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Role of Mycoplasmal Infections in Fatigue Illnesses: Chronic Fatigue and Fibromyalgia Syndromes, Gulf War Illness and Rheumatoid Arthritis

G. L. Nicolson,* PhD, M. Y. Nasralla,* PhD, A. R. Franco,‡ MD, K. De Meirleir,+ MD, N. L. Nicolson,* PhD, R. Ngwenya,† MD and J. Haier,* MD, PhD

**The Institute for Molecular Medicine, Huntington Beach, CA 92647 USA,*
‡Arthritis Center of Riverside, Riverside, CA 92501 USA,
+Internal Medicine, Free University of Brussels, 1090 Brussels, Belgium and
†James Mobb Immune Enhancement, Harare, Zimbabwe

Address correspondence to: Prof. Garth L. Nicolson, The Institute for Molecular Medicine,, Huntington Beach, CA 92647 (Tel: 949-715-5978; Email: gnicolson@immed.org)

SUMMARY. Bacterial and viral infections are associated with several fatigue illnesses, including Chronic Fatigue Syndrome (CFS), Fibromyalgia Syndrome (FMS), Gulf War Illnesses (GWI) and Rheumatoid Arthritis (RA), as causative agents, cofactors or opportunistic infections. We and others have looked for the presence of invasive pathogenic mycoplasmal infections in patients with CFS, FMS, GWI and RA and have found significantly more mycoplasmal infections in CFS, FMS, GWI and RA patients than in healthy controls. Most patients had multiple mycoplasmal infections (more than one species). Patients with chronic fatigue as a major sign often have different clinical diagnoses but display overlapping signs/symptoms similar to many of those found in CFS/FMS. When a chronic fatigue illness, such as GWI, spreads to immediate family members, they present with similar signs/symptoms and mycoplasmal infections. CFS/FMS/GWI patients with mycoplasmal infections generally respond to particular antibiotics (doxycycline, minocycline, ciprofloxacin, azithromycin and clarithromycin), and their long-term administration plus nutritional support, immune enhancement and other supplements appear to be necessary for recovery. Examination of the efficacy of antibiotics in recovery of chronic illness patients reveals that the majority of mycoplasma-positive patients respond and many eventually recover. Other chronic infections, such as viral infections, may also be involved in various chronic fatigue illnesses with or without mycoplasmal and other bacterial infections, and these multiple infections could be important in causing patient morbidity and difficulties in treating these illnesses.

INTRODUCTION

Many debilitating chronic illnesses are characterized by the presence of chronic fatigue (1). Indeed, chronic fatigue is the most commonly reported medical complaint of all patients seeking medical care (2). However, the fatigue syndromes, such as Chronic Fatigue Syndrome (CFS, sometimes called Myalgic Encephalomyelitis), Fibromyalgia Syndrome (FMS) and Gulf War Illnesses (GWI) are distinguishable as separate syndromes that have muscle and overall fatigue as major characteristics, among many other multiorgan signs and symptoms (3-6), including immune system abnormalities (7). These syndromes have complex chronic signs and symptoms, including muscle pain, chronic fatigue, headaches, memory loss, nausea, gastrointestinal problems, joint pain, vision problems, breathing problems, depression, low grade fevers, skin disorders, tissue swelling, chemical sensitivities, among others. Because of the complex nature of these illnesses, many patients are often diagnosed with multiple syndromes. Unfortunately, due to the lack of definitive laboratory or clinical tests that could identify the cause(s) of these illnesses, many patients are diagnosed with somatoforensic disorders. Often these patients have cognitive problems, such as short term memory loss, difficulty concentrating and psychological problems, that in the absence of contrary laboratory tests can result in practitioners diagnosing somatoform disorders rather than organic problems (6). Stress is often portrayed as an important factor in these disorders, and in fact stress can have many effects on the hormonal and immune systems that could be detrimental in virtually any chronic illness (8).

