Role of Chronic Bacterial and Viral Infections in Neurodegenerative, Neurobehavioural, Psychiatric, Autoimmune and Fatiguing Illnesses: Part 2

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ABSTRACT
Chronically ill patients with neurodegenerative and neurobehavioural and psychiatric diseases commonly have systemic and central nervous system bacterial and viral infections. In addition, other chronic illnesses where neurological manifestations are routinely found, such as fatigue and autoimmune diseases, Lyme disease and Gulf War illnesses, also show systemic bacterial and viral infections that could be important in disease inception, progression or increasing the types/severities of signs and symptoms. Evidence of Mycoplasma species, Chlamydia pneumoniae, Borrelia burgdorferi, human herpesvirus-1,-6 and -7 and other bacterial and viral infections revealed high infection rates in the above illnesses that were not found in controls. Although the specific roles of chronic infections in various diseases and their pathogeneses have not been carefully determined, the data suggest that chronic bacterial and/or viral infections are common features of progressive chronic diseases.

ABBREVIATIONS
Ab Beta Amyloid; AD Alzheimer’s Disease; ADHD Attention-Deficit Hyperactivity Disorder; ALS Amyotrophic Lateral Sclerosis; ASD Autism Spectrum Disorders; EBV Epstein-Barr Virus; CFS Chronic Fatigue Syndrome; CFS/ME Chronic Fatigue Syndrome/Myalgic Encephalomyopathy; CI Confidence Interval; CMV Cytomegalovirus; CSF Cerebrospinal Fluid; CNS Central Nervous System; ELISA Enzyme Linked Immunobosrbitant Assay; GS Guillain-Barré Syndrome; GWI Gulf War Illnesses; HHV Human Herpes Virus; HSV Herpes Simplex Virus; MDD Major Depressive Disorder; ME Myalgic Encephalomyelitis; MRI Magnetic Resonance Imaging; MS Multiple Sclerosis; OCD Obsessive-Compulsive Disorder; PANDAS Paediatric Autoimmune Neuropsychiatric Disorders Associated With Streptococci; PCR Polymerase Chain Reaction; PD Parkinson’s Disease; QOL Quality Of Life; TS Tourette’s Syndrome

Introduction
In the first part of this review we considered neurodegenerative and neurobehavioural diseases and the findings that these diseases commonly are associated with systemic and central nervous system bacterial and viral infections.1 In this second part we continue with psychiatric diseases, autoimmune diseases, fatiguing illnesses, and other chronic diseases where chronic infections play an important role.

Psychiatric diseases
Borrelia-associated psychiatric disorders
In addition to neurologic and rheumatologic symptoms Borrelia burgdorferi has been associated with several psychiatric manifestations2-3 (see also below). Such infections can invade the central nervous system and may cause or mimic psychiatric disorders or cause a co-morbid condition. A broad range of psychiatric conditions have been associated with Lyme disease, including paranoia, dementia, schizophrenia, bipolar disorder, panic attacks, major depression, anorexia nervosa and obsessive-compulsive disorder.4-7 For example, depressive states among patients with late Lyme disease are fairly common, ranging from 26% to 66%.3 It is not known whether B. burgdorferi contributes to overall psychiatric morbidity, but undiagnosed chronic Lyme disease caused by this spirochete is considered a differential diagnosis in patients with certain psychiatric symptoms such as depressive symptoms, lack of concentration and fatigue.

The neuropsychiatric sequelae of chronic Lyme disease remains unclear. Studies were performed, some on large numbers of patients, to investigate whether a correlation exists between chronic Lyme disease (defined by seropositivity) and psychiatric disorders.8-11 Interestingly, different results were reported on the association between B. burgdorferi infection and psychiatric morbidity.8-11 For example, Hájek et al.5 compared the prevalence of antibodies to B. burgdorferi in groups of psychiatric patients and healthy subjects. Among the matched pairs, 33% of the psychiatric patients and 19% of the healthy comparison subjects were seropositive. In contrast, Grabe et al.15 did not find an association between Borrelia seropositivity and mental and physical complaints. In 926 consecutive psychiatric patients that were screened for antibodies and compared with 884 simultaneously recruited healthy subjects, seropositive psychiatric patients were found to be significantly younger than seronegative ones, and this was not found in the healthy controls.10 However, none of the psychiatric diagnostic categories used in this study exhibited a stronger association with seropositivity.10 These findings suggest a potential association between B. burgdorferi infection and psychiatric disorders.
morbiditY, but fail to identify any specific clinical 'signature' of
the infection. This might be due to the very low incidence in an
endemic region (0.2%, CI 95% 0.0% to 1.1%) as
demonstrated in 517 patients hospitalized for psychiatric
diseases.8

