

Diagnosis and Treatment of Chronic Mycoplasmal Infections in Fibromyalgia and Chronic Fatigue Syndromes: Relationship to Gulf War Illness

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Summary

Mycoplasma infections are associated with several acute and chronic illnesses, including pneumonia, asthma, rheumatoid arthritis, immunosuppression diseases such as AIDS, genitourinary infections, and Gulf War Illness (GWI). Using forensic Polymerase Chain Reaction blood samples from 132 Chronic Fatigue Syndrome (CFS) (Myalgic Encephalomyelitis) and/or Fibromyalgia Syndrome (FMS) patients were investigated for the presence of mycoplasma infections in blood leukocytes. CFS and FMS patients had completely overlapping signs and symptoms and were grouped for purposes of analysis. There was a significant difference between symptomatic CFS/FMS patients with positive mycoplasma infections (~63%) and healthy positive controls (~9%) ($P < 0.001$). We also examined the incidence of *Mycoplasma fermentans* infections in these CFS/FMS patients (~50%) and controls (0%) ($P < 0.001$). The prevalence of mycoplasma infections in female and male symptomatic patients was similar. Similar to GWI patients with mycoplasma infections (~50%) and with similar signs and symptoms, mycoplasma-positive CFS/FMS patients respond to 6-week cycles of particular antibiotics: doxycycline, minocycline, ciprofloxacin, azithromycin, and clarithromycin. Multiple cycles of these antibiotics plus nutritional support appear to be necessary for recovery.

Keywords: Chronic infections, mycoplasma, antibiotics, nutritional support

Introduction

Chronic illnesses, such as Myalgic Encephalomyelitis or Chronic Fatigue Syndrome (CFS), Fibromyalgia Syndrome (FMS), and Gulf War Syndrome or Gulf War Illnesses (GWI), are similar in that their complex, multi-organ signs and symptoms overlap or are almost identical.¹ These include: chronic fatigue, headaches, memory loss, muscle pain and soreness, nausea, gastrointestinal problems, joint pain and soreness, lymph node pain, short term memory loss, and other signs and symptoms.² The major difference between these illnesses appears to be in the severity of specific signs and symptoms. For example, FMS patients have as their major complaint muscle and overall pain, soreness and weakness, whereas CFS patients most often complain of chronic fatigue and joint pain, stiffness and soreness, but otherwise their complaints overlap. Often these patients have also shown increased sensitivities to various environmental irritants and enhanced allergic responses. Although these illnesses have been known for several years, most patients with CFS, FMS, or GWI have had few treatment options. This may have been due to the imprecise nature of their diagnoses, which are based primarily on clinical observations rather than laboratory tests, and a lack of understanding about the underlying causes of these illnesses.

That the signs and symptoms of FMS and CFS overlap with those found in GWI suggested that these are not separate syndromes, they are CFS-like disorders.³ In the case of GWI, over 100,000 veterans of the Persian Gulf War in 1991 have been found to have this disorder, not including immediate family members. According to one U.S. government

study, GWI has slowly spread to family members,⁴ and it is likely that it has also spread to some degree in the workplace. Although this U.S. Senate committee report was a preliminary study on approximately 1,200 GWI families, the survey indicated that approximately 77% of spouses and a majority of children born after the war had signs and symptoms similar or identical to veterans with GWI.⁴ The official position of the U.S. Department of Defense remains that family members have not contracted GWI, but this U. S. Senate study indicates that at a rather large subset of GWI patients have a transmittable illness.⁴ The official U.S. position is only one of several apparent myths about the Gulf War that appear to defy common sense.⁵

Chronic illnesses, such as CFS, FMS, and especially GWI, have been described as somatoform disorders.⁶ Often patients with CFS, FMS, and GWI have cognitive problems, such as short term memory loss, difficulty concentrating, and other psychological problems. Psychologists or psychiatrists who examine CFS, FMS, or GWI patients find psychological or psychiatric problems in these patients and decide in the absence of contrary laboratory findings that these conditions are somatoform disorders, not organic problems. Stress is often mentioned as an important factor or the important factor in these disorders. In particular, GWI patients are often diagnosed with Post Traumatic Stress Disorder (PTSD) in veterans' and military hospitals.⁶ The evidence that has been offered as proof that stress or PTSD is the source of GWI sickness is the assumption that veterans must have suffered from stress by virtue of the stressful environment in which they found themselves during the Gulf War.⁶ In fact, veterans themselves do not feel that stress-

Specific primers for *M. fermentans* (SB1: forward probe, SB2: reverse probe, SB3: internal probe) were selected from the *tuf* gene¹² (see Table 1).