There is growing awareness that the chronic fatigue illnesses can have an infectious nature that is either responsible (causative) for the illness, a cofactor for the illness or appears as an opportunistic infection(s) responsible for aggravating patient morbidity (9). There are several reasons for this notion (10), including the nonrandom or clustered appearance of the illness, often in immediate family members, and the course of the illness and its response to therapies based on treatment of infectious agents. Since chronic illnesses are often complex, involving multiple, nonspecific, overlapping signs and symptoms, they are difficult to diagnose and even more difficult to treat (9). Most chronic fatigue illnesses do not have effective therapies, and these patients rarely recover from their condition (11), causing in some cases catastrophic economic problems.

SIGNS AND SYMPTOMS ANALYSIS

Some chronic illnesses, such as Rheumatoid Arthritis (RA), are well established in their clinical profile (12), whereas others, such as CFS, FMS and GWI, have rather nonspecific but similar overlapping, multi-organ signs and symptoms. A major difference between these illnesses appears to be in the severity of specific signs and symptoms. For example, CFS patients most often complain of chronic fatigue and joint pain, stiffness and soreness, whereas FMS patients have as their most major

complaint muscle and overall pain, soreness and weakness. For the most part, the clinical profiles of these illnesses are similar, and this can be easily seen when the signs and symptoms of CFS, FMS, and GWI patients are compared (Figures 1A and 1B). Thus although chronic illnesses are considered to be complex, they do display certain similarities, suggesting that these illnesses are related and not completely separate syndromes (6, 9). In addition, these chronic illness patients often show increased sensitivities to various environmental irritants and chemicals and enhanced allergic responses.

Although chronic fatigue illnesses have been known in the literature for many years, most patients with CFS, FMS, GWI and in some cases RA have had few treatment options. This may have been due to the imprecise nature of their diagnoses, which are usually based primarily on clinical observations rather than laboratory tests, and a lack of understanding about the underlying causes of these illnesses or the factors responsible for patient morbidity. Chronic illnesses could have different initial causes or triggers but similar secondary events, such as opportunistic viral and/or bacterial infections that cause significant morbidity (9, 10). With time these secondary events may progress to be the most important in determining overall signs and symptoms and treatment options.

The data presented in Figures 1A and 1B show the most common signs and symptoms found in CFS, FMS and GWI patients and symptomatic GWI family members after the onset of illness. In these figures the data for FMS and CFS have been combined, because previous studies indicated that with the exception of the extent of muscle pain and tenderness, there were essentially no major differences in patient signs (6, 10). Illness Survey Forms were analyzed to determine the most common signs and symptoms at the time when blood was drawn from patients. The intensity of approximately 120 patient 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 into 29 different signs and symptoms groups and were considered positive if the average value after onset of illness was two or more points higher than prior to the onset of illness. CFS/FMS patients had complex signs and symptoms that were similar to those reported for GWI, and the presence of rheumatoid signs and symptoms in each of these disorders indicates that there are also similarities to RA (12, 13). Moreover, it is not unusual to find immediate family members who slowly displayed similar signs and symptoms following the return home of veterans with GWI, suggesting that these civilian patients contracted their illnesses from chronically ill family members with GWI (10). Examination of the increase in signs and symptoms of GWI family members that now have a chronic illness similar to GWI indicates that they have signs and symptoms similar to civilian CFS/FMS patients. The main difference between veterans with GWI and their family members was in the greater breadth and severity of signs and symptoms found in GWI patients than in their symptomatic family members. Since Gulf War veterans were presumably exposed to many more environmental toxic agents compared to nondeployed family members, this is not unexpected. When the signs and symptoms of CFS/FMS/GWI were compared to patients with other chronic illnesses that did not show evidence of chronic infections, there were also notable differences. For example, in contrast to CFS/FMS/GWI patients, this latter chronic illness patient group did not show differences in gastrointestinal problems, coagulation problems, hair loss and scalp problems, night sweats and intermittent fevers (Figures 1A and 1B). This suggests that CFS/FMS/GWI patients with chronic infections may have some unique clinical problems not commonly found in other chronic illness patients.