In addition to serological data, clinical evidence for the
association of psychiatric symptoms and post-Lyme disease has
also been investigated. If mental and physical complaints in
patients were assessed with the von Zerssen’s complaint scale
using multivariate analyses, the data revealed that definitions of
seropositivity were not associated with increased mental or
physical complaints.11 In contrast, if the SF-36 was used to
determine Quality of Life (QOL) in post-Lyme patients, the
average SF-36 physical component summary (40±9, range 29-
44) and mental component summary (39±14, range 23-46) of
the QOL assessment were worse than the general USA
population, and they could be significantly improved by anti-
Lyme antibiotics (46% versus 18%, p=0.007).3 Barr et
al.12 examined the relation between complaints of memory
disturbance and measures of mood and memory functioning in
55 patients with serological evidence of late-stage Lyme
borreliosis. There was a significant correlation between
subjective memory ratings and self-reported depression
(p<0.001) but not with objective memory performance,
indicating memory disturbance in chronic Lyme patients. Using
a structured psychiatric interview, the Positive and Negative
Affect Schedule, the Lyme Symptom Checklist, and a battery of
neuropsychological tests in 30 post-Lyme patients, participants
did not appear to have an elevated incidence of psychiatric
disorders or psychiatric history.13 Their mood, however, was
characterized by lowered levels of positive affect and typical
levels of negative affect that were similar to affect patterns in
individuals with chronic fatigue syndrome (CFS). Similarly,
Hasset et al.3,7 reported on 240 consecutive post-Lyme patients
who were screened for clinical psychiatric disorders, such as
depression and anxiety. After adjusting for age and sex, these
disorders were more common in symptomatic patients than in
the comparison group (Odds Ratio=3.54, CI 95% 1.97-
6.55, p<0.001), but personality disorders were comparable in
both groups.

Although psychiatric co-morbidity and other psychological
factors are prominent in post-Lyme patients, it remains
uncertain whether these symptoms can be directly attributed to
the chronic course of Borrelia infections or to other chronic
illness-related factors.

Schizophrenia

Several microbes have been suspected as pathogenetic factors in
schizophrenia, such as Chlamydia species, toxoplasma, and
various viruses. For example, a number of studies have reported
associations between Toxoplasma gondii infection and the risk of
schizophrenia with an overall hazard ratio of 1.24.14 In addition,
chlamydial infections have been found in 40% of schizophrenic
patients compared to 7% in healthy controls.15 These infections
represented the highest risk factor yet found to be associated
with schizophrenia that was highly significant (Odds
Ratio=9.43, p=1.39 x 10⁻⁹), especially with Chlamyphila
d psittaci (Odds Ratio=24.39, p=2.81 x 10⁻¹). Interestingly,
Schizophrenic carriers of the HLA-A10 genotype were clearly
the most often infected with Chlamydia, especially C.
psittaci (Odds Ratio=50.00, p=8.03 x 10⁻⁸), pointing to a
genetically related susceptibility.16 However, skepticism against
the role of bacterial infection in schizophrenia was also fostered
by the low impact of anti-infectious treatment on the course of
disease progression in schizophrenia.17

