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Article

Multiple Mycoplasmal Infections Detected in Blood of Patients with Chronic Fatigue Syndrome and/or Fibromyalgia Syndrome

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Abstract The aim of this study was to investigate the presence of different mycoplasmal species in blood samples from patients with chronic fatigue syndrome and/or fibromyalgia syndrome. Previously, more than 60% of patients with chronic fatigue syndrome/fibromyalgia syndrome were found to have mycoplasmal blood infections, such as *Mycoplasma fermentans* infection. In this study, patients with chronic fatigue syndrome/fibromyalgia syndrome were examined for multiple mycoplasmal infections in their blood. A total of 91 patients diagnosed with chronic fatigue syndrome/fibromyalgia syndrome and with a positive test for any mycoplasmal infection were investigated for the presence of *Mycoplasma fermentans*, *Mycoplasma pneumoniae*, *Mycoplasma hominis* and *Mycoplasma penetrans* in blood using forensic polymerase chain reaction. Among these mycoplasma-positive patients, infections were detected with *Mycoplasma pneumoniae* (54/91), *Mycoplasma fermentans* (44/91), *Mycoplasma hominis* (28/91) and *Mycoplasma penetrans* (18/91). Multiple mycoplasmal infections were found in 48 of 91 patients, with double infections being detected in 30.8% and triple infections in 22%, but only when one of the species was *Mycoplasma pneumoniae* or *Mycoplasma fermentans*. Patients infected with more than one mycoplasmal species generally had a longer history of illness, suggesting that they may have contracted additional mycoplasmal infections with time.

Introduction

Chronic fatigue is reported by 20% of all patients seeking medical care [1, 2]. Many well-known medical conditions are associated with chronic fatigue [3], and it is often an important secondary condition in many diagnoses. Although chronic fatigue is associated with many illnesses, chronic fatigue syndrome (CFS) and fibromyalgia syndrome (FMS) are distinguishable as separate syndromes based on established clinical criteria [4, 5]. They are characterized by their complex multiorgan chronic signs and symptoms, including muscle pain, chronic fatigue, headaches, memory loss, nausea, gastrointestinal problems, joint pain, and vision and breathing problems, among others. Many patients are diagnosed with both syndromes. Since physical and laboratory results do not usually identify pathogenic agents or other causes, these conditions are often considered somatoforensic disorders. However, in many cases family members of these patients gradually display similar signs and symptoms, suggesting an infectious explanation for the illnesses [6].

Using forensic polymerase chain reaction (PCR) for detection of *Mycoplasma* spp. and *Mycoplasma fermentans* in blood samples from 132 CFS/FMS patients, we previously found that 62.9% and 50% were positive for *Mycoplasma* spp. and *Mycoplasma fermentans* infection, respectively [7]. In healthy controls without clinical signs and symptoms, significantly fewer subjects were positive for *Mycoplasma* spp. (9.6%) or *Mycoplasma fermentans* (0%) infection [7]. We also found that more than 50% of patients with rheumatoid arthritis had mycoplasmal infections, and in 36% of these patients, multiple infections with more than one mycoplasmal species were detected [8]. The PCR tests

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that we used to identify mycoplasmal infections are very sensitive and highly specific. These tests are a dramatic improvement on the relatively insensitive serum antibody tests that are routinely used to assay for systemic mycoplasmal infection [9, 10].

Mycoplasmas are prokaryotes without cell walls of the class *Molecutes*. They are small, free-living, self-replicating organisms [11, 12]. Although mycoplasmas are commonly found in the oral cavity and as symbiotic gut flora, some species can cause acute and chronic illnesses when they penetrate into the blood vascular system and systemically colonize organs and tissues. For example, mycoplasmas such as *Mycoplasma penetrans*, *Mycoplasma fermentans* and *Mycoplasma pirum* can enter a variety of tissues and cells and cause systemic signs and symptoms. Mycoplasmas have also been shown to have a complex relationship with the immune system. They are very effective at evading host immune responses, and synergism with other infectious agents has been seen [13].

The difference between the incidence of infection with any species of *Mycoplasma* and the incidence of infection with *Mycoplasma fermentans* supports the hypothesis that some patients have infections with mycoplasmal species other than *Mycoplasma fermentans*. In the present study, we extended our examination of mycoplasmal infections to include mycoplasmal species other than *Mycoplasma fermentans*. We found the presence of multiple mycoplasmal infections in many patients suffering from CFS and/or FMS.