CHRONIC INFECTIONS IN CFS, FMS AND GWI

As stated above, there exists indirect evidence suggesting the infectious nature in at least certain subsets of chronic illness patients. We have been particularly interested in the association of specific chronic infectious agents with CFS, FMS, GWI and RA, because these microorganisms can potentially cause most or essentially all of the signs and symptoms found in these patients (6, 9, 10, 13). One type of "stealth" infection that could fulfill the criteria of association with a wide range of signs and symptoms are certain microorganisms of the class Mollicutes. This is a class of small bacteria, lacking cell walls, and some species are capable of invading several types of human cells and tissues and are associated with a wide variety of human diseases (14).

We and others have examined the presence of mycoplasmal blood infections in CFS, FMS, GWI and RA patients. The clinical diagnosis of these disorders was obtained from referring physicians according to the patients' major signs and symptoms. Blood was collected, shipped over night at 4°C and processed immediately for Nucleoprotein Gene Tracking (NPGT) after isolation of blood leukocyte nuclei (15, 16) or Forensic Polymerase Chain Reaction (FPCR) after purification of blood leukocyte DNA using a Chelex procedure (6, 13, 17). We used FPCR to determine the species of mycoplasmal infections. The sensitivity and specificity of the PCR methods were determined by examining serial dilutions of purified DNA of *M. fermentans*, *M. pneumoniae, M. penetrans* and *M. hominis*. Amounts as low as 10 fg of purified DNA were detectable. The amplification with genus primers produced the expected fragment size in all tested species, which was confirmed by hybridization with an inner probe 18). Others have also used PCR with single (19, 20) or multiple (21, 22) sets of PCR primers. Using NPGT to analyze the blood leukocytes from GWI patients we found that 91/200 (~45%) were positive for mycoplasmal infections. In contrast, in nondeployed, healthy adults the incidence of mycoplasmal infections was 4/62 (~6%) (15, 16, Table 1). Similarly, using PCR 55% of GWI patients were positive for *Mycoplasma spp*. and 36% were found to have *M. fermentans* infections (22, Table 1). The slight difference in percentage of positive patients is probably due to the differences in sensitivities of these two methods. In comparison, using FPCR or PCR 52-63% of CFS/FMS patients (n~1,000) had mycoplasmal infections (6, 19-23), whereas only 9-15% of controls (n~450) tested positive (Table 1).

Patients with CFS/FMS often have multiple

mycoplasmal infections and probably other chronic infections as well. When we examined CFS/FMS patients for the presence of *M. fermentans*, *M. pneumoniae, M. penetrans, M. hominis* infections, multiple infections were found in over one-half of 93 patients (17, Table 1). CFS/FMS patients had double (>30%) or triple (>20%) mycoplasmal infections, but only when one of the species was *M. fermentans* or *M. pneumoniae* (17). We also found higher score values for increases in the severity of signs and symptoms in CFS/FMS patients with multiple infections. CFS/FMS patients with multiple mycoplasmal infections generally had a longer history of illness, suggesting that patients may have contracted additional infections during their illness (17).

CHRONIC INFECTIONS IN RA

The causes of rheumatic diseases are not known, but RA and other autoimmune diseases could be triggered or more likely exacerbated by infectious agents (24). In some animal species infection by certain species of mycoplasmas can result in remarkable clinical and pathological similarities to RA and other rheumatoid diseases. Aerobic and anaerobic intestinal bacteria, viruses and mycoplasmas have been proposed as important agents in RA (24-29), and there has been increasing evidence that mycoplasmas may play a role in the initiation or progression of RA (13, 29-31). Mycoplasmas have been proposed to interact nonspecifically with B-lymphocytes, resulting in modulation of immunity, autoimmune reactions and promotion of rheumatic diseases (30), and mycoplasmas have been found in the joint tissues of patients with rheumatic diseases, suggesting their pathogenic involvement (28).

When Haier et al. (13) and Vojdani and Franco (22) examined RA patients' blood leukocytes for the presence of mycoplasmas, it was found that approximately one-half were infected with various species of mycoplasmas. The most common species found was *M. fermentans*, followed by *M. pneumoniae* and *M. hominis* and finally *M. penetrans* (13, 22). Similar to what we reported in CFS/FMS patients (17), there was a high percentage of multiple mycoplasmal infections in RA patients when one of the species was *M. fermentans* (13).