Amplification of the target sequence of 717 bp size (850 kb for *M. fermentans*) was performed in a total volume of 50 µl PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 9) containing 0.1% Triton X-100, 200 µM each of dATP, dTTP, dGTP, dCTP, 100 pmol of each primer, 1-2 units of AmpliTaq DNA polymerase and 0.5-1 µg of chromosomal DNA. *Mycoplasma fermentans* DNA (0.5-1 ng of DNA) was used as positive control for amplification. The amplification was carried out in a thermocycler (GeneAmp PCR System). All glassware and pipette tips were decontaminated, and positive displacement pipettes were used. Negative and positive controls were used in each run.^{12,13}

The amplified samples were run on 1% agarose gel containing 5 µl/100 ml of Ethidium bromide in buffer (pH 8.0) for 1 hour at 75 V. The band was visualized with a UV source, and a Polaroid picture was taken. For Southern blotting the gel was denatured in 0.5 M NaOH for 30 minutes and neutralized in 1 M Tris-HCl (pH 8) for 30 minutes. DNA was transferred from the gel to a Nytran membrane using 10 x SSC buffer. After transfer, UV cross-linking was performed. The Nytran membrane was washed with 6 x SSC and 0.2% (w/v) SDS and membranes were prehybridized for 24 hours at 50°C with hybridization buffer consisting of 6 x SSC, 0.2% SDS, 1x Denhardt's blocking solution and 100 µg/ml salmon sperm. Membranes were then hybridized with 32P-labeled UNI- or SB3 probe (10⁷ cpm per bag) in 10 ml hybridization buffer (5 x SSC, 0.2% SDS, 1x Denhardt's blocking solution, 100 µg/ml salmon sperm) for 48 hours at 50°C. After hybridization, the membranes were washed once with 6 x SSC, 0.1% SDS, followed by two washes of 2 x SSC, 0.1% SDS at 42°C for 20 minutes. The membranes were dried and exposed to autoradiograph film for 7 days at -70°C.

Signs/Symptoms	% of Patients
Chronic fatigue	93
Depression	91
Paraesthesia	86
Joint pain, reduced mobility	84
Vision problems, light sensitivity	84
Cognitive problems	84
Muscle spasms, burning muscles	81
Dizziness, balance disturbance	79
Stuttering, difficulty speaking	78
Breathing problems	76
Flatulence, bloating	76
Headache	74
Lack of bladder control; frequent urination	71
Chemical sensitivities	71
Stomach cramps or pain	71
Sinus pain, nasal congestion	69
Changed alimentation	67
Loss of libido, impotence	66
Sore throat	62
Skin rashes	62
Nausea, vomiting, regurgitation	62
Tinnitus, hearing loss	62
Cardiac problems	59
Frequent coughing, thick saliva clearing	59
Night sweats	57
Diarrhea	57
Chest pain or pressure	57
Frequent infections	55
Frequent sores, yeast infection	55
Allergies	53
Dry or itchy eyes	52
Menstrual or genital pain	50

Table 2. Major signs and symptoms of CFS/FMS patients in the study

Results

Illness survey forms were analyzed to determine the most common signs and symptoms at the time when the blood was drawn from patients. The intensity of signs and symptoms prior to and after onset of illness was recorded on a 10-point rank scale (0-10, extreme). The data were arranged by 38 different signs and symptoms and were considered positive if the value after onset of illness was three or more points higher than prior to illness. Since the signs and symptoms of CFS and FMS patients completely overlapped, these data were considered together. The data in Table 2 indicate that patients diagnosed with CFS or FMS had complex signs and symptoms that were similar to those reported previously for GWI.¹