Genetic backgrounds and viral infections and/or reactivations as
well as cytokine-related pathomechanisms have also been
proposed as causative for psychiatric disorders, such as
schizophrenia. Specific genetic patterns of MICB polymorphism
(MHC class I polypeptide-related sequence B, chromosome 6p21)
were identified in patients seropositive for CMV and HSV-1.18
Similar polymorphisms were found for the COMT Val158Met related to serological evidence of HSV-1
infections in individuals with bipolar disorder.19 This serologic
evidence of HSV-1 infection appeared to be associated with
cognitive impairment in individuals with bipolar disorders and
was found to be an independent predictor of cognitive
dysfunction in individuals with schizophrenia.20 In addition,
viral exposure during gestation has been described as a risk
dfactor for schizophrenia. Offspring of mothers with serologic
evidence of HSV-2 infection were at significantly increased risk
for the development of psychoses (Odds Ratio=1.6; CI 95%
1.1-2.3). These results are consistent with a general model of
risk resulting from enhanced maternal immune activation
during pregnancy.21 However, this was not confirmed in another
study.22 Similar contradictory results were observed in a small
group of 8 patients with schizophrenia where reactivation of
herpesviruses (HSV-1, CMV, EBV, varicella-zoster virus and
human HHV-6) and other viruses (measles, rubella, mumps,
influenza A and B and Japanese encephalitis viruses) during
acute onset or exacerbation of schizophrenia was investigated,
but none of these viruses were detected in these patients.23
Also, a search for HSV-1 or varicella zoster virus infection in
postmortem brain tissue from schizophrenic patients did not
reveal evidence of persistent CNS infections with these
viruses.24

Schizophrenic patients show a number of cytokine changes that
may be important in their condition. For example, differences in
interleukin-2, -4 and -6, among other cytokines, have been
seen in schizophrenic patients.25-27 Often these changes in
cytokines or cytokine receptors have been linked to associated
genetic changes found in schizophrenia.28-30 Monji et
al.31 recently reviewed the evidence for neuroinflammation,
increases in pro-inflammatory cytokines and genetic changes in
schizophrenia and concluded that these changes are closely
linked to activation of microglia. Although the microglia
comprise only about 10% of the total brain cells, they respond rapidly to even minor pathological changes in the brain and may contribute to neurodegeneration through the production of pro-inflammatory cytokines and free radicals. CNS infections could also activate microglia and cause similar events.

Neuropsychiatric Movement Disorders

Gilles de la Tourette’s syndrome (TS) is a neurological condition that usually begins in childhood and results in involuntary sounds or words (vocal tics) and body movements (movement tics). An association between infection and TS has been repeatedly described.32 Abrupt onset of the disease, usually after infection, was noted in up to 11% of these patients.33-34 A role for streptococcal infections (PANDAS, see below) as causative or mediating agent in TS was established several years ago.35 Additionally, the involvement of other infectious agents, such as B. burgdorferi or M. pneumoniae, has been described in case reports and small studies. For example, comparing 29 TS patients with 29 controls revealed significantly elevated serological titers in TS patients (59% versus 3%). This higher proportion of increased serum titers, especially IgA titers, suggested a putative role for M. pneumoniae in a subgroup of patients with TS.36 In predisposed persons, infection with various agents including M. pneumoniae should be considered as at least an aggravating factor, but an autoimmune reaction has to be taken into account in TS patients. In addition, co-infections with toxoplasmosis have been described in a few case reports of obsessive-compulsive disorder (OCD).37 As mentioned above, streptococcal infections are likely to play a pivotal role in these syndromes.38

The pathogenic mechanism may be secondary to an activation of the immune system, resulting in an autoimmune response. This will be discussed in the next section.

Autoimmune Diseases

Infections are associated with various autoimmune conditions.39-40 Autoimmunity can occur when infections like cell-wall-deficient bacteria are released from cells containing parts of cell membranes that are then seen as part of a bacterial antigen complex, or bacteria can synthesize mimicry antigens (glycolipids, glycoproteins or polysaccharides) that are similar enough in structure (molecular mimicry) to stimulate autoimmune responses against similar host antigens. Alternatively, viral infections can weaken or kill cells and thus release cellular antigens, which can stimulate autoimmune responses, or they can incorporate molecules like gangliosides into their structures.