The precise role of mycoplasmas in RA and other rheumatic inflammatory diseases is under investigation; however, mycoplasmas could be important cofactors in the development of inflammatory responses in rheumatic diseases and for progression of RA. As an example of the possible role of mycoplasmas in rheumatic diseases, *M. arthritidis* infections in animals can trigger and exacerbate autoimmune arthritis (31, 32). This mycoplasma can also suppress T-cells and release substances that act on polymorphonuclear granulocytes, such as oxygen radicals, chemotactic factors and other substances (32). Mycoplasmal infections can increase proinflammatory cytokines, such as Interleukin-1, -2 and –6 (33), suggesting that they are involved in the development and possibly progression of rheumatic diseases such as RA.

A variety of microorganisms have been under investigation as cofactors or causative agents in rheumatic diseases (9, 24, 25). The discovery of EB virus (26) and cytomegalovirus (27) in the cells of the synovial lining in RA patients suggested their involvement in RA, possibly as a cofactor. There are a number of bacteria and viruses that are candidates in the induction or progression of RA or its progression (9, 24). In support of a bacterial involvement in RA, antibiotics like minocycline can alleviate the clinical signs and symptoms of RA (34). This and similar drugs are likely suppressing infections of sensitive microorganisms like mycoplasmas in rheumatic diseases, although they could also have immunoregulatory effects.

MYCOPLASMAL INFECTIONS IN OTHER DISEASES

Mycoplasmas have been associated with the progression of autoimmune and immunosuppressive diseases, such as HIV-AIDS (35). In some cases these infections have been associated with terminal human diseases, such as an acute fatal illness found with *M. fermentans* infections in non-AIDS patients (36). Importantly, mycoplasmal infections are now thought to be a major source of morbidity in HIV-AIDS (37). On this basis, Blanchard and Montagnier (37) have proposed that certain mycoplasmas like *M. fermentans* are important cofactors in the progression of HIV-AIDS, accelerating disease progression and accounting, in part, for the increased susceptibility of AIDS patients to additional opportunistic infections. Since most studies on the incidence of mycoplasmal infections in HIV-AIDS patients have employed relatively insensitive tests, it is likely that the occurrence of mycoplasmal infections in HIV-AIDS is much greater than previously thought and may be associated with a rapid fatal course of the disease. In HIV-AIDS mycoplasmas like *M. fermentans* can cause renal and CNS complications (38), and mycoplasmas have been found in various tissues, such as the respiratory epithelial cells of AIDS patients (39). Other species of mycoplasmas have been found in AIDS patients where they have also been associated with disease progression (40). In addition to immune suppression, some of this increased pathogenecity may be the result of mycoplasma-induced host cell membrane damage from toxic oxygenated products released from intracellular mycoplasmas (41). Also, mycoplasmas may regulate the HIV-1 virus, such as HIV-LTR-dependent gene expression (42), suggesting that mycoplasmas may play an important regulatory role in HIV expression.

There is some preliminary evidence that mycoplasmal infections are associated with various autoimmune diseases. In some mycoplasma-positive GWI cases the signs and symptoms of Multiple Sclerosis (MS), Amyotrophic Lateral Sclerosis (ALS), Lupus, Graves' Disease and other complex autoimmune diseases have been seen. 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. The autoimmune signs and symptoms could be the result of intracellular pathogens, such as mycoplasmas, escaping from cellular compartments and incorporating into their own structures pieces of host cell membranes that contain important host antigens that can trigger autoimmune responses. Alternatively, mycoplasma surface components, sometimes called 'superantigens,' may

directly stimulate autoimmune responses (43). Perhaps the most important event, the molecular mimicry of host antigens by mycoplasma surface components, may explain, in part, their ability to stimulate autoimmune responses (44).

Asthma, airway inflammation, chronic pneumonia and other respiratory diseases are known to be associated with mycoplasmal infections (45). For example, *M. pneumoniae* is a common cause of upper respiratory infections (46), and severe Asthma is frequently associated with mycoplasmal infections (47).

Cardiopathies can be caused by chronic infections, resulting in myocarditis, endocarditis, pericarditis and others. These are often due to chronic infections by *Mycoplasma spp*. (48), *Chlamydia spp*. (49) and possibly other infectious agents.

