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Chronic fatigue syndrome (CFS) associated with *Staphylococcus* spp. bacteremia, responsive to potassium arsenite 0.5% in a veterinary surgeon and his coworking wife, handling with CFS animal cases

W. Tarello*

CP 42, 06061 Castiglione del Lago, Perugia, Italy

Abstract

Chronic fatigue syndrome (CFS) in human patients remain a controversial and perplexing condition with emerging zoonotic aspects. Recent advances in human medicine seem to indicate a bacterial etiology and the condition has already been described in horses, dogs, cats and birds of prey in association with micrococci-like organisms in the blood. To evaluate the possibility of a chronic bacteremia, a veterinary surgeon (the author) and his coworking wife, both diagnosed with CFS and meeting the CDC working case definition, were submitted to rapid blood cultures and fresh blood smears investigations. Blood cultures proved *Staph*-positive and micrococci-like organisms in the blood were repeatedly observed in the 3-year period preceding the arsenical therapy, during which several medicaments, including antibiotics, proved unsuccessful.Following treatment with a low dosage arsenical drug (potassium arsenite 0.5%, im., 1 ml/12 h, for 10 days) both patients experienced complete remission. At the post-treatment control made 1 month later, micrococci had disappeared from the blood, and the CD4/CD8 ratio was raising.

Keywords: Chronic fatigue syndrome (CFS); Micrococci; Fatigue; *Staphylococcus*; Zoonosis; Potassium arsenite; Arsenic

Resumé

L'étiologie du Syndrome de Fatigue Chronique (SDF) chez l'homme reste entourée de nombreuses spéculations et perplexités. Apparemment il s'agit d'une zoonose émergente qui a déjà été décrite chez les chevaux, les chiens, les chats et les oiseaux de proie, associée à la présence

* European Veterinary Center, PO Box 51751, Jumeirah Tower, Sheick Zayed Road, Dubai, United Arab Emirates.

E-mail address: wtarello@supereva.it (W. Tarello).

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de bactéries types micrococciques trouvées dans le sang. Récente publications humaines semblent indiquer une étiologie bactériologique. Afin d'évaluer la possible association avec une infection chronique à bactéries, un vétérinaire practicien (l'auteur) e sa femme, avec un diagnostic de CFS et correspondant à la définition humaine pour cette maladie, on été soumis à hémocultures et à la recherche microscopique directe de germes dans le sang. Le hémocultures furent positives pour *Staphylococcus spp.* Chez les deux suject examinés, des bactéries type micrococciques ont été maintes fois retrouvées sur le globules rouges pendant les trois ans qui on précédé la thérapie arsenicale, et leur présence était associée aux symptomes de fatigue/douleur et à des anomalies biochimiques correspondantes. Au cours de la meme periode l'utilisations d'autre médicaments, y inclus des antibiotiques, n'avait sorti aucun résultat thérapheutique. En revanche, les symptômes ont disparu après traitement par l'arsenite de potassium à 0,5% (liqueur de foulère 1 ml/12 h pendant 10 jours). Cette thérapeutique a permis une guérison complète et durable du syndrome chez les deux sujets et l'augmentation du rapport CD4 CD8.

Mots cléf: Syndrome de fatigue chronique (CFS); Micrococci; Fatigue; *Staphylococcus*; Zoonose; Arsenite potassique; Arsenic

1. Introduction

Chronic fatigue syndrome (CFS) as originally defined by the American Centers for Disease Control [1] and as recently redefined [2] is a human illness in which patients experience severe, debilitating fatigue and a variety of multiple nonspecific symptoms for >6 months. Despite multidisciplinary investigations into the cause of CFS, its etiology remains unknown [3] and no consistent cellular or biochemical alteration has been found which could be used to differentiate the condition from similar fatigue-related diseases [4].

CFS in people is characterized by highly variable patterns of symptoms including myalgias, sore throat, headaches, adenopathy, low-grade fever, loss of libido, irritable bowel, poor functional status and neurocognitive disorders [5]. To identify people with CFS, physicians evaluate patients with persistent fatigue of undetermined cause using the CFS definition developed by the International CFS Study Group and published in the *Annals of Inrernal Medicine* in December 1994 [2], replacing the first research case definition published 6 years earlier [1].

