

IMMUNOLOGICAL ANOMALIES AND THROMBOCYTOPENIA IN 117 DOGS AND CATS DIAGNOSED WITH CHRONIC FATIGUE SYNDROME (CFS)⁺

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Retrospective analysis of immune dysfunctions found in 55 dogs and 62 cats diagnosed with Chronic Fatigue Syndrome (CFS), revealed leukopenia in 11% of dogs (n=6) and 22.5% of cats (n=14), lymphopenia in 14.5% of dogs (n=8) and 10% of cats (n=6), hypogammaglobulinaemia in 9% of dogs (n=5) and 13% of cats (n=8) and thrombocytopenia in 20% of dogs (n=11) and 68% of cats (n=42). All patients had creatine kinase enzyme levels above the normal range (CK = 5–100 IU/L) and carried micrococcus-like organisms on erythrocytes. Blood cultures proved positive for *Staphylococcus* spp. in 16 cases. After low-dosage arsenic-based therapy (thiacetarsamide sodium) all animals experienced complete clinical remission. Subsequent controls demonstrated immune restoration in 4 representative FIV-FeLV negative cats, previously diagnosed with CFS associated with leukopenia, lymphopenia, hypogammaglobulinaemia and thrombocytopenia. The main conclusion is that a CFS-like disease in dogs and cats, characterised by the common hallmarks of high CK levels, absence of known causes of chronic fatigue in animals and presence of micrococcus-like organisms in the blood, can be associated with humoral and/or cellular immune deficiencies in 9–22.5% of cases and with thrombocytopenia in 20–68% of cases. Considerations are made on the possible role of micrococci in the aetiology of the condition and on the similarities with CFS in humans.

Key words: Chronic fatigue syndrome, CFS, dog, cat, lymphopenia, leukopenia, hypogammaglobulinaemia, thrombocytopenia

Chronic Fatigue Syndrome (CFS) is a debilitating illness in humans (Fukuda et al., 1994), complicated by the fact that its diagnosis is largely based on subjective complaints and the absence of reliable tests (Chaudhuri et al., 2000). The condition is also called Chronic Fatigue and Immune Dysfunction Syndrome (CFIDS) due to the frequency by which autoimmune defects (Bell, 1994), cellular (Johnson, 1996) and humoral deficiencies (Hilgers and Frank, 1994) are recorded. Prolonged and unexplained fatigue associated with nonspecific symptoms, such as sore throat, myalgia, arthralgia, adenopathy, allergic reactions,

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sleep disorders and neurocognitive anomalies characterise the disease (NIAID, 1996).

The pathogenesis of CFS remains unknown (Chaudhuri et al., 2000).

Epstein-Barr virus, cytomegalovirus, human herpesviruses type 1, 2 and 6, parvovirus, Coxsackie virus and echovirus have been suspected, at different times, to be the causative agent(s) of CFS in humans. Today it is acknowledged that no direct evidence links any of these viruses to the cause of CFS or its symptoms and their concurrent reactivation or occurrence do not rule out a diagnosis of CFS (NIAID, 1996).

CFS is not widely known to affect animals, although there have been a number of scientific reports indicating that the condition may have zoonotic (Glass, 2000a) and veterinary implications (Ricketts et al., 1992; Tarello, 2001a-g). In the past, leukopenia has been reported in 79% of 32 horses diagnosed with 'Equine Fatigue Syndrome' (Ricketts et al., 1992) and severe humoral immunodeficiencies have been observed in birds of prey diagnosed with CFIDS (Tarello, 2001b). It has been recently confirmed that a subset of animals owned by CFIDS sufferers may show abnormal signs, thrombocytopenia and immunological dysfunctions which mimic CFS in humans (Glass, 2000b).

It seemed thus important to report the retrospective analysis of immunological anomalies found in 117 canine and feline cases diagnosed with CFS during 1993–94 (Tarello 2001c) in which the gamma globulin levels, white blood cells, total lymphocytes and platelet counts were regularly recorded.

Materials and methods

Patients

The immunological status of 55 dogs and 62 cats diagnosed with Chronic Fatigue Syndrome during 1993–94 in Italy (Tarello, 2001c) was studied retrospectively with particular reference to the gamma globulin values, white blood cell (WBC), total lymphocyte (TL) and platelet counts.

Criteria for selection of cases

All animals have been diagnosed with Chronic Fatigue Syndrome on the basis of: (a) clinical presentation dominated by chronic fatigue/lethargy and symptoms and signs similar to those of CFS in humans (Tarello, 2001c, d, f), (b) cytological observation of micrococcus-like organisms in the blood (Fig. 1), (c) serum creatine kinase activity higher than normal ranges (CK = 5–100 IU/L) (Willard et al., 1994), (d) exclusion of other/concurrent causes of chronic fatigue in animals (Tarello, 2002), (e) response to an arsenical drug (thiacetarsamide sodium) in low dosages.