Mycoplasmal infections are also associated with a variety of illnesses, such as *M. hominis* infections in patients with hypogammaglobulinemia (29), and *M. genitalium* with nongonococcal urethritis (50). Mycoplasmas can exist in the oral cavity and gut as normal flora, but when they penetrate into the blood and tissues, they may be able to cause or promote a variety of acute or chronic illnesses. These cell-penetrating species, such as *M. penetrans*, *M. fermentans*, *M. hominis* and *M. pirum,* among others, can cause infections that result in complex systemic signs and symptoms. Mycoplasmal infections can also cause synergism with other infectious agents. Similar types of chronic infections caused by *Chlamydia, Brucella, Coxiella* or *Borriela* may also be present either as single agents or as complex, multiple infections in many chronic illnesses (9).

MYCOPLASMA TREATMENT

Although mycoplasmal infections are often misdiagnosed or inappropriately treated (45), they can be successfully treated using antibiotics and nutritional support (51, 52). Appropriate treatment with antibiotics should result in patient improvement and even recovery, and this has been seen in GWI, CFS, FMS and RA patients (Table 2). The recommended treatments for mycoplasmal blood infections require long-term antibiotic therapy, usually 12 months or more or multiple 6-week cycles of doxycycline (200-300 mg/day), ciprofloxacin (1,500 mg/day), azithromycin (500 mg/day) or clarithromycin (750-1,000 mg/day). Multiple cycles are required, because only a few patients recovered after a few cycles, possibly because of the intracellular locations of pathogenic mycoplasmas, the slow-growing nature of these microorganisms and their relative drug sensitivities. For example, of 87 GWI patients that tested positive for mycoplasmal infections, all patients relapsed after the first 6-week cycle of antibiotic therapy, but after up to 6-7 cycles of therapy 69/87 patients responded and eventually recovered and returned to active duty (15, 16, Table 2). Similarly, the majority of CFS/FMS patients who tested positive for mycoplasmal infections also responded to the antibiotic therapy (53, Table 2). Although these clinical studies were not placebo-controlled, blinded studies, double-blind, placebo-controlled antibiotic trials using minocycline versus placebo treatment of RA patients indicates that this antibiotic is clinically effective in RA (34, 54, Table 2).

The clinical responses that were seen in mycoplasma-positive chronic illness patients were not due to placebo effects, because administration of some antibiotics, such as penicillins, resulted in patients becoming more not less symptomatic, and they were not due to immunosuppressive effects that can occur with some of the recommended antibiotics (6, 9, 16). 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 these illnesses were caused by psychological problems or solely by environmental exposures rather than infections, 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, and this is not what has been found. CFS, FMS, RA or GWI patients also have nutritional and vitamin deficiencies that must be corrected (52, 53). In addition, a fully functional immune system may be essential to overcoming these infections, and supplements and immune enhancers appear to be effective in helping patients recover (52, 53).

Although we have proposed that chronic infections are an appropriate explanation for the morbidity seen in a rather large subset of CFS, FMS, GWI and RA patients, and in a variety of other chronic illnesses, not every patient will have this as a diagnostic explanation or have the same types of chronic infections. Additional research will be necessary to clarify the role of multiple infections in chronic diseases, but these patients could benefit from appropriate antibiotic and neutraceutical therapies that alleviate morbidity.

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FIGURE LEGENDS

Figures 1A and 1B (same legend). Incidence of increase in severity of signs and symptoms in 260 chronic illness patients. Severity of illness was scored using 117 signs and symptoms on a 10-point scale (0, none; 10 extreme) prior to and after the onset of illness. Scores were placed into 29 categories containing 3-9 signs/symptoms and were recorded as the sum of differences between values before and after onset of illness divided by the number of questions in the category. Changes in score values of 2 or more points were considered relevant. Patient groups were CFS/FMS (■), GWI (❒) GWI symptomatic family members (❒) and chronic illness patients not in the above groups that did not show evidence of chronic bacterial infection (❒). Asterisk (*) indicates score = 0.

Table 1. Summary of mycoplasmal infections in patient groups and controls.