Patients and Methods

Patients and Specimens. Blood samples from 91 patients (67 female, 24 male) who were positive for mycoplasmal infections by PCR using the mycoplasma genus-specific primers and who were clinically diagnosed with CFS and/or FMS according to consensus criteria [4, 14, 15] were investigated for multiple mycoplasmal infections. The mean age of all patients was 43 ± 14 years, while the illness history averaged 145 ± 140 months. Voluntary healthy controls ($n = 32$) without the clinical signs and symptoms described for patients were selected from comparable geographical areas. They were chosen after a routine clinical examination. Age (45 ± 12 years) and gender (19 female, 13 male) of control subjects were comparable to that of patients. Their blood samples were transported freshly under the same conditions as patients' blood, as described below. Control samples were run together with patient specimens at the same time. Mycoplasma tests were performed on all specimens in a blinded matter.

Blood was collected in citrate-containing tubes and immediately brought to ice bath temperature as described previously [7, 8]. Samples were shipped refrigerated or on wet ice by overnight courier for analysis. Whole blood (50 µl) was used for preparation of DNA using Chelex (BioRad, USA) as follows. Blood cells were lysed with nanopure water (1.3 ml) at room temperature for 30 min. After centrifugation at $13\,000 \times g$ for 2 min, the supernatants were discarded. Chelex solution (200 µl) was added, and the samples were incubated at 56 °C and at 100 °C for 15 min each. Aliquots from the centrifuged samples were used immediately for PCR or stored at –70 °C until use. Multiple mycoplasma tests were performed on all patients.

Severity Score of Signs and Symptoms. Illness survey forms that analyzed the most common signs and symptoms of chronic illnesses at the time the blood sample was drawn, before and after the onset of illness, were given to each patient. Patients marked the intensity of 114 signs/symptoms prior to and after the onset of illness on a 10-point self-rating rank scale (0, none; 10, extreme). The 114 questions were then grouped into 28 categories containing three to nine questions each. An average score for each category was calculated as the average change in the intensity of all signs and symptoms in the category (score = sum of differences between self-rating values prior to and after onset of illness / number of questions in the category). The data from prior to the onset of illness and after the onset as well as within the last week before the blood was drawn were compared. A significant difference was obtained if the score after onset/within the last week was three or more points higher than that prior to the illness [9]. Additionally, the average score change with illness and the duration of illness were correlated with different mycoplasmal species identified. Surveys of 51 patients with negative mycoplasma test results were used to compare score values. Survey data were statistically analyzed using Spearman Rank correlation and Mann-Whitney tests (StatMost32; Dataxiom, USA).

Amplification of Gene Sequences. Amplification of the target gene sequences (Table 1) was performed in a total volume of 50 µl of PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 9) containing 0.1% Triton X-100, 200 µm each of dATP, dTTP, dGTP and dCTP, 100 pmol of each primer, and 0.5–1 µg of chromosomal DNA. Purified mycoplasmal DNA (0.5–1 ng of DNA) was used as a positive control for amplification. The amplification was carried out for 40 cycles with denaturing at 94 °C and annealing at 60 °C (genus-specific primers and *Mycoplasma penetrans*) or 55 °C (*Mycoplasma pneumoniae*, *Mycoplasma hominis*, *Mycoplasma fermentans*). Extension temperature was 72 °C in all cases. Finally, product extension was performed at 72 °C for 10 min. Negative and positive controls were present in each experimental run [8, 16–18].

Southern Blot Confirmation. The amplified samples were run on a 1% agarose gel containing 5 µl/100 ml of ethidium bromide in TAE buffer (0.04 M Tris-acetate, 0.001 M EDTA, pH 8.0). After denaturing and neutralization, Southern blotting was performed as follows. The PCR product was transferred to a Nytran membrane (Schleicher & Schuell, Germany). After transfer, UV cross-linking was performed. Membranes were prehybridized with hybridization buffer consisting of 1 × Denhardt's solution and 1 mg/ml salmon sperm as blocking reagent. Membranes were then hybridized with ^{32}P-labeled internal probe (10^7 cpm per bag). After hybridization and washing to remove unbounded probe, the membranes were exposed to autoradiography film for 7 days at –70 °C.