In addition to molecular mimicry, autoimmunity involves several other complex relationships within the host, including inflammatory cytokines, Toll-like receptor signalling, stress or shock proteins, nitric oxide and other stress-related free radicals, among other changes that together result in autoimmune disease.39, 40

Guillain-Barré syndrome

Guillain-Barré syndrome (GB) is a demyelinating autoimmune neuropathy often associated with bacterial infections.40 Symptoms include pain, muscle weakness, numbness or tingling in the arms, legs and face, trouble speaking, chewing and swallowing. Of the types of infections found in GB, Campylobacter jejuni, Mycoplasma pneumonia and Haemophilus influenzae are often found.39 For example, Taylor et al.41 found serological evidence of C. jejuni in 5 of 7 patients with GB and other motor neuropahties, and Gregson et al.42 found anti-ganglioside GM1 antibodies that cross-reacted with C. jejuni liposaccharide isolates. When infections were examined in GB cases in India, Gorthi et al.43 found that 35% and 50% of GB patients had serological evidence of C. jejuni and M. pneumoniae infections, respectively, while one-third of cases showed evidence of both infections. In Japan Mori et al.44 found that 13% of GB patients had antibodies against Haemophilus influenzae. Autoantibodies stimulated by infections found in GB patients can cross-react with nerve cell gangliosides (anti-GM1, anti-GM1b, anti-GD1a, among others), and these are thought to be important in the pathogenesis of GB.45 Indeed, injection of C. jejuni lipo-oligosaccharide into rabbits induces anti-gangliosides and a neuropathy that resembles acute motor axonal neuropathy.46

Viruses have also been found to be associated with GB.47 Examples are: CMV,48 HIV,49 herpes simplex virus,50 West Nile virus,50 and HHV-6.51

Paediatric autoimmune neuropsychiatric disorders associated with Streptococci (PANDAS)

Streptococcal infections in children are usually benign and self-limited. In a small percentage of children, however, prominent neurologic and/or psychiatric sequelae can occur. Post-streptococcal basal ganglia dysfunction has been reported with various manifestations, all of which fall into a relatively well-defined symptom complex or syndrome called paediatric autoimmune neuropsychiatric disorders associated with streptococcal infection (PANDAS).52

Evidence from past studies indicates that adults and children with a symptom course consistent with PANDAS experience subtle neuropsychological deficits similar to those of primary psychiatric diagnosis of OCD and TS.53 PANDAS are now considered as a well-defined syndrome in which tics (motor and/or vocal) and/or OCD are consistently exacerbated in temporal correlation to a group A beta-hemolytic streptococcal infection. However, the pathological relationship between OCD or tics/TS in childhood to antecedent group A Streptococci is still not fully understood.52
In an epidemiological investigation Leslie et al. assessed whether antecedent streptococcal infection(s) increase the risk of subsequent diagnosis of OCD, TS, other tic disorders, attention-deficit hyperactivity disorder (ADHD) or major depressive disorder (MDD). Children with newly diagnosed OCD, TS, or tic disorder were more likely than controls to have had a diagnosis of streptococcal infection in the previous year (Odds Ratio=1.54, CI 95% 1.29-2.15). Previous streptococcal infection was also associated with incident diagnoses of ADHD (Odds Ratio=1.20, CI 95% 1.06-1.35) and MDD (Odds Ratio=1.63, CI 95% 1.12-2.30). Similar results were found in a retrospective, cross-sectional, observational study of 176 children and adolescents with tics, TS, and related problems. In a case-control study of children 4 to 13 years old patients with OCD, TS, or tic these disorders were more likely than controls to have had prior streptococcal infection (Odds Ratio=2.22; CI 95% 1.05-4.69) in the 3 months before onset date. The risk was higher among children with multiple streptococcal infections within 12 months (Odds Ratio=3.10; CI 95% 1.77-8.96). Having multiple infections with group A beta-hemolytic Streptococcus within a 12-month period was associated with an increased risk for TS (Odds Ratio=13.6; CI 95% 1.93-51.0). Similar results were found in patients with typical symptoms of Tourette’s syndrome. The frequency of elevated anti-streptolysin O titers was also significantly higher (p=0.04) in patients with attention-deficit hyperactivity disorder (64%) than in a control group (34%).