	Percentage of Subjects Positive for Mycoplasmal Infections									
Reference	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)	(J)
Method	NPGT	NPGT	FPCR	FPCR	FPCR	PCR	PCR	PCR	PCR	FPCR
Gulf War										
Illness *(n)*	(30)	(170)							(60)	
M. spp.	47	45							55	
M. fermentans	31	30							36	
M. pneumoniae										
M. hominis									5	
M. penetrans									3	
			(132)	(91)*						
CFS/FMS *(n)*			63	100*		(200)	(50)	(100)	(140)	(565)
M. spp.			50	48*			54	54	52-54	53
M. fermentans				59*		67	36	36	32-35	25
M. pneumoniae				31*						
M. hominis				19*					8-9	
M. penetrans									4-6	
					(28)					
Rheumatoid										
Arthritis *(n)*					53				(60)	
M. spp.					29				49	
M. fermentans					18				23	
M. pneumoniae					21					
M. hominis					4				11	
M. penetrans									7	
			(32)	(33)	(32)					
Controls *(n)*	(14)	(41)	9		9	(77)	(50)	(100)	(160)	(71)
M. spp.	0	4.8	0	0	0	16.8	14	15	15	9.9
M. fermentans	0			0	0		8	8	8	2.8
M. pneumoniae				0	0					
M. hominis				0	0			3	3	
M. penetrans								2	2	

Method: NPGT, Nucleoprotein Gene Tracking; FPCR, Forensic Polymerase Chain Reaction; PCR, Polymerase Chain Reaction. **References:** A, Nicolson and Nicolson, 1996 (15); B, Nicolson et al., 1998 (16); C, Nicolson et al., 1998 (6); D, Nasralla et al., 1999 (17); E, Haier et al., 1999 (13); F, Huang et al., 1999 (20); G, Vojdani et al., 1998 (19); H, Choppa et al., 1998 (21); I, Vojdani and Franco, 1999 (22); J, Nasralla et al., 1999 (23). *Only patients that were positive for *M. spp.* were enrolled in the study.

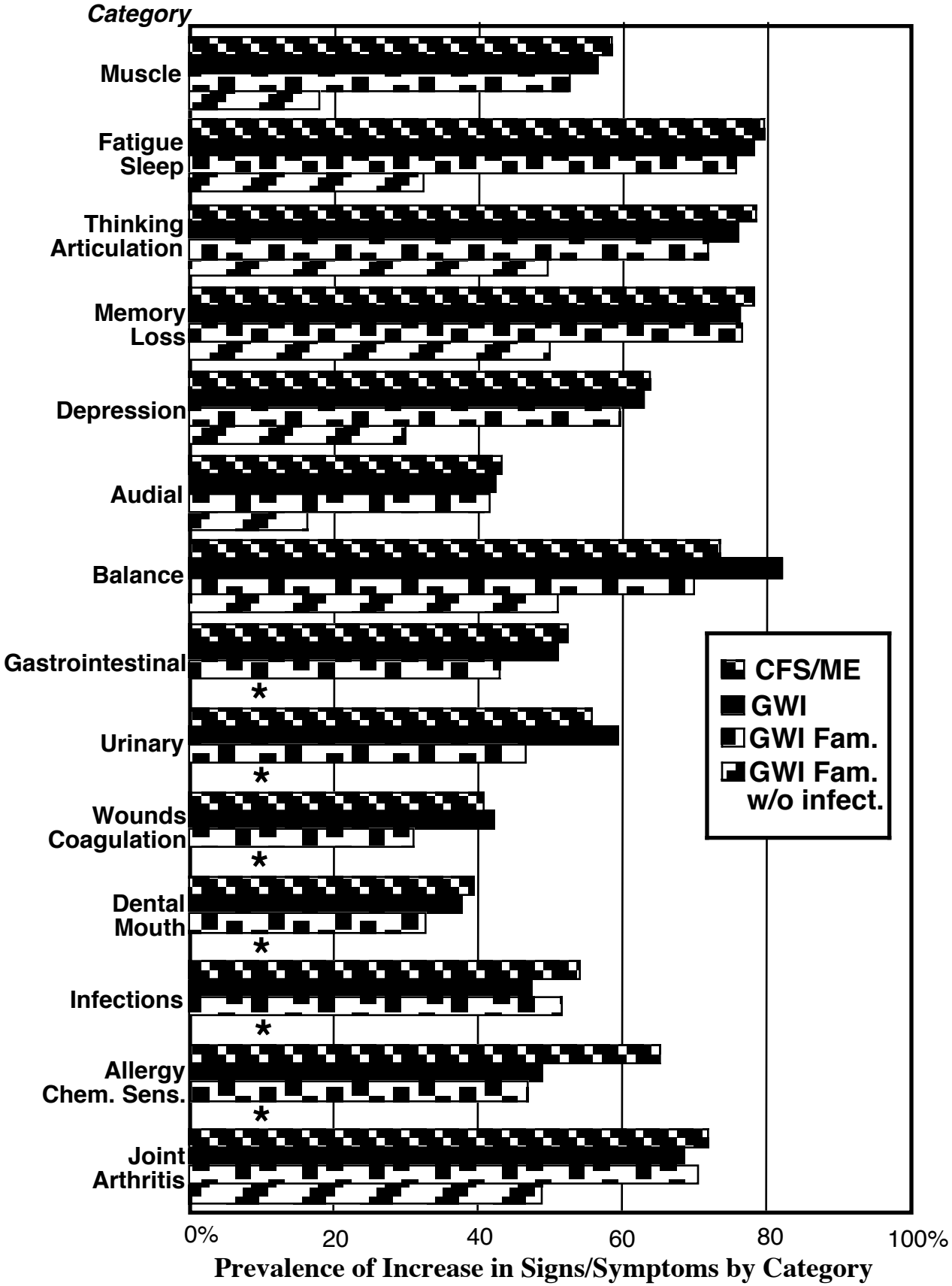
Table 2. Summary of chronic illness patients' antibiotic treatment results.