Most CFS cases are sporadic. Occasionally, close contacts, including family members, become ill with CFS at about the same time [5], suggesting a possible contagiousness. Nonetheless, no published data implicate a peculiar virus as the cause of CFS [5]. Borna disease virus (BDV), a neurotropic RNA virus affecting humans, sheeps and horses, has been recently discharged as a cause of fibromyalgia [6], a condition with many symptoms overlapping those of CFS. These two closely related illnesses, commonly coexist in the same patient and a diagnosis of fibromyalgia does not exclude one of CFS [5].

A zoonotic transmission have been suggested [7] and, additionally, 2.9 and 7.5% of veterinary surgeons, respectively < and > than 40 years old have been found to suffer from chronic fatigue in Switzerland [8,46], a percentage significantly higher than the 0.2–0.5% estimated prevalence of CFS in the population [9].

Recent advances in human medicine seem to indicate a staphylococcal ethiology [4] and the condition has already been described in horses [10], dogs [11], cats [12] and birds of

prey [13] in association with micrococci-like organisms in the blood and *Staph*-positive blood cultures (*S. xilosus*, *S. intermedius*) [11–13].

The symptomatology in animals with CFS can be superimposed on that of the human disease and some animals cases have been found to fulfil also the current human criteria for the diagnosis [12]. Consequently, numerous checks were carried out when the author and his wife both fell ill at the same time with CFS in September 1992, shortly after they had begun research on animals affected by an unknown syndrome, characterized by the presence of micrococci on the red blood cells, and always responsive to arsenical drugs.

The primary purpose of this study was to report on the serological and blood microbiological findings in two persons diagnosed with CFS (the author and his wife) and to compare them with those obtained from CFS animals cases. An additional objective was to report on how the syndrome was responsive to an arsenical drug, potassium arsenite 0.5% (Fowler's solution 1/2) given intramuscularly in low dosage (1 ml/12 h, for 10 days; thus 7.5 mg of arsenic/day), as previously experienced with success in animals treated with another arsenical drug (thicetarsamide sodium, *Caparsolate,* Abbott Laboratories) [10–13] and inferred from the Merck Index [14] and other ancient veterinary [15] and medical sources [16].

2. Materials and methods

2.1. Patients investigation

In September 1992, two persons—a 38-year-old veterinary surgeon and his 32-year-old co-working wife—experienced a sudden acute flu-like onset of a syndrome characterized by common symptoms dominated by chronic fatigue, headache, muscle and joint pain, sleep disturbances, sore throat and cognitive impairment. Since June 1992, both subjects were increasingly used to collect blood animal samples for haematologic and serologic analysis on animals showing an unusual illness, characterized by chronic weakness, presence of micrococci in the blood and responsiveness to arsenical medicaments. Particular precautions were not adopted in handling with these subjects.

In the absence of spontaneous recovery, a panel of laboratory tests was performed in February 1993, 6 months after the acute onset of the condition, leading to a diagnosis of chronic fatigue syndrome (CFS) in both human patients. Blood samples were successively collected again in November 1995 and March 1996 for further analysis.

2.2. Hematology

Complete blood counts (CBC) were performed on samples collected in February 1993, November 1995 and March 1996. A CD4/CD8 ratio examination was performed in November 1995 and in March 1996.

Two fresh blood smears from each subject, stained with May—Grunwald—Giemsa and Wright techniques, were prepared every about 6 months from February 1993 to March 1996, and checked for emoparasites, bacteria and others blood anomalies (× 100, Leitz Biomed). In both patients, blood smears were also performed at day 0, 4, 10 and 40 during and following the potassium arsenite treatment (10–20 February 1996).

2.3. Serology

Human blood specimens collected in February 1993 were used for serologic testing for circulating EBV antibodies (IgG–IgM) and for the Weil–Felix reaction. Specimens collected in November 1995 were tested for HIV-1 and Hepatitis B.

2.4. Biochemistry

Serum creatine kinase (CK) and lactate dehydrogenase activities (LDH) were calculated at rest in both patients, on the basis of the musculo-skeletal pain/fatigue symptoms, in November 1995 and March 1996.

2.5. Microbiology

In sterile laminary-flux hood conditions (*Mini Securitas, PBI*) rapid blood-cultures (1–2 min for sampling, insemination and incubation at 37°C) were carried out on Columbia plates under CO2 enriched atmosphere, on 27 February 1993 and on 20 March 1996. Representative colonies of bacteria were then submitted to Gram stain and Catalase test. Species identification was not performed.