Sydenham’s chorea is one manifestation of post-streptococcal neuropsychiatric movement disorders. A pathogenic similarity between Sydenham’s chorea, TS and other PANDAS has been suggested since some patients can present with one diagnosis and then evolve with other neuropsychiatric conditions. These observations support a role of group A streptococcal infection and basal ganglia autoimmunity. Anti-basal ganglia antibodies that are associated with serologic evidence of recent streptococcal infection were found as potential diagnostic markers for this group of disorders, which includes Sydenham’s chorea as the prototype.

However, contradictory results were also reported. For example, an association between symptom exacerbations and new group A beta-hemolytic streptococcus infections among 47 pediatric patients with TS and/or OCD was not observed. In addition, the failure of immune markers for streptococcal infections to correlate with clinical exacerbations in a small study of children with pediatric autoimmune neuropsychiatric disorders raised concerns about the viability of autoimmunity as a pathophysiological mechanism in these syndromes. However, in a second study the same group reported that patients who fit published criteria for pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections represented a subgroup of those with chronic tic disorders and OCD. These patients may be vulnerable to group A beta-hemolytic Streptococcus infection as a precipitant of neuropsychiatric symptom exacerbations.

Taken together, these findings provide epidemiologic evidence that some pediatric-onset neuropsychiatric disorders, including OCD, tic disorders, ADHD, and MDD, may be, at least partially, related to prior streptococcal infections. Group A beta-hemolytic Streptococcus infections are likely not the only event associated with symptom exacerbations for PANDAS patients, but they appear to play a role at least in a subgroup of these children. A potential genetic susceptibility for these post-infectious complexes has been recently proposed.

The recent recognition that these pediatric neurobehavioural syndromes have infectious and/or immunologic triggers has pointed to important new avenues for their management.

**Fatiguing illnesses**

**Chronic fatigue syndrome/myalgic encephalomyelitis**

Chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) is a fatiguing illness characterised by unexplained, persistent long-term disabling fatigue plus additional signs and symptoms, including neurophysiological symptoms. Brain imaging studies have shown that CFS/ME patients are dysfunctional in their ventral anterior cingulate cortex, and they also have other brain MRI abnormalities. In addition, CFS/ME patients also have immunological and inflammation abnormalities, such as alternations in natural killer cell function, and cytokine profiles. In addition, the hypothalamo-pituitary-adrenal axis, which plays a major role in stress responses, appears to be altered in CFS/ME.

Most, if not all, CFS/ME patients have multiple chronic bacterial and viral infections. For example, when patients were examined for evidence of multiple, systemic bacterial and viral infections, the Odds Ratio for this was found to be 18 (CI 95% 8.5-37.9, p< 0.001). In this study CFS/ME patients had a high prevalence of one of four Mycoplasma species (Odds Ratio=13.8, CI 95% 5.8-32.9, p< 0.001) and often showed evidence of co-infections with different Mycoplasma species, C. pneumoniae (Odds Ratio=8.6, CI 95% 1.0-71.1, p< 0.01) and HHV-6 (Odds Ratio=4.5, CI 95% 2.0-10.2, p< 0.001). In a separate study the presence of these infections was also related to the number and severity of signs and symptoms in CFS/ME patients, including neurological symptoms. Similarly, Vojdani et al. found Mycoplasma species in a majority of CFS/ME patients, but this has not been seen in all studies. Interestingly, when European CFS/ME patients were examined for various Mycoplasma species, the most common species found was M. hominis, whereas in North America the most common species found was M. pneumoniae, indicating possible regional differences in the types of infections in CFS/ME patients. In addition to Mycoplasma species, CFS/ME patients...
are also often infected with *B. burgdorferi*, as mentioned above, *C. pneumoniae*,75, 77, 83