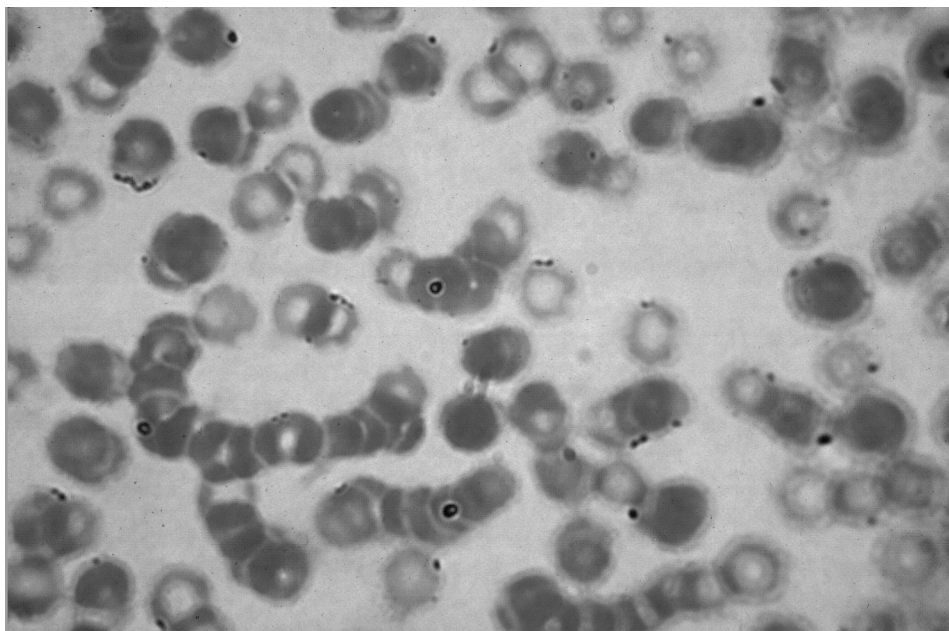


Fig. 1. Micrococcus-like organisms on some erythrocytes in a fresh blood smear from a reported case diagnosed with CFS ($\times 100$, Leitz Biomed)

Serology

Eleven cats with typical symptoms of an underlying immunodeficiency were tested for feline retroviruses FIV and FeLV (Cyte-Combo Elisa, IDDEX) and five cats and seven dogs for serum *Dirofilaria immitis* antigens (Diro-CHECK). Antibodies against *Borrelia burgdorferi* (Cite-Lyme ELISA, IDDEX), *Leishmania infantum* (Leishcan 16), and *Bartonella vinsonii* subsp. *berkhoffii* (indirect immunofluorescence antibody assay, IFA) were searched for respectively in 3, 3 and 5 representative dogs evidencing symptoms which matched the clinical presentation of diseases caused by these aetiological agents.

Biochemistry

Serum values of total protein (TP) and creatine kinase (CK) were calculated in all subjects using a commercial biochemical analyser (Simplicity Macomb).

Haematology

Fresh blood smears, stained with the May-Grünwald-Giemsa or Wright technique, were prepared from each patient and checked for haemoparasites (*Haemobartonella felis*, *Haemobartonella canis*, *Babesia* spp., *Ehrlichia* spp., *Hepatozoon canis*) and other anomalies ($\times 100$, Leitz Biomed).

A Knott test for the search of microfilariae (*Dirofilaria immitis*, *Dirofilaria repens*) was performed on 7 dogs and 5 cats with a clinical picture indicative of heartworm disease or subcutaneous dirofilariosis (Tarello, 2002). WBC count was calculated both automatically (QBC Veterinary Analyser; Becton Dickinson) and manually (Malassez cell with Unopette dilution method) in order to provide the most reliable results. Platelet count was determined uniquely with the QBC Veterinary Analyser. Total lymphocyte count was based on the microscopic ($\times 100$) differential identification of 200 consecutive WBCs. The normal ranges for white blood cell count (WBCs in cats = 5,500–19,500/mcl; WBCs in dogs = 6,000–17,000/mcl), total lymphocyte count (TL in cats = 1,500–7,500/mcl; TL in dogs = 1,000–6,000/mcl), platelet count (Pt in cats = 300–700,000/mcl; Pt in dogs = 200–500,000/mcl) and total gamma globulin (T γ G in cats = 1.5–3.0 gr/dl; T γ G in dogs = 0.8–1.8 gr/dl) were inferred from Bush (1991).