Results

Mycoplasma pneumoniae, *Mycoplasma fermentans*, *Mycoplasma penetrans and Mycoplasma hominis Infections.* For the determination of any mycoplasmal infection, we used primer sets for the rRNA gene (GPO-1 and UNI-) [5]. Although the GPO-1 and UNI-sequences (*Mycoplasma* spp.) are capable of some possible cross-reactions with mycoplasma-related organisms, the conditions used yielded specific products for mycoplasmas, as shown by van Kuppeveld et al. [5] and Dussurget et al. [19]. That the patients we examined had mycoplasmal infections was confirmed

Table 1 Sequences, target genes and size of amplified products from mycoplasmal DNA used for mycoplasma genus-specific and species-specific PCR

Sequence name	Sequence	Target	Size (bp)	Source, year [reference]
GPO1 primer MGSO primer UNI-probe	ACT CCT ACG GGA GGC AGC AGT A TGC ACC ATC TGT CAC TCT GTT AAC CTC TAA TCC TGT TTG CTC CCC AC	16S mRNA genus	717	van Kuppeveld et al., 1992 [5]
SB 1 primer SB 2 primer SB 3 probe	CAG TAT TAT CAA AGA AGG GTC TT TCT TTG GTT ACG TAA ATT GCT TTT TTC AGT TTC GTA TTC GAT G	*tuf* gene *M. fermentans*	850	Berg et al., 1995 [16]
MP5-1 primer MP5-2 primer MP5-4 probe	GAA GCT TAT GGT ACA GGT TGG ATT ACC ATC CTT GTT GTA AGG CGT AAG CTA TCA GCT ACA TGG AGG	unknown gene *M. pneumoniae*	144	Bernet et al., 1989 [24]
Mhom1 primer Mhom2 primer GPO1 probe	TGA AAG GCG CTG TAA GGC GC GTC TGC AAT CAT TTC CTA TTG CAA A ACT CCT ACG GGA GGC AGC AGT A	16S mRNA *M. hominis*	281	van Kuppeveld et al., 1992 [5]
IMM-7 primer IMM-5 primer IMM-3 probe	GGA AAC GGG AAT GGT GGA ACA GAT TTC TGC TAA TGT TAC AGC AGC AGG AGG GAA TCT GTG ATC TTA TTC	P35 gene (lipoprotein) *M. penetrans*	704	Haier et al., 1999 [8]

by species analysis. Using the *Mycoplasma fermentans*-specific primers SB1 and SB2 from the *tuf* gene, we found a single band of 850 bp size that hybridized only with the ^{32}P-labeled internal probe SB3. Similar results were obtained for the other mycoplasmal species. To examine the reliability of the method, we performed multiple assays (repeated 3–7 times) on 40 samples. All results were completely reproducible. In three cases, the sixth and seventh repeat of an initial positive result produced only a weak but positive signal due to degradation of DNA from repeated freezing and thawing.

The sensitivity of mycoplasma detection by the method described was assessed by the detection of control mycoplasmal DNA and by internal Southern blot hybridization using mycoplasma-specific probes. Using serial dilutions of mycoplasmal DNA, the method was able to detect as low as 10 fg of DNA [7, and unpublished data]. In other experiments, *Mycoplasma fermentans* was added to control blood samples at various concentrations. We were able to detect specific products down to 10 ccu/ml blood. Thus, with the use of specific Southern blot hybridization, the PCR procedure can result in specific test results of high sensitivity, down to the presence of a few microorganisms in a clinical sample. In our experience, conventional PCR yields results similar to forensic PCR with extracellular mycoplasma, but not with clinical samples that contain intracellular mycoplasmas. The reason for this is not known, but it could be due to inhibitors present in the clinical samples or to loss of mycoplasmal DNA in the conventional extraction procedures due to protein complexing or degradation by cellular nucleases.