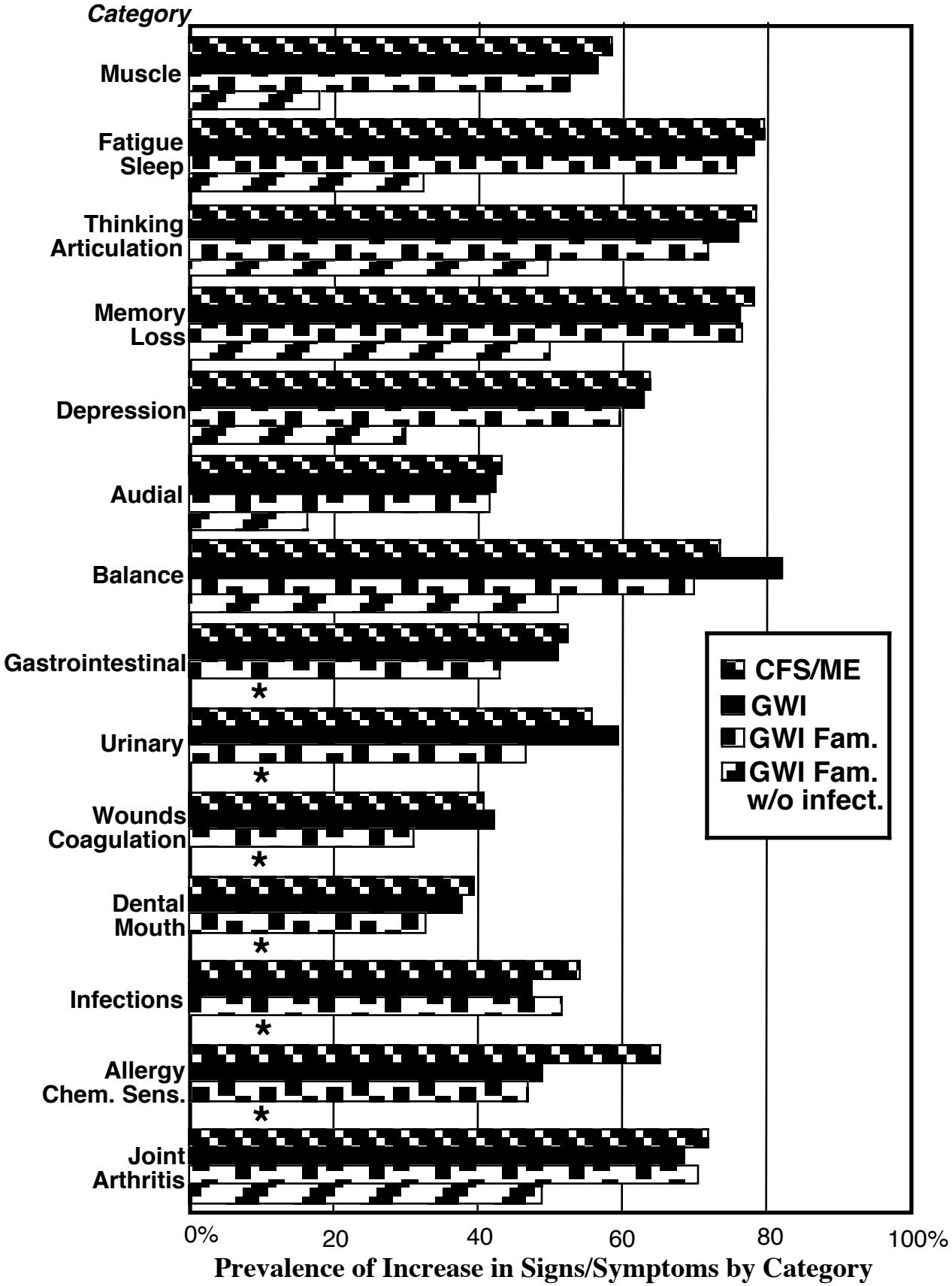
	Percent patients mycoplasma-positive or responding to therapy				
Reference	(A)	(B)	(C)	(D)	(E)
Gulf War Illness *(n)*	(30)	(170)			
Blinded, controlled study (Y/N)	(No)	(No)			
Mycoplasma-positive pts	47	46			
Clinical Response*	*ND*	*ND*			
Clinical Recovery*	78	80			
CFS/FMS *(n)*			(30)		
Blinded, controlled study (Y/N)			(No)		
Mycoplasma-positive pts			66		
Clinical Response*			80		
Clinical Recovery*			50		
Rheumatoid Arthritis *(n)*				(219)	(46)
Blinded, controlled study (Y/N)				(Yes)	(Yes)
Mycoplasma-positive pts				*ND*	*ND*
Clinical Response				54	50
Clinical Recovery				*ND*	40