Microbiology

Twenty-two canine and feline cases out of 117 diagnosed with CFS were submitted to rapid blood cultures using sterile equipment (gloves, needles) and conditions (asepsis of the skin). Time as short as possible was required for sampling and culturing, insemination on Columbia agar plates with 5% ram's blood (bioMerieux) under laminar-flux hood (Mini-Securitas, PBI) and incubation at 37 °C in CO₂-enriched atmosphere for 2–3 days. Representative colonies were then submitted to Gram stain, catalase test and speciation (API-Staph, bioMerieux). Similar blood cultures were also performed in a control group of ten animals (five healthy and five differently diseased) in order to assess the risk of contamination (Tarello, 2001c, e).

Electrophoresis

Serum protein electrophoresis was performed using a Cellomatic 2 reader and freshly drawn samples from diseased animals diagnosed with CFS, without delay or refrigeration.

Controls

Checks on the WBC, TL, platelet counts and gamma globulin levels were made on 4 cats 56–135 days after therapy (Table 1).

Results

General

All cats proved *Haemobartonella felis* and *Ehrlichia* spp. negative and 11 representative feline cases out of 62 diagnosed with CFS were found to be FIV and FeLV negative. In five cats the search for antibodies against *Dirofilaria immitis* proved negative as was the search for *D. immitis* and *D. repens* microfilariae.

Table 1

Four cats diagnosed with CFS: laboratory examination results before and after treatment with thiacetarsamide sodium

Test	Cat no. 41		Cat no. 66		Cat no. 67		Cat no. 218			Reference values
	Day 0	Day 135	Day 0	Day 120	Day 0	Day 56	Day 0	Day 64	Day 84	
WBC	1,400*	8,500	7,400	7,300	4,920*	18,400	1,100*	16,400	18,200	5,500–19,500/mcl
Lymphocytes	900*	1,700	3,600	2,400	1,350*	3,000	800*	4,900	3,500	1,500–7,000/mcl
Platelets	110,000*	322,000	318,000	335,000	290,000*	415,000	236,000*	373,000	379,000	300–700,000/mcl
Total protein	6.76	7.20	5.7*	7.2	6.20	6.96	8.90	7.38	8.00	5.8–8.0 g/dl
Albumin	3.31	2.97	3.30	3.44	2.30*	2.70	3.40	3.05	3.08	2.5–5.0 g/dl
α_1 -globulin	0.88	0.82	0.5	0.60	0.96	0.65	0.85	0.62	0.80	0.2–1.1 g/dl
α_2 -globulin	0.82	0.68	0.4	0.62	0.83	0.77	2.05	1.35	1.05	0.4–0.9 g/dl
β -globulin	0.93	0.74	1.1	0.88	1.4	1.28	1.70	0.92	1.15	0.7–1.6 g/dl
γ -globulin	0.82*	1.96	0.4*	1.66	0.70*	1.55	0.90*	1.45*	1.92	1.5–3.5 g/dl
RBCs affected by micrococci	20–25	0	25–30	0	30–40	0	40–50	0	0	%

*Low value

All dogs proved negative for *Babesia* spp., *Ehrlichia* spp., *Haemobartonella canis*, *Hepatozoon canis*, and *Dirofilaria* spp. (Tarello, 2002).

Representative canine cases out of 55 diagnosed with CFS were found to be antibody-negative against *Borrelia burgdorferi* (n=3), *Leishmania infantum* (n=3) and *Bartonella vinsonii* (n=5). All animals had creatine kinase levels above the normal range (CK = 5–100 IU/L), carried micrococcus-like organisms on the erythrocytes (Fig. 1) and found complete clinical remission after a low-dosage treatment (0.1 ml/Kg/day, during three days, i.v.) with an arsenic-based medicament, thiacetarsamide sodium.

Blood cultures from 5 cats and 11 dogs proved *Staphylococcus* spp. positive, since all isolates appeared as Gram positive and catalase-positive cocci (Tarello, 2002c, e). Representative colonies from 11 out of 16 positive plates were submitted to speciation (API-Staph, bioMerieux) producing the following identifications: *S. xylosus* (2 dogs and 1 cat), *S. intermedius* (2 dogs and 1 cat), *S. chromogenes* (2 dogs), *S. epidermidis* (1 dog), *S. cohnii* (1 dog) and *S. lugdunensis* (1 dog). Similar blood cultures performed in a control group of ten animals showed negative results after 5 days of incubation. This group included 4 healthy dogs, 1 dog diagnosed with parvovirus infection, 1 dog with rodenticide intoxication, 2 cats with kidney failure, 1 cat with haemobartonellosis and 1 cat (n=66) formerly diagnosed with CFS associated with *Staphylococcus*-positive blood culture and successfully treated with thiacetarsamide sodium (Tarello, 2001c, e)

