lethal in some patients (about 7% of patients can have disseminated intravascular coagulation, acute respiratory distress syndrome and heart failure), but the majority of patients with *Babesia* spp. have the chronic form of the infection. In *Babesia* infections patients can show mild to severe hemolytic anemia (probably correlating with the protozoan colonization of erythrocytes, which can be seen by experienced individuals in blood smears) and a normal to slightly depressed leukocyte count [56]. However, this is usually not seen in patients who have progressed to the chronic phase of the disease.

LD patients are at risk for a variety of other opportunistic infections, including other bacterial infections as well as viral and fungal infections. These can complicate diagnosis and treatment, but they may be principally a problem in the late, chronic phase of the disease. Late stage patients with neurological manifestations, meningitis, encephalitis, peripheral neuropathy and other signs and symptoms may have complicated co-infections that are not recognized or treated by their physicians.

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