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Mycoplasma blood infection in chronic fatigue and fibromyalgia syndromes

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Abstract Chronic fatigue syndrome (CFS) and fibromyalgia syndrome (FMS) are characterised by a lack of consistent laboratory and clinical abnormalities. Although they are distinguishable as separate syndromes based on established criteria, a great number of patients are diagnosed with both. In studies using polymerase chain reaction methods, mycoplasma blood infection has been detected in about 50% of patients with CFS and/or FMS, including patients with Gulf War illnesses and symptoms that overlap with one or both syndromes. Such infection is detected in only about 10% of healthy individuals, significantly less than in patients. Most patients with CFS/FMS who have mycoplasma infection appear to recover and reach their pre-illness state after long-term antibiotic therapy with doxycycline, and the infection can not be detected after recovery. By means of causation and therapy, mycoplasma blood infection may permit a further subclassification of CFS and FMS. It is not clear whether mycoplasmas are associated with CFS/FMS as causal agents, cofactors, or opportunistic infections in patients with immune disturbances. Whether mycoplasma infection can be detected in about 50% of all patient populations with CFS and/or FMS is yet to be determined.

Keywords Chronic fatigue syndrome · Fibromyalgia · Mycoplasma infection · Persian Gulf syndrome · Polymerase chain reaction

Introduction

Several rheumatic conditions such as chronic fatigue syndrome (CFS) and fibromyalgia syndrome (FMS) are

characterised by the lack of consistent laboratory and clinical abnormalities. They are similar in that they are complex multiorgan signs and symptoms that overlap or are almost identical, and their differences are related to the severity of specific signs and symptoms. Although CFS and FMS are distinguishable as separate syndromes based on established criteria, it has been reviewed that up to 70% of patients with FMS have coexisting CFS and, conversely, that 35–70% of patients with CFS have FMS [1]. Among patients with FMS, 58% of females and 80% of men are reported to have CFS, suggesting that both syndromes would be better understood in a further subclassification by means of causation and treatment [2].

The Gulf War illnesses (GWI), collectively termed Gulf War syndrome, have affected thousands of veterans of the Persian Gulf War in 1990/1991. In many GWI patients, chemical, radiological, or biological exposures or combinations of these have explained various signs and symptoms. However, in a rather large proportion of patients, the symptoms are unexplained and similar to those noted after other major conflicts. They are often consistent with CFS and/or FMS [3, 4, 5, 6]. Since a high proportion of immediate family members of such patients have also presented with symptoms that overlap with CFS and/or FMS, the involvement of transmittable biological agents has been suggested. It was argued that in several patients mycoplasma infection can be responsible for such transmission and thereby associated with these syndromes [3]. Some possible explanations are that the veterans received their infection through vaccines contaminated with mycoplasmas or that vaccines in unexplained ways made them vulnerable to such infection [3, 7]. The infection may have been transmitted to spouses and children later on by prolonged close contact with infected GWI veterans, perhaps by airborne transmission [3]. These are currently very controversial medical and political issues, especially in the USA. The aim of this review is to focus on mycoplasma infection in CFS/FMS.

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Detection of mycoplasmas

Mycoplasmas are the smallest free-living self-replicating bacteria known. They possess a triple-layered limiting membrane but no rigid bacterial cell wall. Their primary habitats in humans are the mucous surfaces of the respiratory and urogenital tracts, alimentary canal, joints, eyes, and mammary glands. They are often found as extracellular parasites attached to the external surfaces of host cells, but some species may invade host cells and tissues and replicate intracellularly.

Although most of the roughly 15 mycoplasma species described in humans are nonpathogenic, some may penetrate blood vessels and colonize major organs, causing acute and chronic illnesses. Probably the best known is *Mycoplasma pneumoniae*, which can cause primary atypical pneumonia and other airway disorders such as tracheobronchitis and pharyngitis. Though definite proofs are lacking, mycoplasmas have been incriminated as pathogens in other disorders such as septic, reactive, and rheumatoid arthritis, Crohn's disease, immunodeficiency disorders, urogenital tract diseases, and chronic asthma. Furthermore, mycoplasmas may serve as B cell and T cell mitogens and induce autoimmunity through the activation of antiseif T cells or polyclonal B cells [8, 9, 10].