Dogs

Leukopenia (WBCs < 6,000/mcl, ranging from 2,500 to 5,900/mcl) was reported in 6/55 dogs (11%), lymphopenia (TL < 1,000/mcl, ranging from 400 to 950/mcl) in 8/55 dogs (14.5%), thrombocytopenia (Pt < 200,000/mcl, ranging from 128 to 198,000/mcl) in 11/55 dogs (20%) and hypogammaglobulinaemia (total γ -globulin < 0.8 g/dl, ranging from 0.5 to 0.7 g/dl) in 5/55 dogs (9%) diagnosed with CFS. In all remaining cases WBC, TL, platelets and γ -globulin were within the normal ranges.

Cats

Leukopenia (WBCs < 5,500/mcl, ranging from 1,100/mcl to 5,500/mcl) was reported in 14/62 cats (22.5%), lymphopenia (TL < 1,500/mcl, ranging from 900 to 1450/mcl) in 6/62 cats (10%), thrombocytopenia (Pt < 300,000/mcl, ranging from 110 to 290,000/mcl) in 42/62 cats (68%) and hypogammaglobulinaemia (total γ -globulin < 1.5 g/dl, ranging from 0.4 to 1.1 g/dl) in 8/62 cats (13%) diagnosed with CFS. In all remaining cases WBC, TL, platelets and γ -globulin were within the normal ranges.

Controls

Post-therapy controls performed 56–135 days later on three cats (n=41, 67 and 218) showing concurrent leukopenia, lymphopenia, thrombocytopenia and hypogammaglobulinaemia, and one cat (n=66) showing severe hypogammaglobulinaemia and a *Staphylococcus*-positive blood culture, lead to the finding of a complete immune restoration (Table 1). Specific biochemical and microbiological anomalies found in cats no. 66, 67 and 218 have already been published in a previous paper from this author (Tarello, 2001c).

Discussion

Results from the present study indicate that both humoral (hypogammaglobulinaemia) and cell-mediated (leukopenia, lymphopenia) immunodeficiencies occur in a subset (9–22.5%) of dogs and cats diagnosed with CFS.

Thrombocytopenia, ranging from mild to medium, was frequent in both dogs (20%) and cats (68%) with CFS.

Representative animal patients thus tested proved also negative for infection with *Haemobartonella felis* and *canis*, *Bartonella vinsonii*, *Babesia* spp., *Ehrlichia* spp., *Dirofilaria* spp., *Borrelia burgdorferi*, *Leishmania infantum* and FIV and FeLV viruses. Search for enteroviruses and herpesviruses was not attempted in the present cases, because today it is acknowledged that no direct evidence links any of these viruses to the cause of CFS or its symptoms (NIAID, 1996). On the other hand, in the author's experience, the presence of micrococcus-like organisms in the blood seems to be a diagnostic criterion for CFS diagnosis in many species including humans (Tarello, 2001a–g). In fact, all patients in this study were found to be carriers of micrococcus-like organisms in the blood and 16 of them produced *Staphylococcus* spp. positive blood cultures (Tarello 2001c, e). These results, coupled with the high frequency with which thrombocytopenia was detected, are compatible with the observation that unconventional forms of *Staphylococcus epidermidis* have already been noted in the circulating blood of human thrombocytopenic patients suffering from immunological disorders (Tedeschi et al., 1976). In these pioneering researches, the multiplication of Gram-positive cocci originating from L-forms carried by platelets of autoimmune thrombocytopenic patients was attributed to the primary platelet damage enhanced following interaction with the bacteria (Tedeschi et al., 1975). Recent confirmation on the involvement of toxin-secreting coagulase-negative *Staphylococcus* spp. in CFS (Dunstan et al., 2001) is also in accordance with the results reported here.

In this study, leukopenia was recorded in 11% of dogs and 22.5% of cats, and lymphopenia in 14.5% of dogs and 10% of cats.

These findings are compatible with the observation that leukopenia has already been reported in a subgroup of Swedish cats affected by a similar syndrome, named 'staggering disease' (Kronevi et al., 1974) and in 79% of horses from UK diagnosed with 'Equine Fatigue Syndrome' (Ricketts et al., 1992). It is interesting also to note that leukopenia (Bell, 1994) and lymphopenia (Johnson, 1996) frequently occur in a subset of human patients diagnosed with CFS (Gupta and Vayuvegula, 1991).