Using species-specific primers and forensic PCR, the incidence of various mycoplasmal species in CFS/FMS patients was examined. *Mycoplasma pneumoniae* infections were observed in 54 of 91 patients with CFS/FMS. *Mycoplasma fermentans* infection occurred in 44 of 91 patients, whereas infection with *Mycoplasma hominis* (28/91) or *Mycoplasma penetrans* (18/91) was found at a lower incidence (Figure 1A). In 28 cases that were positive for the general mycoplasma test (*Mycoplasma* spp.), none of the four species examined were found. Previously, some healthy controls without any clinical signs and symptoms were found to be positive (~9%) for mycoplasmal infection (*Mycoplasma* spp.). The difference between patients and the control group was significant ($P<0.001$). Similarly, in a previous control study using the technique of nucleoprotein gene tracking, we found four of 62 (~6%) normal healthy adults were positive for mycoplasmal infection [7, 9]. In the present study, all 32 control subjects were negative for *Mycoplasma fermentans*, *Mycoplasma pneumoniae*, *Mycoplasma hominis* and *Mycoplasma penetrans* infections. The incidence of infection with *Mycoplasma pneumoniae, Mycoplasma penetrans* or *Mycoplasma hominis* was similar in female and male patients, whereas a significant difference ($P<0.01$) in the incidence of infection with *Mycoplasma fermentans* was found between females (23/67) and males (12/24).

Multiple Mycoplasmal Infections. Single infections with one of the four mycoplasmas tested were observed in 28 of the 91 patients. The most commonly observed single infection was *Mycoplasma pneumoniae* (18/91 patients). Single infections with the other species were detected in only a few cases (*Mycoplasma fermentans* in 3 patients, *Mycoplasma hominis* in 5 patients, *Mycoplasma penetrans* in 2 patients). Multiple mycoplasmal infections were detected in 48 of the 91 patients, appearing as double infections in 28 patients or triple infections in 20 patients. We did not find any patient positive for all four of the mycoplasmal species tested. All patients with multiple infections showed combina-