References: A, Nicolson and Nicolson, 1996 (15); B, Nicolson et al., 1998 (16); C, Nicolson, 1999 (54); D, Tilley et al., 1995 (34); (E), O'Dell et al., 1999 (55). *, Data only for mycoplasma-positive patients; *ND*, not determined.

FIGURE LEGENDS

Figures 1A and 1B (same legend). Incidence of increase in severity of signs and symptoms in 260 chronic illness patients. Severity of illness was scored using 117 signs and symptoms on a 10-point scale (0, none; 10 extreme) prior to and after the onset of illness. Scores were placed into 29 categories containing 3-9 signs/symptoms and were recorded as the sum of differences between values before and after onset of illness divided by the number of questions in the category. Changes in score values of 2 or more points were considered relevant. Patient groups were CFS/FMS (■), GWI (❒) GWI symptomatic family members (❒) and chronic illness patients not in the above groups that did not show evidence of chronic bacterial infection (❒). Asterisk (*) indicates score = 0.


Category
Muscle
Fatigue
Sleep
Thinking
Articulation
Memory
Loss
Depression
Audial
Balance
Gastrointestinal
*
Urinary
*
Wounds
Coagulation
*
Dental
Mouth
*
Infections
*
Allergy
Chem. Sens.
*
Joint
Arthritis
CFS/ME
GWI
GWI Fam.
GWI Fam.
w/o infect.
0%
20
40
60
80
100%
Prevalence of Increase in Signs/Symptoms by Category


Category
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Chem. Sens.
*
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CFS/ME
GWI
GWI Fam.
GWI Fam. w/o infect.
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20
40
60
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100%
Prevalence of Increase in Signs/Symptoms by Category