2.6. Therapy

Potassium arsenite 0.5% (Fowler's solution 1/2) was administered intramuscularly at low dosages (1 ml/12 h for 10 days; thus 7.5 mg of As/day) from 10 to 20 February 1996. No other medicament was contemporary given. Relapses had occured in both patients after previous treatment with magnesium, selenium and carnitine supplementation (March 1993), tetracyclines (*Bassado,* 200 mg/8 h., os., for 21 days, August 1995) and pirimetamine + sulphametopirazine (*Metakelfin,* 2 tablets/week, 4 times, October 1995).

2.7. Questionnaires

Information on the presence and severity (from 0 to 10) of 12 symptoms related to CFS were collected in two questionnaires and a radial plot, as suggested by Dr David S. Bell in his book [17]. To calculate the radial plot, the patient fills out the questionnaire to determine the severity of each of 12 symptoms, with a range from 0 (no pain or problem) to 10 (very severe) for each symptom noted. The patient completed this questionnaire prior to an appointment with a physician. The answers given by the patient should be representative of a typical day over the past month [17]. The 12 questions were: (1) from 0 to 10, how much fatigue, tiredness, or exhaustion do you experience? (2) How much of a problem is sore throat? (3) How severe are headaches? (4) How much of a problem is aching of the eyes, blurry vision, or light sensitivity? (5) How much of a problem is abdominal pain, bloating, or gas? (6) How much of a problem is pain in your lymph nodes? (7) How much of a problem is depression, mood changes or panic attacks? (8) How much of a problem is pain or aching in your muscles? (9) How much of a problem is memory loss or difficulty concentrating? (10) How much of a problem is poor sleep, insomnia, or waking unrefreshed? (11) How much concern is numbness, tingling, dizziness, or balance problems? (12) How much of a problem is pain in your joints?

2.8. Controls

In order to verify the supposedly CFS-related blood anomalies and to assess the risk of contamination, two co-living healthy relatives non self-reporting fatigue and not having animal contact (the father and brother of the author) spontaneously underwent identical fresh blood smears examinations and blood cultures on 3 March 1993.

3. Results of clinic cases

At first examination (10 February 1993), the results of Weil–Felix reaction were consistent with low serologic titers, respectively 1:50 in patient #1 and 1:100 in patient #2, against *Proteus vulgaris* OX-19 strain, but not against OXK and OX-2. CBC count results were unremarkables. Contemporary, the IgG EBV titers were 1/160 in patient #1 and 1/640 in patient #2. At that time, chronic mononucleosis was thought to be the cause of CFS, and the CFS-like illness were popularly termed 'chronic EBV'. Consequently the two patients were discharged by the physician with a diagnosis of CFS and a therapy based on magnesium, selenium and carnitine supplementation, which was performed in March 1993 and did not produced any benefit. Results coming from the veterinary practice and CFS animal cases suggested a self-made laboratory testing. From February 1993 to March 1996, fresh blood smears taken from patients were examined about every 6 months at light microscopy (× 100), showing the constant presence of numerous micrococci 0.3–0.5 μm in diameter on the surface of some red blood cells (Fig. 1), apparently similar to those already seen in animals with CFS [10–13].

Two blood cultures on Columbia plates, performed on 27 February 1993 on both patients, and immediately incubated into CO2 enriched atmosphere at 37°C, generated

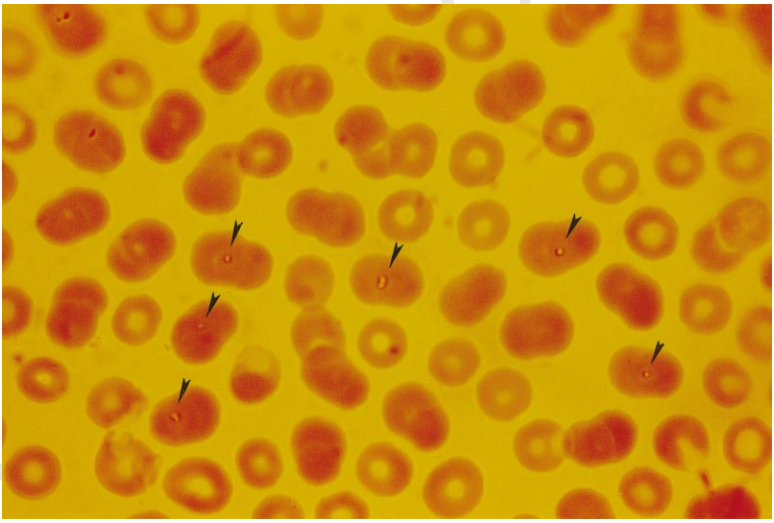

Fig. 1. A person with CFS (the author) and micrococci on the external surface of some red blood cells, before treatment with low dosage Potassium arsenite 0.5% (Fowler's solution 1/2).