Patients' blood was analyzed for the presence of mycoplasmal infections in blood leukocytes. Positive PCR results were confirmed if the PCR product was 717 base pairs in size using the genus-specific primers (or 850 base pairs for *M. fermentans* specific primers) along with a positive control of the same size in the same gel, and if a visible band obtained after hybridization with the internal probe. Distilled water and buffers were also used as negative controls, and these showed no amplification product. The sensitivity and specificity of the PCR method were determined by examining serial dilutions of purified DNA of *M. fermentans*, *M. pneumoniae*, *M. penetrans*, *M. hominis*, and *M. genitalium*. Amounts as low as 1 fg of purified DNA were detectable for all species using the genus primers. The amplification with GPO-1 and MGSO produced the expected fragment size in all tested species, which was confirmed by hybridization with the inner UNI-probe.

Mycoplasma tests were performed on all patients as described above either from Chelex-purified DNA or DNA prepared from whole blood using the Promega kit. The targeted *Mycoplasma* spp. sequence was amplified from DNA extracted from the peripheral blood of 83/132 patients (62.9%). No specific sequence could be detected in 49

patients (37.1 %). In 32 healthy subjects positive results for *Mycoplasma spp.* were obtained in 3 samples (-9%). The difference between patient and control groups was significant ($p < 0.001$). The tests for *M. fermentans* using the primer set SB1 and SB2 yielded specific PCR products with the expected size and confirmation by hybridization using the inner SB3 probe in 50.0% of CFS/FMS patients compared to none in the 32 control subjects. The differences between positive and negative patients and between positive patients and control subjects were significant ($p < 0.001$). The ratio between positive and negative patients was comparable in female and male both for the rest of *Mycoplasma spp.* (60.7 % vs. 70.0 % positive) and *M. fermentans* (48.9% vs. 52.6% positive).

Two different methods for preparation of DNA from blood were used to evaluate the influence of DNA preparation on PCR sensitivity and specificity. We compared the Chelex and the Promega kit preparation methods, and our results demonstrated that DNA prepared by Chelex allowed better recovery than the DNA prepared by the Promega kit. In some samples positive results from DNA prepared by Chelex were negative when DNA was prepared with the Promega kit. Additionally, we studied the possible interference of DNA by inhibition of PCR. There was no inhibition of PCR product formation by DNA prepared by Chelex method or Promega kit. We also examined the effects of temperature and time of blood shipment on the results. Storage at 0-4°C resulted in reproducible assay results, whereas storage at room temperature resulted in loss of PCR signal over time. Within 1-2 days at room temperature, some of the positive samples reverted to negative results. Also, blood drawn in tubes (blue-top) containing citrate and kept at 0-4°C before the assay yielded better results than other anticoagulants.

Discussion

Certain species of mycoplasmas are associated with human diseases, such as an acute fatal illness found with certain *Mycoplasma fermentans* infections.¹⁵ In addition, *M. fermentans* can cause renal

and CNS complications in patients with AIDS.¹⁶ *M. fermentans* (incognitus strain) was shown in recent studies to be an unusually invasive mycoplasma found within respiratory epithelial cells.¹⁷ Other species of mycoplasmas are also associated with human illnesses: *M. pneumoniae* is a common cause of upper respiratory infection in humans,¹⁸ *M. hominis* infections were first found in patients with hypogammaglobulinemia, *Ureaplasma urealyticum*, *M. pneumoniae*, and *M. salivarium* have been localized in joint tissues of patients with rheumatoid diseases,¹⁹ *M. hominis* and *U. urealyticum* infections are common in patients following organ transplantation^{20, 21} and immunosuppressive chemotherapy,²² and *M. genitalium* was first isolated from the urogenital tracts of patients with nongonococcal urethritis.^{23, 24} Although mycoplasmas can exist in the oral cavity and gut as normal flora, when they penetrate into the blood and tissues, they can cause acute or chronic illnesses. These cell-penetrating species, such as *M. penetrans*, *M. fermentans*, and *M. pirum* among others, can cause complex systemic signs and symptoms.¹⁰ They can also have specific or nonspecific stimulatory or suppressive effects on the immune system, and they can induce cytokine secretion.²⁵ Mycoplasmas are very effective at evading the immune system, and synergism with other infectious agents can occur.¹⁰