Other infections are also found in CFS/ME patients, such as viral infections: CMV,84 parvovirus B19,85 enterovirus86 and HHV-6.75, 77, 85, 88 For example, Ablashi et al.84 found that 54% of CFS/ME patients had antibodies against HHV-6 early protein, compared to 8% of controls. Similarly, Patnaik et al.84 found that 77% of CFS/ME patients were positive for HHV-6 early antigen IgG or IgM antibodies, whereas only 12% of control subjects had IgG or IgM antibodies to HHV-6 early antigen. Recently a new retrovirus, XMRV, was found in mononuclear blood cells of 67% of 101 chronic fatigue syndrome patients compared to only 3.7% of healthy controls. Cell culture experiments determined that the patient-derived virus was infectious and could possibly be transmitted.89

**Gulf War illnesses**

GWI is a syndrome similar to CFS/ME.90 In most GWI patients the variable incubation time, ranging from months to years after presumed exposure, the cyclic nature of the relapsing fevers and the other chronic signs and symptoms, and their subsequent appearance in immediate family members, are consistent with an infectious process.90, 91 GWI patients were exposed to a variety of toxic materials including chemicals, radiochemicals and biologicals so not all patients are likely to have infections as their main clinical problem. Neurological symptoms are common in GWI cases.90 Baumzweiger and Grove92 have described GWI as neuro-immune disorder that involves the central, peripheral and autonomic nervous systems as well as the immune system. They attribute a major source of the illness to brainstem damage and central, peripheral and cranial nerve dysfunction from demyelination. They found GWI patients have muscle spasms, memory and attention deficits, ataxia and increased muscle tone.92

Bacterial infections were a common finding in many GWI patients.90 Mycoplasmal infections were found in about one-half of GWI patients, and more than 80% of these cases were PCR positive for *M. fermentans*.90, 91, 93-95 In studies of over 1,500 U.S. and British veterans with GWI, approximately 45% of GWI patients have PCR evidence of such infections, compared to 6% in the non-deployed, healthy population. Other infections found in GWI cases at much lower incidence were *Y. pestis*, *Coxiella burnetii* and *Brucella* species.90

When we examined the immediate family members of veterans with GWI who became sick only after the veteran returned to the home, we found that >53% had positive tests for mycoplasmal infections and showed symptoms of CFS/ME. Among the CFS/ME-symptomatic family members, most (>80%) had the same *Mycoplasma fermentans* infection as the GWI patients compared to the few non-symptomatic family members who had similar infections (Odds Ratio=16.9, CI 95% 6.0-47.6, p<0.001).91 In contrast, in the few non-symptomatic family members that tested *Mycoplasma*-positive, the *Mycoplasma* species were often different from the species found in the Gulf War Illness patients (*M. fermentans*). The most sensible conclusion is that veterans came home with *M. fermentans* infections and then transmitted these infections to immediate family members.91

### Some other infectious diseases with neurological aspects

#### Lyme Disease

Lyme disease is caused by a tick bite and the entry of the spiral-shaped spirochete *B. burgdorferi* as well as other co-infections.90 Lyme disease is the most common tick-borne disease in North America. After incubation for a few days to a month, the *Borrelia* spirochete and co-infections migrate through the subcutaneous tissues into the lymph and blood where they can travel to near and distant host sites, including the central nervous system.3, 97-99 Transplacental transmission of *B. burgdorferi* and co-infections can occur in pregnant animals, including humans, and blood-borne transmission to humans by blood transfusion is likely but unproven. The tick-borne co-infections associated with Lyme disease can and usually do appear clinically at the same time, complicating clinical diagnoses.100

Lyme disease signs and symptoms eventually overlap with the signs and symptoms of other chronic illnesses, and patients are often diagnosed with illnesses like CFS/ME, chronic arthritis or a neurological disease.80, 97, 100 About one-third of cases with Lyme disease start with the appearance of a round, red, bullseye skin rash (*erythema migrans*) at the site of the tick bite, usually within 3-30 days.100 Within days to weeks mild flu-like symptoms can occur that include shaking chills, intermittent fevers and local lymph node swelling. After this localised phase, which can last weeks to months, the infection can spread to other sites resulting in disseminated disease. In the disseminated (late) phase patients present with malaise, fatigue, fever and chills, headaches, stiff neck, facial nerve palsies (Bell’s palsy) and muscle and joint pain, and other signs and symptoms.100-104