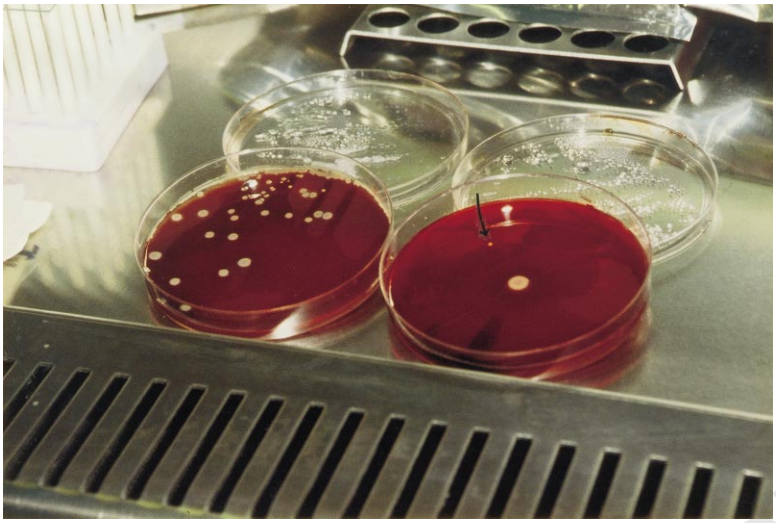

Fig. 2. Rapid blood-culture on Columbia plates, in carbon dioxide enriched atmosphere, from the author (right) and his wife (left). Positive growth of nonpigmented nonhemolitics *Staphylococcus spp.* colonies became evident 3 days after incubation at 37°C. This picture was take at day 5, because initially the colonies were like pin-heads, slow-growing and difficult to see.

slow-growing nonpigmented nonhemolytic small pin-heads-like colonies 3 days later (Fig. 2; right: patient #1; left: patient #2). Picture 2 was taken at day 5, when the colonies had considerably grown, under constant carbon dioxide supplementation. Cocci gram positive (Fig. 3) and catalase positive where then identified in both plates, so the strains were expected to be staphylococci. Unfortunately, identification to the species level could not be performed.

Similar blood cultures from two co-living healthy relatives (father and brother of patient #1), performed in the same conditions on 3 March 1993, did not produced any bacterial growth within 10 days. Repeated blood smears from the two control subjects proved to be micrococci-negative.

3.1. Patient #1

In March 1993, CFS in patient #1 proved to be resistant to magnesium, selenium and carnitine supplementation. The low positive titer (1/50) observed against OX-19 strain in the Weil–Felix reaction (OX2 and OXK resulted negative) suggested a specific treatment with doxycycline (*Bassado*, 200 mg/8 h, os., for 21 days) which was performed in August 1995, without showing any clinical improvement. However small micrococci-like organisms, 0.3–0.5 μm in diameter, were still found attached to the external surface of red blood cells (RBCs) in quantity varying from 10 to 15%. During October 1995, a therapy with an antimalarian drug (*Metakelfin*, pirimetamine + sulfametopirazine; two tablets a week for 4 times) was also attempted and proved unsuccessful.

One month later, in November 1995, a new panel of blood test was carried out: the