The pathogenic potential of mycoplasma species is related to their hiding within cells. The mechanism of cell entry is unclear but not restricted to certain cell types such as phagocytosing neutrophils or monocytes/macrophages. Inside cells, they facilitate DNA rearrangements which promote genetic diversity and maximise the coding potential of their own small genomes. Immune recognition can therefore often be evaded, and the antibody response usually does not occur until the infection has progressed. An infection may also circumvent normal immune mechanisms, as illustrated by the high reinfection rates of patients with pneumonia caused by *M. pneumoniae*. Normal results of specific antibody assays therefore do not necessarily rule out a potential mycoplasma infection. The infection may also be overlooked because mycoplasmas do not grow on conventional solid culture media but require specific nutrient media, not least because they are often contaminants of cell and tissue cultures and other biologic material. The cultivation of mycoplasmas is also difficult and time-consuming and most often requires several weeks [8, 9, 10].

The use of polymerase chain reaction (PCR) methods enables the sensitive and specific detection of mycoplasmas and other micro-organisms and the discrimination between different mycoplasma species by their DNA products. The PCR methods for mycoplasmas have to eliminate contamination and cross-reactions with other micro-organisms as well as the potential of detecting only mycoplasma DNA fragments derived from sources such as gut micro-organisms or the diet [8, 9]. The detection of a micro-organism by PCR or other methods can be equally distributed among patients

with a certain medical condition and control population, and the general need of using defined clinical controls should be noted [8, 9].

Detection of mycoplasmas in CFS, FMS, and GWI

By means of PCR methods applied to the nuclear fraction of blood leukocytes, several groups of investigators have detected mycoplasma DNA products in about 50% (44.7–68.6%) of several hundred CFS, FMS, and GWI patients, respectively [11, 12, 13, 14, 15, 16, 17, 18, 19]. In contrast, such products were found only in about 10% (0–15.0%) of several hundred healthy asymptomatic age and sex comparable individuals, in each study significantly lower than in patients (Table 1). The percentage differences of patients and healthy controls are most likely due to differences in the PCR procedures used. The GWI patients studied had clinical signs and symptoms that overlap with CFS and/or FMS [13, 14, 15]. In several studies, the diagnoses of CFS and FMS overlapped, and the patients were therefore considered together [16, 17, 18] (Table 1).

These studies have apparently shown that blood leukocytes are infected with mycoplasmas and not only the carriers of mycoplasma DNA products that might have been taken up by phagocytosing leukocytes. They have evidently also ruled out the potential of contamination and cross-reactions with other micro-organisms [11, 12, 13, 14, 15, 16, 17, 18, 19]. Also, contamination with mycoplasmas that might have occurred during the procedures should have led to a more random distribution of results.

Single blood infection of mycoplasmas may consist of species such as *M. fermentans*, *M. pneumoniae*, *M. penetrans*, *M. hominis*, or *M.* genus and have been found in 44.7–68.6% of patients [11, 12, 13, 14, 15, 16, 17, 18, 19] (Table 1). Multiple infections have been detected in 52.7% of patients, appearing as double infections in 30.8% and triple infections in 22.0%. All multiple infections have been found as combinations of *M. fermentans* and/or *M. pneumoniae*, with or without other species [17]. Thus, *M. fermentans* and *M. pneumoniae* appear to be the mycoplasma species most often encountered in patients with CFS, FMS, and GWI.

It has recently been argued that *M. hominis* is the most predominant species among CFS patients in Europe, whereas *M. pneumoniae* is most often detected in North American CFS patients [19]. However, to extrapolate such findings in patients from Belgium to the whole of Europe [19] is incorrect, and such demographic differences have yet to be established. The incidence of mycoplasma infection is similar in female and male patients, and no special mycoplasma species has been sorted out as predominant in blood cells from healthy individuals [11, 12, 13, 14, 15, 16, 17, 18, 19].

Important observations are that CFS/FMS patients infected with more than one mycoplasma species generally have longer histories of illness and greater severity of

Table 1 Mycoplasma products in peripheral white blood cells of patients with chronic fatigue syndrome (CFS), fibromyalgia syndrome (FMS), and Gulf War illness (GWI) detected by polymerase chain reaction methods

Reference	Diagnosis	N patients	Percentage of patients positive for mycoplasma	N healthy controls	Percentage of controls positive for mycoplasma
[11]	CFS	100	52.0	100	15.0
[12]	CFS	100	52.0	50	14.0
[13]	CFS	100	52.0	160	2.0
	FMS	40	54.0	–	–
	GWI	60	55.0	–	–
[14]	GWI	30	46.6	21	0.0
[15]	GWI	170	44.7	41	4.9
[16]	CFS and/or FMS	132	62.9	32	9.3
[17]	CFS and/or FMS	91	59.3	32	2.0
[18]	CFS and/or FMS	565	53.1	71	9.9
[19]	CFS	261	68.6	36	5.6

signs and symptoms than patients infected with a single species. It has therefore been suggested that patients may acquire additional mycoplasma infections with time [17].