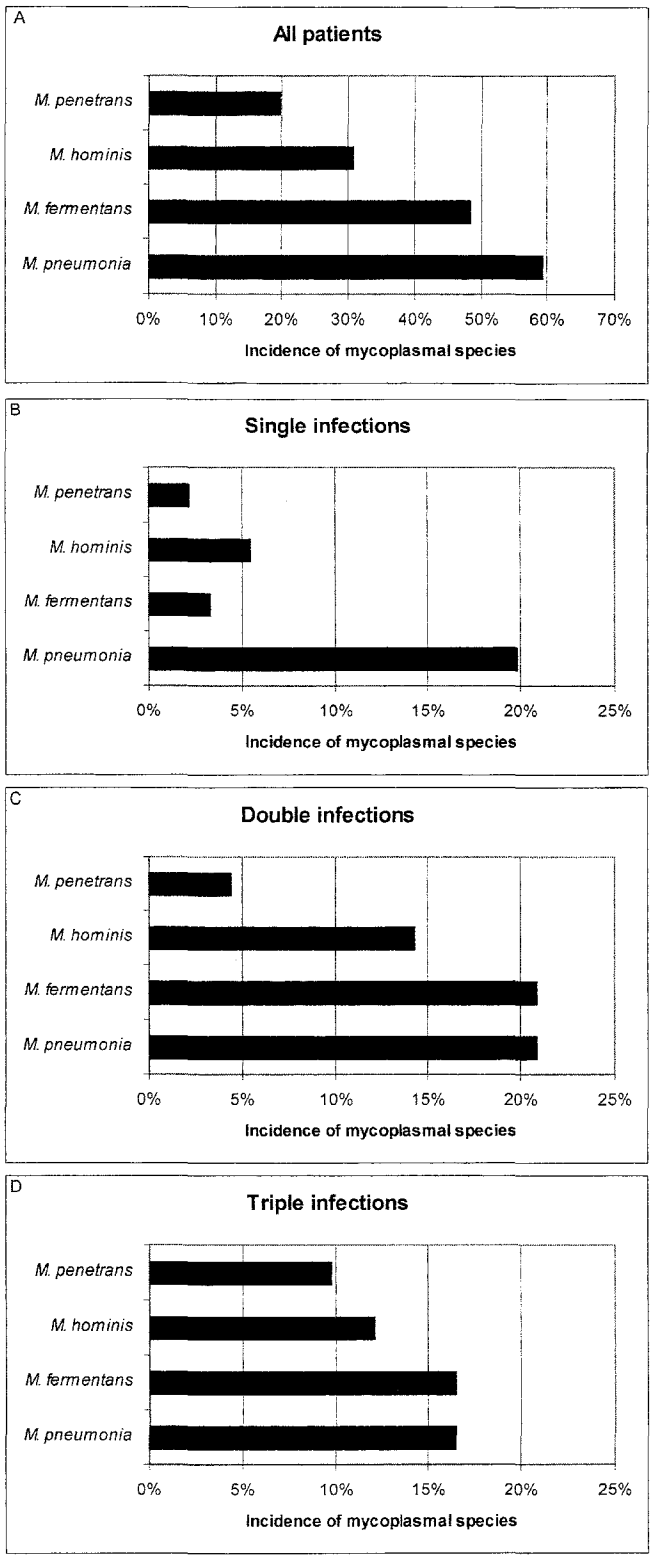

Figure 1 Incidence of multiple infections with different mycoplasmal species in mycoplasma-positive CFS/FMS patients. (**A**) Overall incidence of mycoplasma species detected; incidence of (**B**) single, (**C**) double, or (**D**) triple infections with different mycoplasmal species. Data are shown as a percentage of all mycoplasma-positive patients ($n=91$); species were not identified in 30.8% of patients

tions of *Mycoplasma pneumoniae* and/or *Mycoplasma fermentans* (with or without other species). The combination of *Mycoplasma hominis* and *Mycoplasma penetrans* was not observed (Figures 1A–D).

Mycoplasma Test Results and Signs and Symptoms Severity Score. We found that the severity of signs/symptoms was independent of the time of onset of illness, and the average of severity score was similar during the week the blood was drawn compared with the values after the onset of the disease. All categories for signs and symptoms showed an increase in score values (3 or more points) after the onset of illness in more than half of the patients. Patients with negative test results for mycoplasmas had an illness history of 153 ± 112 months, whereas patients with mycoplasmal infections had a duration of illness of 165 ± 134 months. Although the difference was not significant ($P=0.11$), patients infected with three different mycoplasmal species tended to have a longer illness history (211 ± 235 months) than patients infected with one (126 ± 144 months) or two (150 ± 154 months) species. There were no differences in the duration of illnesses relative to the different mycoplasmal species detected (Table 2).

To evaluate the influence of specific species or multiple infections on the severity of illness, we compared the average increase in the various scores prior to the onset of illness with the values after the onset of illness and during the week the blood was drawn. The highest increases in score values were found in fatigue/sleep problems, depression, memory loss, balance disturbances, muscle and joint pain or problems, head/neck aches and night sweats/fevers. Differences between infected patients relative to the different mycoplasmal species were not found. Tendencies for higher increases in the severity of signs and symptoms were found in patients with double infections compared to the other patients (Table 3).

Discussion

Patients with CFS and FMS exhibit complex overlapping chronic signs and symptoms. In these patients clinical conditions other than fatigue that can explain the signs and symptoms, such as malignancies or autoimmune diseases, are absent [4]. In contrast, FMS patients have muscle and overall pain as primary complaints, but they exhibit most if not all of the signs and symptoms commonly found in CFS patients [15]. The major difference between these illnesses appears to be in the severity of specific signs and symptoms.

A majority of CFS and FMS patients have systemic mycoplasmal infections that may explain most of their chronic signs and symptoms [6, 7]. Mycoplasmal infec-

Table 2 Patient age and duration of illness compared with *Mycoplasma* spp. detected in blood specimens

	Mycoplasma spp.		No. of different *Mycoplasma* spp. identified				*Mycoplasma* species identified			
	Negative	Positive	None	One	Two	Three	*fermentans*	*pneumoniae*	*penetrans*	*hominis*
Mean age (years)	40.8	40.9	47.5	31.6	41.8	45.0	42.6	41.6	34.3	44.9
Mean duration of illness (months)	153.3	167.5	199.5	127.6	150.3	211.7	183.7	170.0	121.1	197.5

Table 3 Changes in average severity of signs and symptoms in patients with different mycoplasma species in their blood after the onset of illness. Severity of signs and symptoms was assessed using a Patient Illness Survey Form that included 114 signs and symptoms. The intensity of signs and symptoms were marked by patients on a 10-point scale (0, none; 10, extreme) prior to and after the onset of illness. Scores were determined in each category (3–9 questions) as the sum of differences between values prior to and after the onset of illness/number of questions in the category