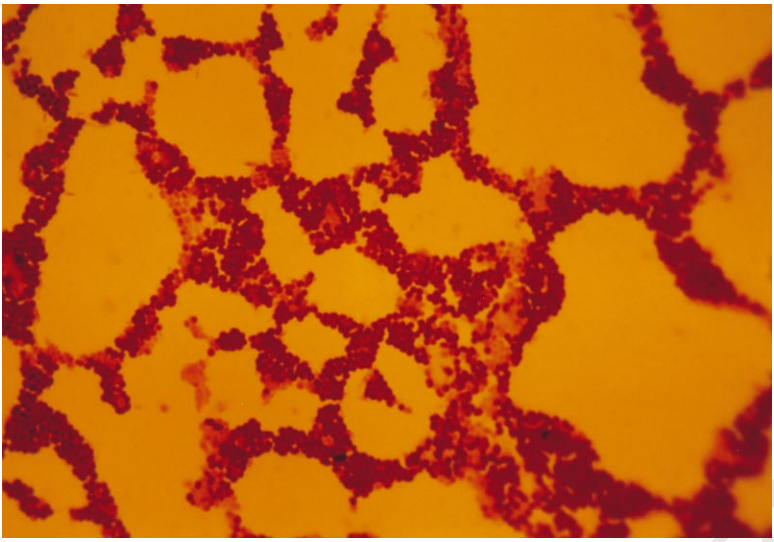

Fig. 3. Staphylococci in a Gram stained smear from a colony derived from the above mentioned Columbia plates.

HIV-1 and Hep. B tests resulted negative and the CD4/CD8 ratio was found to be low (1.74) if compared to the normal mean value (2.0) in humans.

The creatine-kinase blood serum activity at rest (CK = 287 IU/l) was above the normal ranges for humans (24–195 IU/l). The lactate dehidrogenase activity (LDH = 473 IU/l) too, was above the upper normal limit (225–450 IU/l).

The Bell's Questionnaire produced a radial plot score of 138.5, which is in the average range for patients with CFS. Blood smears showed the presence of micrococci over 8–10% of RBCs.

The arsenical treatment with Potassium arsenite 0.5% was performed 3 months later, from 10 to 20 February 1996, at the dosage described in Section 2. At day 0, blood smears still revealed the presence of micrococci upon 8–10% of red blood cells (Fig. 1). In a few days, the weakness decreased and the exercise tolerance improved markedly. At day 4, two fresh blood smears revealed a decreasing percentage of micrococci upon RBCs (2–5%).

The clinical response appeared satisfactory at day 10, when muscular and joint pain had ceased and a reduced percentage of RBCs (1–2%) was carrying micrococci.

Control made at day 40 on fresh blood smears showed the presence of micrococci over only 0.5% of RBCs, blood culture of control proved negative after 5 days, and the Bell's Questionnaire gave a score of 44, supporting the subjective feeling of complete recovery. The creatine kinase (CK = 151 IU/l) and the lactate-dehidrogenase (LDH = 156.3 IU/l) activities were now between the ranges. The CD4/CD8 ratio was slightly increased (1.77). The clinical improvement was evident and during the following 5 years (1996–2001) patient #1 did not suffer from relapses. A great improvement in mood, intellectual function, memory and sexual interest was also noted.

3.2. Patient #2

As in the previous case, different medicament had already been tried without success by patient #2, including magnesium and carnitine supplementation and tetracyclines. In November 1995, serological testing proved negative for HIV-1 and Hep. B and the CD4/CD8 ratio was found to be lower (1.33) than the normal mean value (2.0).

All other laboratory examinations gave results within normal limits, including CBC, CK and LDH activities at rest. Fresh blood smears showed that about 6% of RBCs had micrococci on their surface and the Bell's questionnaire produced a radial plot score of 141.5 points.

The treatment with potassium arsenite 0.5% was performed from 10 to 20 February 1996 at the dosage described and contemporary to the patient #1 therapy. At day 0, blood smears still revealed the presence of micrococci upon 6% of RBCs.

The first control made at day 4 led to the findings of a decreased number of RBCs carrying micrococci (4%), and of disappearance of symptoms related to premenstrual syndrome. At day 10, weakness and joint pain had completely disappeared and a minor number of RBCs (1%) appeared parasitazed by micrococci.

Control made at day 40 revealed a complete recovery from neuro-cognitive disfunctions and exercise intolerance. A reduction of weight without diet changes was also noted. Fresh blood smears resulted negative for micrococci and a blood culture proved also negative 5 days later. The Bell's Questionnaire produced a radial plot score of 8 points, which matches with a healthy status.

During the following 5 years, patient #2 did not suffer from relapses of CFS or premenstrual syndrome, nor received any other medical treatment.