We have begun to examine patients with chronic illnesses for the presence of systemic mycoplasmal infections. For example, our studies on GWI showed that 45% of Gulf War veterans with chronic signs and symptoms similar to CFS and FMS are positive for *M. fermentans* infections in their blood leukocytes.^{26, 27} The most common complaints of these veterans are chronic fatigue, muscle and joint pain, and other signs and symptoms which they share with patients diagnosed with CFS and FMS.¹ Chronic fatigue is reported by 20% of all patients seeking medical care^{28, 29} and is associated with many well-known conditions.³⁰ Although fatigue is an important secondary condition in several chronic illnesses, CFS and FMS are distinguishable as separate syndromes based

on established clinical criteria.^{31, 32}

The complex signs and symptoms found in CFS, FMS, and GWI patients could be due to system-wide or systemic chronic infections that can penetrate various tissues and organs, including the central and peripheral nervous systems.³ When such infections occur, they can cause the complex signs and symptoms seen in CFS, FMS, and GWI, including immune dysfunction. Interestingly, as chronic illnesses, such as GWI (and in some cases CFS and FMS) progress, there are a number of accompanying clinical problems, particularly autoimmune problems. These include in some patients the signs and symptoms of multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS or Lewy Gehrige's Disease), lupus, Graves' disease, arthritis, and other complex autoimmune diseases. Such usually rare autoimmune responses are consistent with certain chronic infections, such as mycoplasmal infections that penetrate into nerve cells, synovial cells, and other cell types. We speculate that these autoimmune signs and symptoms are caused when intracellular pathogens, such as mycoplasmas, escape from cellular compartments and incorporate into their own structures pieces of host cell membranes that contain important host membrane antigens that can trigger autoimmune responses. Alternatively, the antigens on the mycoplasma cell surface may directly stimulate an autoimmune response (Dr. J. Baseman, personal communication). Thus patients with such infections may be responding to microorganism antigens as well as their own membrane antigens, producing unusual autoimmune signs and symptoms.

Treatment Suggestions

Once mycoplasmal infections have been identified in the white blood cell fractions of subsets of CFS, FMS, GWI, and other patients, they can be treated. If such infections are important in these disorders, then appropriate treatment with antibiotics should result in improvement and even recovery. This is what has been found.^{3, 26, 27} The recommended treatments for mycoplasmal blood infections require long-term

antibiotic therapy,³³ usually multiple 6-week cycles of doxycycline (200-300 mg/day), ciprofloxacin or Cipro[®] (1,500 mg/day), azithromycin or Zithromax[®] (500 mg/day) or clarithromycin or Biaxin[®] (750-1,000 mg/day).³⁴ Multiple cycles are required, because few patients recover after only a few cycles,^{26,27} possibly because of the intracellular locations of mycoplasmas like *M. fermentans* and *M. penetrans*, and the slow-growing nature of these microorganisms. For example, 87 GWI patients that tested positive for mycoplasmal infections were treated with antibiotics. All patients relapsed after the first 6-week cycle of therapy, but after up to 6 cycles of therapy 69/87 patients recovered and returned to active duty.^{26,27} The clinical responses that are seen are not due to placebo effects, because administration of some antibiotics, such as penicillins, resulted in patients becoming more or less symptomatic, and they are not due to immunosuppressive effects that can occur with some of the recommended antibiotics. Interestingly, CFS, FMS, and GWI patients that slowly recover after several cycles of antibiotics are generally less environmentally sensitive, suggesting that their immune systems may be returning to pre-illness states. If such patients had illnesses that were caused by psychological or psychiatric problems or solely by chemical exposures, they should not respond to the recommended antibiotics and slowly recover. In addition, if such treatments were just reducing autoimmune responses, then patients should relapse after the treatments are discontinued.