Sign/symptom	Score									
	Mycoplasma spp.		No. of *Mycoplasma* spp. identified				*Mycoplasma* species identified			
	Negative	Positive	None[a]	One	Two	Three	*ferment-ans*	*pneu-moniae*	*pene-trans*	*hominis*
Fatigue/sleep problems	3.44	3.38	3.69	2.69	3.66	3.52	3.67	3.51	3.68	2.78
Depression	3.59	4.15	3.47	4.00	4.70	4.04	4.40	4.08	4.50	4.21
Memory problems	2.89	3.31	3.71	3.17	3.57	2.93	3.18	3.31	2.86	3.33
Balance problems	2.86	3.81	4.06	3.65	4.00	3.61	3.79	3.80	3.29	4.03
Muscle pain/ache	4.11	4.37	3.75	4.18	4.88	4.31	4.61	4.19	4.69	4.67
Joint pain/ache	2.26	2.24	2.31	1.92	2.78	1.86	2.32	2.23	2.07	2.09
Infections	4.34	3.22	4.34	3.10	3.53	2.32	2.96	3.10	2.73	2.53
Skin disorders	1.96	2.32	1.90	2.51	2.53	2.13	2.24	2.40	2.13	2.56
Hair/scalp disorders	1.36	1.59	1.88	1.22	1.94	1.39	1.64	1.24	2.24	1.42
Skin rash/sensitivity	1.57	2.10	2.66	2.04	1.94	2.02	1.91	1.77	2.36	2.19
Genital disorders	1.48	1.87	2.72	2.12	1.55	1.52	1.50	1.55	1.36	2.13
Alimentation	0.94	1.27	1.38	1.34	1.67	0.69	1.08	1.19	1.54	0.87
Sensory disorders	2.97	2.53	3.29	2.18	2.88	2.02	2.51	2.52	1.90	2.22
Head/neck ache	2.08	1.95	2.60	2.00	2.06	1.40	1.75	1.79	1.56	1.74
Swelling (tissue)	3.10	2.77	3.56	2.46	2.81	2.57	2.68	3.00	2.46	2.15
Night sweats/fever	2.70	2.25	2.53	2.29	2.26	2.04	2.11	2.10	2.04	2.45
Gastrointestinal problems	1.58	2.57	3.19	1.85	3.19	2.18	2.41	2.54	2.46	2.62
Urinary problems	2.12	2.48	2.97	2.09	2.66	2.36	2.38	2.32	2.56	2.57
Bleeding	2.40	1.96	2.13	1.50	2.13	2.11	2.11	1.91	2.32	1.82
Mouth cavity problems	1.28	1.50	0.63	1.62	2.00	1.33	1.54	1.58	1.81	1.67
Visual disorders	1.32	1.30	1.79	1.18	1.23	1.21	1.14	1.25	1.40	1.12
Auditory disorders	2.12	1.90	1.77	1.83	2.31	1.55	1.77	1.89	2.06	1.85
Eye problems	2.28	1.96	2.69	2.31	1.63	1.61	1.66	1.64	1.71	1.94
Taste/smell problems	2.26	2.46	3.00	2.44	2.81	1.76	2.36	2.11	2.43	2.16
Nasopharyngeal problems	1.72	2.18	2.54	2.18	2.19	1.95	2.12	1.92	2.26	2.10
Breathing problems	1.93	2.64	2.38	2.10	2.94	2.95	2.85	2.74	3.64	2.24
Heart problems	1.97	2.26	2.18	1.86	2.64	2.24	2.35	2.09	2.73	2.38
Chemical sensitivity/allergy	2.65	2.29	2.58	1.97	2.52	2.17	2.29	2.29	2.81	1.76

[a] Test for all four species negative

tions are often associated with night sweats, intermittent fevers, chronic fatigue, skin rashes, increased dermal sensitivity, joint and muscle pain, swelling and reduced mobility of joints, heart palpitations, pain and arrhythmia, stomach cramps and regurgitation, loss of vision, double vision and other problems, depending on the organ or tissue system infected [20]. Although most mycoplasmal species were previously considered as relatively benign microorganisms with a low pathogenic potential, recent studies have shown that mycoplasmal infections can cause a variety of illnesses, including a large percentage of pneumonia and asthma at high frequencies [21], and they can lead to fatal illness [22].