4. Discussion

Two human patients, professionally involved with CFS animal cases and meeting the CDC criteria for CFS diagnosis, were found to be carriers of micrococci in the blood and produced *Staph*-positive blood cultures. Complete recovery and lasting remission, confirmed by 5 years of follow-up (1996–2001), were obtained by intramuscular treatment with low dosage potassium arsenite 0.5% (Fowler's solution 1/2, 1 ml/12 h., for 10 days; thus 7.5 mg As/day), a trivalent inorganic arsenical, administered as single drug. Furthermore, patient #1 (the author) self-reported a great improvement in mood, intellectual function, memory and sexual interest. In patient #2 (wife) symptoms linked to premenstrual syndrome, which is frequently associated with CFS, contemporary disappeared. The differential diagnosis of CFS is potentially vast, and all patients need a thorough history and physical examination to exclude alternative diagnosis. Both patients in this study fulfilled these requirements and relapsed after extensive prior therapy, including tetracyclines at rickettsial dosages (*Bassado*), pirimetamine + sulphametopirazine (*Metakelfin*), magnesium, selenium and carnitine supplementation. This is not in contrast with recent advances in human medicine reporting that serum carnitine deficiency does not contribute to or causes the symptoms of CFS [18], and that Mg deficient CFS patients do not improve after oral supplementation with Mg [19].

A link between CFS and Rickettsial diseases has been suspected [20], due to their similar clinical presentation, but not proved.

The reactivation of a chronic infection due to *Rickettsia prowazeki* (Brill–Zinsser syndrome) is the only rickettsial condition in which negative titers against OX-2 and OX-K and low titers against OX-19 *Proteus vulgaris* strains may be observed [21], as in the two cases described here. However, the very low values obtained and the absence of dermatological lesions during the last 6 months led to the exclusion of the *R. prowazeki* involvement and to a diagnosis of CFS (February 1993) based on symptoms pattern and on high IgG EBV titers (1/160 and 1/640 respectively in patient #1 and #2). Lack of therapeutical response to a tetracycline course performed at rickettsial dosages (*Bassado,* 200 mg/8 h, for 21 days) in August 1995, apparently seem to confirm the exclusion of *R. prowazeki* previously made and, also, of any other doxycycline-responsive etiologic agent, in the two patients concerned.

On the other hand, it is acknowledged in human medicine that persistent infection with a close phylogenetically related microrganism, *Bartonella* (*Rochalimae*) *henselae*, is unlike to be the cause of CFS [22]. In a recent report [20], people originally (a.k.a. wrongly) diagnosed with CFS, tested positive to Rickettsial strains by means of the Giroud Micro-Agglutination test and were successfully treated with tetracyclines, apparently demonstrating how misleading can be a diagnosis of CFS based on clinical presentation only, in the absence of specific test and of an accurate exclusion of alternative causes.

In veterinary medicine also, the aspecific symptoms (weakness, poor appetite, lymphadenopathy) observed in cats with *Haemobartonella felis* infection [23,24], a rickettsial disease, can be superimposed to those of CFS in cats [12]. The two feline conditions may be discriminate on the basis of the blood smears examinations and biochemical and microbiological findings [12,23]. Furthermore, like the close related *Mycoplasma* genus, *H. felis* is always susceptible to doxycyclines [24] and no alternative therapies are indicated in recent literature [25] nor resistances to the specific treatment have ever been reported.

Metakelfin treatment in both patients (October 1995), based on the assumption that CFS is rarely diagnosed in tropical areas and that micrococci-like organisms in the blood may be human babesiae-like *Babesia microti*, proved also unsuccessful. The therapeutic role of malaria chemoprophylaxis is today acknowledged against the immune-mediated Crohn's disease in humans [26] but not against CFS, a condition also associated with several autoimmune aspects but dominated by a different pattern of symptoms.

The use of Potassium arsenite 0.5% as a drug of secondary choice in the treatment of CFS was suggested by its striking effectiveness against a similar condition previously observed in horses [10], birds of prey [13] and dogs and cats [11,12], sharing with the two human cases of this study the presence of micrococci in the blood and *Staph*-positive blood cultures.

Diagnosis of CFS was first made in February 1993, based on exclusion of other known fatigue-related diseases, on high IgG EBV titers and on the presence of criteria meeting the working case definition [1], and confirmed in November 1995 by means of the Bell's Questionnaire [17], based on the revised description of the syndrome [2].