In addition to antibiotics, patients with CFS, FMS, or GWI usually have nutritional and vitamin deficiencies that must be corrected.³⁴ For example, these patients are often depleted in vitamins B, C, and E, and certain minerals. Unfortunately, patients with these chronic illnesses often have poor absorption. Therefore, high doses of some vitamins must be used, and others, such as vitamin B complex, cannot be easily absorbed by the gut, so sublingual natural B-complex vitamins in small capsules or liquids should be used instead of oral capsules. General vitamins plus extra

C, E, CoQ-10, beta-carotene, folic acid, bioflavoids, and biotin appear to work best. L-cysteine, L-tyrosine, L-carnitine and malic acid can also be useful. Certain minerals are also often depleted in GWI/CFS/FMS patients, such as zinc, magnesium, chromium, and selenium. Antibiotics that deplete normal gut bacteria can result in over-growth of less desirable flora, so *Lactobacillus acidophilus* supplementation is recommended. In addition, a number of natural remedies that boost the immune system, such as herbal teas, whole lemon/olive extract drink, or an extract of olive leaves with antioxidants, milk proteins, among others, are available and are potentially useful, especially during or after antibiotic therapy has been completed.³⁴ Although these products appear to help some patients, their clinical effectiveness in GWI/CFS/FMS patients has not been carefully evaluated. They appear to be useful during therapy to boost the immune system or after antibiotic therapy in a maintenance program to prevent relapse of illness.

Chronic infections, such as those caused by mycoplasmas, are likely to be an important underlying cause of morbidity in a rather large subset of CFS, FMS, GWI, and arthritis patients.^{3, 26, 27} Before systemic mycoplasmal infections can be considered important in causing disease, certain criteria must be fulfilled:³⁵ [a] The incidence rate among diseased patients must be higher than in those without disease. This has been found for *M. fermentans*. Although this mycoplasma has been found in asymptomatic adults, the incidence is low, usually only a few percent compared to about one-half of Gulf War Illness patients.^{26,27} [b] More of the mycoplasma must be recoverable from diseased patients than from those without disease. This has been found.^{26,27} [c] An antibody response should be found at higher frequency in diseased patients than in those without disease. This has been seen, but usually not until the disease has progressed. According to I.o et al.^{15, 36, 37} *M. fermentans* hides inside cells and does not elicit a strong immune response until near death. [d] A clinical response should be accompanied by elimination of the

mycoplasma. This is exactly what has been found.^{26, 27} [e] Clinical responses should be differential, depending on the type of antibiotic. Only antibiotics that are effective against the pathogenic mycoplasmas result in recovery, and some antibiotics, such as penicillins, can worsen the condition.^{26, 27} [f] The mycoplasma must cause a similar disease in susceptible animals. The best description comes from Lo et al.,²⁶ where injection of *M. fermentans* into monkeys resulted in development of a fulminant disease that leads to death. These animals display initially many chronic signs and symptoms. [g] The mycoplasma must cause a similar disease when administered to volunteers. This has not been done, because of ethical considerations. [h] A specific anti-mycoplasma antibody reagent or immunization protects against disease. This has not yet been done to our knowledge. Therefore, six out of eight of the above criteria have been fulfilled, at least in cases of *M. fermentans*.

We have proposed that chronic infections are an appropriate explanation for the morbidity seen in a rather large subset of CFS, FMS, GWI, and some arthritis patients, but certainly not every patient will have this as a diagnostic explanation or have the same types of chronic infections.³ Some patients may have chemical exposures or other environmental problems as the underlying reason for their chronic signs and symptoms. In these patients, chronic infections may be opportunistic. In others, somatoform disorders or illnesses caused by psychological or psychiatric problems may indeed be important. However, in these patients antibiotics should have no effect whatsoever, and they should not recover on antibiotic therapies. The identification of specific infectious agents in the blood of chronically ill patients may allow many patients with CFS, FMS, GWI, or arthritis to obtain more specific diagnoses and effective treatments for their illnesses.

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