In one study of CFS patients, the number of mycoplasma cells per amount of human genomic DNA could be determined [11]. Even so, no culture studies of blood specimens from current patient groups with mycoplasma infection have been performed to find viable micro-organisms. Not least because such culture studies are likely to imply the cocultivation of leukocytes [9], it is not clear whether they can be accomplished. Thus, it should be kept in mind that blood infection with mycoplasmas is not a septicemia but the presence of micro-organisms within circulating leukocytes which do not survive or replicate independently of these host cells [8, 9, 10].

By means of serologic procedures, other investigators have failed to detect *M. fermentans* in GWI patients [20, 21]. It remains conjectural whether these findings can be ascribed to the lack of a normal antibody response to *M. fermentans* [8, 9, 10] or if the patients studied had contracted their illnesses by chemical or radiological exposure during the Persian Gulf War [3].

Potential implications of mycoplasma infection in CFS and FMS

It is not clear whether mycoplasma infection is associated with CFS/FMS as causative agents, cofactors, or opportunistic superinfections in patients with immune disturbances. Mycoplasma infection with species like *M. fermentans* and *M. penetrans* has been implicated as a cofactor in the progression and morbidity in HIV-AIDS [8, 22, 23]. Although immunologic abnormalities in CFS are a matter of controversy, it has been argued that immune disturbances occur episodically in an increasing and decreasing temporal pattern consistent with episodic immune dysfunction [24, 25, 26]. Such transient disturbances may be difficult to demonstrate, but they may occur just as easily in FMS. In comparison to HIV-AIDS, episodic immune dysfunction can be a precipitating factor for the increased susceptibility to mycoplasma infection in patients with CFS/FMS.

Mycoplasma infection is likely to cause coinfections or even synergism with other infectious agents such as chlamydia and brucella species, enteric bacteria such as proteus, and various viruses such as parvovirus, Epstein-Barr virus, cytomegalovirus, hepatitis viruses, and herpes viruses, among others [9, 27]. Thus, patients with these syndromes or illnesses may have multiple bacterial and viral infections. In spite of the potential roles of other micro-organisms and immune disturbances, chronic mycoplasma infection is suggested as an explanation for much of the morbidity and illness progression encountered in many CFS/FMS patients [17].

Mycoplasmas and mycoplasma-derived surface components have been shown to modulate the activities of human monocytes/macrophages and NK cells and trigger the production of a wide variety of up- and downregulating cytokines and chemokines. For instance, mycoplasma-mediated secretion of proinflammatory cytokines such as tumour necrosis factor alpha, interleukin-1 (IL-1) and IL-6 by macrophages, and the attraction and activation of human neutrophils and monocytes may significantly contribute to the inflammatory responses during mycoplasma infection [9, 27, 28]. In addition, surface-located lipoproteins of pathogenic mycoplasmas [29, 30], which are also present on other micro-organisms [31], are likely to initiate innate, IL-12-mediated host defense mechanisms against various infectious pathogens including mycoplasmas [29, 30, 31]. However, whether such mycoplasma-derived activities [9, 27, 28, 29, 30] are able to explain symptoms such as fatigue and pain and the lack of overt laboratory abnormalities in CFS/FMS patients with mycoplasma infection [11, 12, 13, 14, 15, 16, 17, 18, 19] remains unclear.

Lack of laboratory abnormalities is also often encountered in patients with pneumonia due to *M. pneumoniae* [8, 9, 32, 33], which is an airway disease and not a mycoplasma blood infection. Although this cold agglutinin-associated disease can be combined with extrapulmonary manifestations such as rashes and cardiac abnormalities such as pericarditis and also have an epidemic pattern [8, 9, 33], there does not appear to be a link between this pneumonia or its other potential manifestations and CFS/FMS.

Treatment of mycoplasma blood infection in CFS, FMS, and GWI

In CFS, FMS and GWI patients who have mycoplasma blood infection by PCR testing, long-term therapy with 200 mg daily of doxycycline for at least 6 months given in 6-week cycles with 2 weeks in between appears effective. Although such cycle therapy is empirical and not evidence-based, it seems that about 80% of a total of ca. 160 such patients studied until now have recovered by this treatment [14, 16] (Table 1).