In recent years, it has become clear that elevated EBV antibody titers are not diagnostic

for CFS: some healthy people have high EBV titers and some people with CFS do not [5]. Currently, it is not considered useful to test for antibodies to EBV in a patient with symptoms suggestive of CFS and the etiologic role of Epstein–Barr virus has been ruled out [5]. Today, a case of the chronic fatigue syndrome is defined by the presence of the following:

1. clinically evaluated, unexplained, persistent or relapsing chronic fatigue;
2. the concurrent occurrence of four or more of the following symptoms, all of which must have persisted or recurred during 6 or more consecutive months of illness and must not have predated the fatigue: (a) self-reported impairment in short-term memory or concentration; (b) sore throat; (c) tender cervical or axillary lymph nodes; (d) muscle pain and/or multijoint pain; (e) headaches of a new type, pattern or severity; (f) unrefreshing sleep; (g) post-exertional malaise lasting more than 24 h.

In November 1995, the condition had already had a 3 year history, lacking spontaneous recovery and response to other therapies, with patient #1 meeting the criteria 1 and 2a, 2b, 2d, 2f and 2g, an patient #2 meeting the criteria 1 and 2a, 2c, 2d and 2f, according to current human definition [2,5]. The method used to establish the presence and severity of these and other symptoms has been the Bell's CFIDS Questionnaire (1994) [17]. This is a modification of the method developed by Dr Holmes and his New Zealand coworkers to evaluate abnormalities in laboratory evaluation, presented at the London Myalgic Encephalomyelitis conference in April 1990 [17]. This method is a subjective evaluation of certain symtoms and their severity and requires a certain diagnostic pattern of symptoms to produce the high scores (>50) characteristically seen in CFS [17]. Therefore, it is theoretically possible to differentiate CFS from other illnesses such as depression and somatization, in which the pattern would appear visually different from CFS and the radial plot score would be much less than in typical CFS (<50).

In this report, the patients referred as having CFS apparently matched the clinical picture of CFS in humans [5] and animals [10–13] and had a radial plot score of 138.5 (patient #1) and 141.4 (patient #2) 3 months before the potassium arsenite treatment (November 1995). One month after the therapy (March 20, 1996) the radial plot scores were respectively 44 and 8, and the patterns were visually different from those of depression, somatization or CFS, suggesting a clinical recovery. These subjective findings were accompanyed by the objective observation that, during the same span of time, CK and LDH serum activities returned within the normal ranges in patient #1 and the CD4/CD8 ratios slightly increased in both patients. The presence of micrococci-like organisms in blood smears examined before treatment was suggestive of a chronic low-grade bacteremia and the microrganisms observed were similar to those previously detected in animals with CFS [10–13].

The coincidental finding led to the suspect of a possible link between CFS and micrococci in humans also. This was apparently confirmed by the recovery of two *Staph*-positive rapid blood cultures, producing slow-growing (72 h) nonpigmented nonhemolytic small colonies in both patients. Picture 2 was taken at day 5 when colonies had grown considerably. At day 3, all colonies were far smaller, looking like pin-heads, as the one indicates by the arrow. Concomitantly, 11 blood cultures performed in dogs, cats [12] and

birds of prey [13] diagnosed with CFS also required 2–3 days for bacterial growth in carbon dioxide enriched atmosphere and the colonies were similar to those here reported, small, white or grey-pearl, and produced little if any detectable haemolysis [12]. It has to be noted that carbon dioxide requirement [27–29] and slow growth on solid medium, taking more than 18 h for colonies to be apparent [30], are characteristically linked to staphylococcal small-colony variants (SCVs), which have the same appearance on Gram stain (Fig. 3) [30] and are defined by colony size 10 times smaller than the parent strain [31]. It may be observed that colonies in Fig. 2 are big and do not exactly match the definition, but this picture was taken at day 5, 2 days after the first pin-head-like appearance of all the colonies, which were initially too small, like the one indicates by the arrow, to be photographed.

Now, the major suspect is that the unorthodox procedure, including rapid culture on solid blood medium and carbon dioxide supply, may have favorized the growth of SCVs of *Staphylococcus spp.* in both human cases here described as in the animals with CFS previously described [10–13]. This is not without importante, because recent advances in microbiology show that coagulase-negative [32,33] and *Staphylococcus aureus* [34–36] small-colony variants (SCVs), characterized by nonpigmented nonhemolytic slow-growing pin-head-like colonies, may be be linked to persistent and recurrent infections [30,35–37], such as CFS, and are more resistant to antibiotics than the parent population from wich they arose [30], including some coagulase-negative vancomycin resistant gram-positive cocci [11,38]. The clinical presentation of these infections is readily explained by a reduction in electron transport [35], resulting in a decrease elettrochemical gradient and reduced quantities of adenosine triphosphate (ATP) at disposal [30]. The consequence is an abnormal ion channel function that may be explain the symptoms of chronic fatigue [3].