In our studies of patients with Gulf War illness, we found mycoplasmal infections in about one-half of over 200 patients; these patients were found to have principally *Mycoplasma fermentans* or *Mycoplasma pneumoniae* infections [9, 10]. Similar results were recently reported by Choppa et al. [23]. Moreover, in over one-half of the civilians with CFS/FMS, we have found a variety of pathogenic mycoplasmal species in the leuko-

cyte fractions of blood samples [7]. In the present study, we examined patients with CFS/FMS for the presence of multiple systemic mycoplasmal infections. CFS/FMS patients were tested for the cell-penetrating species *Mycoplasma fermentans* and *Mycoplasma penetrans,* as well as for *Mycoplasma pneumoniae* and *Mycoplasma hominis*. We found that the majority of the patients had multiple infections, with two or more mycoplasmal species predominating. We detected mainly combinations of *Mycoplasma fermentans* and *Mycoplasma pneumoniae* infections in these patients.

The tests that we used to identify mycoplasmal infections, based on PCR, are very sensitive and highly specific [10]. The sensitivity of mycoplasma detection by the method described was assessed by the detection of control mycoplasmal DNA and by internal hybridization using mycoplasma-specific probes. Using serial dilutions of mycoplasmal DNA, the method was able to detect as low as 10 fg of DNA by specific Southern blot hybridization. The improved handling of blood samples, including DNA preparation, allowed specific detection of mycoplasmal infections in human blood with high sensitivity and reliability. Previously, we found that blood samples deteriorate if stored or shipped at room temperature [7]. Thus, the rapid processing of blood samples is particularly important in obtaining reliable data.

Although we used genus primers to determine whether patients had mycoplasmal infections, the UNI- and GPO-1 primers (*Mycoplasma* spp.) are not totally genus-specific. To overcome the problems in specificity, we confirmed the results for the *Mycoplasma* spp. assay with highly species-specific assays. We were able to identify at least one mycoplasmal species in 73 of 91 patients in whom the general test was positive. In the remaining 28 patients, it is likely that other mycoplasmal species were responsible for the positive amplification signal, such as *Mycoplasma arthritidis* or *Mycoplasma pirum*, but cross-reactions with other closely related microorganisms are possible. The specificity of the general test cannot completely rule out such cross-reactivity. Future studies will examine additional mycoplasmal species using highly species-specific PCR primers. In addition, contamination during sample preparation is an important issue that needs to be considered. We used several procedures to confirm the specificity of our results. Samples obtained from patients and healthy controls were tested simultaneously, and positive and negative controls were used with each sample preparation. Using the technique described, blinded blood samples were investigated in a recent study sponsored by the U.S. Department of Defense. These samples contained live organisms from mycoplasmal cultures seeded in control, negative blood samples for independent tests run by four different laboratories. The results were the same in all laboratories (unpublished results).

Infections with different mycoplasmal species and possibly other infections may explain, in part, the complex signs and symptoms found in CFS/FMS patients [6]. In a previous study on Gulf War illness patients with mycoplasmal infections, long-term antibiotic treatment using doxycycline, ciprofloxacin, azithromycin or clarithromycin led to significant decreases in the severity of signs and symptoms in about 70% of the patients [7, 9, 10]. Multiple treatment cycles were necessary, probably because of the intracellular locations of mycoplasmas like *Mycoplasma fermentans* and *Mycoplasma penetrans*, their inherent insensitivity to antibiotics and the slow-growing nature of these microorganisms [10]. After recovery, these patients were no longer positive for mycoplasmal blood infections [9, 10].

Although illnesses such as CFS and FMS may not be initially caused or triggered by chronic infections, our results suggest that chronic infections may be an appropriate explanation for much of the morbidity seen in many CFS/FMS patients. Although the differences were not significant, multiple infections with different mycoplasmal species appear to occur after the onset of illness, suggesting that mycoplasma-positive patients contracted additional mycoplasmal infections with time. We also found that the severity of major signs and symptoms may be related to the type of mycoplasmal infection. Infections with more than one mycoplasmal species were associated with greater increases in severity of signs and symptoms than infections with a single species. Although significant differences were not found due to a high variance of the score values, there was a tendency for patients with mycoplasmal infections to have higher intensity scores of signs and symptoms than patients without such infections. Obviously, these chronic infections are not sufficient to explain all of the signs and symptoms found in every patient, and chemical exposures, psychological problems and other environmental toxic events may also be important sources of morbidity. It remains unclear whether mycoplasmal infections are causative, cofactors or opportunistic in CFS/FMS patients. The identification of these microorganisms in blood leukocytes does, however, offer an opportunity for more specific diagnosis and treatment of these chronic illnesses.

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