The majority of these patients seem to relapse after only a few cycles of therapy, and up to 1 year of treatment can be required to reach complete recovery and the pre-illness state [14, 16]. The reasons for relapses are probably the intracellular location of pathogenic mycoplasmas, their slow-growing nature, and inherent insensitivity to most antibiotics [8, 9, 10, 33]. After recovery, the patients are negative for mycoplasma blood infection by PCR testing [14, 16]. It has been noted that if such patients had illnesses caused only by psychological or psychiatric problems or by environmental toxic events, they would not respond to doxycycline therapy and recover to the pre-illness state [14, 16].

Other broad-spectrum antibiotics generally suited for mycoplasma infections include ciprofloxacin, azithromycin, chlorithromycin, and the semisynthetic tetracycline derivative minocycline. Because mycoplasmas lack a rigid cell wall, they are not susceptible to antibiotics such as penicillin which target that structure [8, 14, 16, 33].

A major criticism of the antibiotic regimens with doxycycline is their lack of controlled trials, and as yet only one research group [14, 16] has presented them. A recent report on the initiation of a 1-year randomised placebo-controlled trial with continual 200 mg daily of doxycycline in about 500 North American veterans with GWI, mycoplasma blood infection by PCR testing, and clinical symptoms of CFS/FMS is therefore welcomed [34]. Its authors speculate on whether mycoplasma infection can account for as much as 40–50% of US veterans' GWI, and the trial is sponsored by the USA Department of Defense. They also state that the ideas about mycoplasma infection in GWI are their private views and do not reflect the official policy of the US Government or Department of Defense [34]. Such statements may indicate that these issues are highly controversial and perhaps that US authorities prefer not to answer for the possibility of causing infections through administration of vaccines during the Persian Gulf War. Nevertheless, the most interesting part of this trial will be its result.

Conclusions

It seems noteworthy that a high magnitude of about one half of patients with CFS and/or FMS and GWI

patients with symptoms that overlap with CFS/FMS may have chronic mycoplasma blood infection [11, 12, 13, 14, 15, 16, 17, 18, 19]. It also appears significant that, in the majority of these patients, such infection and various symptoms and signs may be overcome by long-term antibiotic therapy [14, 16]. Taken together, these findings provide indirect evidence that mycoplasmas are viable micro-organisms playing a pathogenic role in some CFS/FMS patients. By means of causation and treatment, mycoplasma infection may thereby permit a further subclassification of CFS and FMS. The argument for this subclassification would be strengthened if epidemics of pneumonia due to *M. pneumoniae* reported over the years were associated with an increased prevalence of CFS/FMS, but this association probably does not exist. Thus, mycoplasma blood infection responding to therapy appears to be the only mycoplasma-associated disorder that can be linked to CFS/FMS.

Other infections, especially with viruses, are shown to be risk factors for transient or more persistent CFS/FMS, although there is no direct evidence of a causal association between infectious agents and these syndromes [35, 36, 37]. Chronic blood infection with mycoplasmas, however, appears closely related to the maintenance of CFS/FMS. This concept is supported by the results of in vitro studies which are consistent with the ability of pathogenic mycoplasmas to reside and replicate intracellularly over extended periods of more than 6 months, circumventing antibiotic therapy and immune surveillance and establishing chronic infections [10]. This would probably also be supported by electron microscopic studies of mycoplasmas in CFS/FMS, but such studies are lacking.

Polymerase chain reaction (PCR) methods are not generally available, and normal results of specific antibody assays do not necessarily rule out a potential mycoplasma infection [8, 9, 10]. Although PCR methods for the detection of mycoplasmas can be inaccessible, physicians may start long-term antibiotic therapy of patients with CFS/FMS using doxycycline or another antibiotic suited for mycoplasma infection. However, to initiate such therapy without confirming the presence of infection can not be recommended. The treatment can only suppress, not eradicate mycoplasmas and may have adverse effects and cause antimicrobial resistance [33]. Results of antibiotic treatment in controlled clinical trials in CFS/FMS are also lacking, and the potential role of mycoplasmas in these syndromes is far from clear.

The great similarities in the prevalence of mycoplasma infection between North American CFS/FMS patients [11, 12, 13, 16, 17, 18] and Belgian CFS patients [19] should be noted. One must be careful in extrapolating these results [11, 12, 13, 16, 17, 18, 19] to all patient populations with CFS/FMS. Therefore, the findings of mycoplasma infection in about one-half of CFS and/or FMS patients need confirmation.

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