Antibiotics are not particularly effective against SCVs within endothelial cells. A decrease in electron transport activity account for their resistance to several antibiotics as well as provide a mechanism for persisting within host tissues [30], producing long-standing antibiotic resistant infection.

The intracellular position shields SCVs from host defenses and decreases exposure to antibotics [36]. Frequently, the microbiological diagnosis of these infections remains ambiguous and often these strains are not detectable by conventional microbiological techniques [33]. The use of special microbiological media and prolonged cultivation permit also to stimulate the growth of staphylococcal L-form types from the blood [39]. These bacteria do not have cell walls and can invade the tissues of the hosts avoiding treatment by conventional antibiotics.

The multiple antibiotic resistances and unusual persistent infections due to these staphylococcal variants are not in contrast with recent advances in human medicine indicating a sharp association between toxin-producing *Staphylococus spp.* and CFS [4], a chronic condition that appear no or moderately responsive to prolonged multi-drug antibiotic treatments, frequently followed by relapses [40].

In this study, the isolation of Gram and catalase positive *Staphylococcus* strains from people with CFS handling with CFS animal cases was a picture resembling previously reported associations between toxin producing coagulase-negative and positive *Staphylococcus spp.* and chronic fatigue/pain disorders and CFS in humans [4,41,42], and also

between *Staphylococcus spp.* isolation and CFS in animals with micrococci in the blood [11–13].

In combination with these findings, micrococci observed in fresh blood smears before therapy with potassium arsenite 0.5%, disappeared progressively during the 30 days and three controls made during and following the treatment. The contemporary remission of CFS-related symptoms do not differed from the results obtained using low-dosage arsenical drugs against CFS-resembling human [14,16] and veterinary conditions in recent [10–13] and ancient times [14,15].

In this author experience, the presence of such microrganisms is the only remarkable difference between fresh blood smears taken from healthy and chronically fatigued animals, and apparently was the same in these two human cases. This findings seem to confirm the Dr Luther Lindner (Texas A&M University) report of a newly-identified human blood bacterium (HBB) which is claimed to be present in high number in the blood of persons who have CFS and that cannot be completely eliminated using the standard FDA approved antibiotics. In the two patients with CFS reported here, a treatment with low dosage arsenical medicament caused a reduction in the number of micrococci observed on blood smears in association with clinical improvement. These bacteria were not found in the blood of two healthy relatives, taken as controls and living with patients #1 and #2. Their blood-cultures proved also negative. Taken together, these results apparently exclude a risk of contamination of the Columbia's plates during procedure and seem to confirm the bacterial nature of the micrococci-like organisms observed in the blood (Fig. 1).

In this report, hight CK (>195 IU/l) and LDH (>450 IU/l) serum activity at rest, were detected at first examination in patient #1, but no more at controls made 30 days after arsenical treatment, when the symptoms had disappeared. Increased levels of CK and LDH, the principal muscular enzymes, are occasionally observed respectively in 8% [43,44] and 0.3% [45] of CFS patients, particularly in the acute phase of the illness and in the most affected people.

The laboratory results of patient #1 seem to indicate a possible systemic myopathy and are not in contrast with the frequency with which high CK and LDH levels are observed in horses [10], dogs, cats [11,12] and falcons [13] diagnosed with CFS/CFIDS.

5. Conclusions

In summary, a human cluster of chronic fatigue syndrome experienced complete clinical and hematological remission 30 days after treatment with potassium arsenite 0.5%, an inorganic trivalent arsenical given intramuscularly in low dosages (1 ml/12 h) for 10 days.

The presence of micrococci-like organisms in the blood was associated with CFS-related symptoms and the recovery of *Staph*-positive blood cultures. High muscular enzymes at rest were found in patient #1 before the arsenical treatment, but not more after the clinical recovery, as previously reported in similar animal cases. Although serologic test for CFS do no exist on the market, it seems worthy to suggest that the presence of micrococci in the blood could be used as a coadjutor tool in the diagnosis of CFS, because they apparently are the main hematological difference observed between the blood of healthy and chronically fatigued animals and humans.

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