The disseminated (late) phase of Lyme disease is a chronic, persistent disease with ophthalmic, cardiac, musculoskeletal, central nervous system and internal organ invasion. When it involves the central and peripheral nervous systems, it is often termed neuroborreliosis.100, 104 At this late stage, arthritis, neurological impairment with memory and cognitive loss, cardiac problems (such as myocarditis, endocarditis causing palpitations, pain, bradycardia, hypertension) and severe chronic fatigue are usually apparent.80, 100-102 The signs and symptoms of the chronic (late) phase of the disease usually overlap with other chronic conditions, such as CFS/ME, chronic arthritis, as well as neurodegenerative diseases, causing confusion in the diagnosis and treatment of the chronic phase in patients with Lyme Disease.80, 97, 100, 105 Patients with late stage neuroborreliosis exhibit neuropathologic and
neuropsychiatric disease similar to some of the neurodegenerative diseases discussed in previous sections.\textsuperscript{1}

Diagnostic laboratory testing for Lyme disease at various clinical stages is not fool-proof, and experts often use a checklist of signs and symptoms and potential exposures, along with multiple laboratory tests to diagnose Lyme disease.\textsuperscript{104} The laboratory tests include serology, Western blot analysis of \textit{B. burgdorferi} associated bands, PCR analysis of blood and the nonspecific decrease in CD-57 natural killer cells. Unfortunately, similar to other intracellular bacteria, \textit{Borrelia} spirochetes are not always released into the blood circulation or other body fluids, making the very sensitive PCR method less than reliable for diagnosing Lyme \textit{Borrelia} with blood samples. Lebech and Hansen\textsuperscript{106} found that only 40\% of cerebrospinal fluid samples from patients with Lyme neuroborreliosis were positive for \textit{B. burgdorferi} by PCR.

Co-infections in Lyme disease are important but, in general, have not received the attention that \textit{B. burgdorferi} attracts. Some of the Lyme Disease co-infections on their own, such as \textit{M. fermentans}, have been shown to produce signs and symptoms comparable to \textit{B. burgdorferi} infections.\textsuperscript{80,102}

The most common co-infections found in Lyme disease are species of \textit{Mycoplasma}, mostly \textit{M. fermentans}, present in a majority of cases.\textsuperscript{80,103,107} In some cases multiple mycoplasmal infections are present in patients with Lyme disease,\textsuperscript{103} while other common co-infections include \textit{Ehrlichia} species, \textit{Bartonella} species and \textit{Babesia} species. Such co-infections are present in 10-40\% of cases.\textsuperscript{103,104,108-112} \textit{Ehrlichia} and \textit{Bartonella} species are usually found along with \textit{Mycoplasma} species in Lyme disease.\textsuperscript{94,98,108-111} \textit{Babesia} species, such as \textit{B. microti},\textsuperscript{113} which also causes cat-scratch disease,\textsuperscript{113} are often found in neurological cases of Lyme disease.\textsuperscript{100,111}

Protozoan co-infections have been found with \textit{B. burgdorferi}, such as intracellular \textit{Babesia} species.\textsuperscript{100,108,109,112,114} The combination of \textit{Borrelia}, \textit{Mycoplasma} and \textit{Babesia} infections can be lethal in some patients, and -7\% of patients can have disseminated intravascular coagulation, acute respiratory distress syndrome and heart failure.\textsuperscript{109}

\section*{Brucellosis}

\textit{Brucellosis} is a nonspecific clinical condition characterized by intracellular \textit{Brucella} species infection.\textsuperscript{115} Approximately 40\% of patients with \textit{Brucella} spp. infections have a systemic, multi-organ chronic form of brucellosis that is similar to CFS/ME in its multi-organ signs and symptoms.\textsuperscript{115,116} \textit{Brucella} infections can invade the central nervous system and cause neurological symptoms.\textsuperscript{117}

\textit{Brucella} species cause infections in animals, and often humans get the infections from prolonged contact with infected animals. Thus these bacteria are zoonotic, they are capable of being transmitted from animals to humans. Although there are at least eight species of \textit{Brucella} that are pathogenic, only \textit{B. melitensis}, \textit{B. abortus}, \textit{B. suis} and \textit{B. canis} have been reported to be pathogenic in humans.\textsuperscript{116}

When CFS/ME patients were examined for the presence of \textit{Brucella} spp. infections, approximately 10\% showed evidence by PCR of \textit{Brucella} spp. infections (Odds Ratio=8.2, CI 95\% 1-66, \textit{p}<0.01).\textsuperscript{118} Interestingly, urban CFS/ME patients with \textit{Brucella} infections were not as prevalent as rural patients with \textit{Brucella} infections (Odds Ratio=5.5, CI 95\% 3-23.5, \textit{p}<0.02), while control subjects had very low (1.4\%) rates of infection. Co-infections with \textit{Mycoplasma} species were also found in \textit{Brucella}-positive CFS/ME patients.\textsuperscript{114}

\section*{Final comments to part 2}

The progression, and in some cases, the inception of many chronic diseases are probably elicited by various bacterial and viral infections.\textsuperscript{1,39,40,119} Even if infections are not directly involved in the pathogenesis of these diseases, patients with chronic conditions are at risk of a variety of opportunistic infections that could result in co-morbid conditions or promote disease progression. Infections can complicate diagnosis and treatment, and patients with late-stage disease with complex neurological manifestations, such as meningitis, encephalitis, peripheral neuropathy, psychiatric conditions, or with other signs and symptoms could have infections that are not recognized or treated.

Patients with chronic diseases are particularly difficult to treat using single modality approaches, and this is particularly true for patients who also have multiple chronic infections.\textsuperscript{105,107} The multi-focal nature of chronic diseases and the fact that often treatments are given to suppress signs and symptoms, rather than treat causes of the disease or its progression, have resulted in incomplete or ineffective treatments. On the other hand, even if the causes of chronic diseases are known, by the time therapeutic intervention is undertaken, it may be entirely too late to use approaches that should work on the disease if chronic infections were not present. Moreover, if complex, chronic infections are ignored or left untreated, recovery may be difficult, if not impossible to achieve.

At the moment the evidence that particular or specific types of infections are responsible for the inception or pathogenesis of chronic diseases is inconclusive.\textsuperscript{115} One of the problems that arises in trying to prove this hypothesis is that not all patients appear to have similar chronic infections. Some individuals can harbour chronic infections without any observable signs or symptoms. Although the incidence of chronic infections of the types discussed in this review in symptom-free individuals is generally very low, usually only a few percent,\textsuperscript{116-118} that does not prove that they are important in pathogenesis. Since patients with chronic diseases have been identified that do not have easily diagnosed chronic infections, most researchers have
concluded that infections are not involved in the pathogenesis of chronic diseases. Unfortunately, the tools available to find chronic infections are not optimal, and many patients are likely go undiagnosed with chronic infections for purely technical reasons.1,2,9

In the history of medicine animal models of disease have provided useful information that could not be obtained through clinical studies alone. Indeed, the field of chronic diseases could benefit from the greater use of relevant animal models. We suggest that to be useful, the pathogenesis of the animal models of disease must be similar to the pathogenesis of human disease and the animal models must have a similar response to therapy as humans. Thus such models are only relevant if they closely mimic human disease and its response to treatment. For example, the infection of non-human primates with neuropathologic microorganisms, such as *Mycoplasma fermentans*, resulted in brain infections and fatal diseases with clinically typical neurological signs and symptoms.12,22 These primates also respond to therapies that have been used successfully to treat humans.9,12,35 Thus this particular model may be useful if it can be reproducibly infected with specific microorganisms and later develop neurological signs and symptoms that closely mimic chronic human neurological diseases. Future efforts to determine the relationship between specific infections and the pathogenesis of various chronic diseases may well depend on the further development of relevant animal models.

**Competing Interests**
None declared

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