It is acknowledged, in fact, that about 1/10 of human CFS patients shift naturally to the condition called idiopathic CD4+ lymphocytopenia or ICL (Johnson, 1996), which is characterised by decreased CD4+ T cell count in the absence of HIV infection (Smith et al., 1993) and occasional association with leukopenia and pan-hypogammaglobulinaemia (Famularo et al., 1994).

Most cases of ICL, also called HIV-negative AIDS, fulfil the CDC criterions for CFS (Johnson, 1996) and the two conditions appear today as variations in severity of a single disease (Bell, 1994). Decreased CD4+ cell counts lead to a decreased CD4/CD8 ratio, which is of common occurrence in CFS (Bell, 1994). This anomaly has also been observed in two persons diagnosed with CFS in association with *Staphylococcus* spp. bacteraemia responsive to potassium arsenite (Tarello, 2001*d*). Clinical recovery was associated with disappearance of micrococci from the blood and increased CD4/CD8 ratio.

In four FIV-FeLV negative cats (Table 1) with CFS in this study, leukopenia, lymphopenia, thrombocytopenia and hypogammaglobulinaemia produced an immunological picture similar to the human ICL and these anomalies disappeared in 56–135 days after arsenical treatment. Remission of CFS-associated symptoms and negative results from the search of micrococci in the blood were simultaneously observed.

As a consequence of this, the cellular/humoral immunodeficiencies and thrombocytopenia, observed before treatment, appear to be secondary and acquired immune dysfunctions responsive to arsenical drugs in low dosages. Similar results have been obtained in the past using the same medicament in birds of prey diagnosed with CFIDS and affected by severe pan-hypogammaglobulinaemia (Tarello, 2001*b*). Cats with CFS meeting the current human definition (NIAID, 1996) and relapsed after previous unsuccessful treatments, showed increased total protein values when treated with another arsenical medicament, potassium arsenite, in low dosages (Tarello, 2001*f*).

These results do not contrast with the recent observation that treatment with sulphur-arsenic-ferrous water produces an increased activity of immunoglobulin A secretory portion in the nose (Marullo and Abramo, 1999) and with the old knowledge of the therapeutic efficacy of arsenical medicaments against 'asthenia' and 'general debility' in both humans and animals (The Merck Index, 1976).

The unexpected presence of micrococcus-like organisms adhering to the external surface of red blood cells (Fig. 1) was a hallmark in the 117 patients reported here, 16 of which showed slow-growing, small colonies *Staphylococcus* spp. (*S. intermedius*, *S. xylosus*, *S. epidermidis*, *S. cohnii*, *S. chromogenes* and *S. lugdunensis*) positive blood cultures (Tarello, 2001c, e).

In accordance with these observations, human beings suffering from immunological disorders (Tedeschi et al., 1975; Tedeschi et al., 1976) or diagnosed with CFS (Tarello, 2001d) were found to harbour *Staphylococcus* spp. in the circulating blood.

Underestimated and dismissed as mere opportunistic infections without any aetiologic value, *Staphylococcus* spp. infections are nonetheless recorded also in human ICL patients associated with hypogammaglobulinaemia (common variable immunodeficiency: CVID), toxin production and fatal outcomes (Katial et al., 1997). Recent advances indicate that 92% of 678 Russian patients with both cellular and humoral immunodeficiency had a generalised infection manifested by bacterial shock and sepsis (unknown authors, 2001). Furthermore, all the genital *Staphylococcus aureus* strains isolated from 30 patients diagnosed with toxic shock syndrome were found to be toxin-producing and the most striking laboratory finding in this group was the association with a profound lymphocytopenia (Chow et al., 1984). Staphylococcal enterotoxins induce and maintain T-cell anergy (Stark Aroeira et al., 1997).

The loss of B and CD4 cells especially occurring during sepsis and mitochondria-dependent lymphocyte apoptosis are suggested as a contributing factor to the immunosuppression seen in bacterial sepsis (Hotchkiss et al., 2001).

Taken together, all these observations agree with the results reported here and with recent advances in human research that implicate a possible causative role of toxin-producing staphylococci in chronic pain/fatigue disorders (Butt et al., 1998) as well as in CFS (Dunstan et al., 2001).

Toxin-secreting staphylococci can be considered secondary immunodeficiency-causing agents. In fact, some of them produce a group of proteins called superantigens, which are toxins capable of inducing a polyclonal T-cell activation and high interleukin-2 level secretion (Howard et al., 1991).

In the long run, this mechanism may induce autoimmunity, stimulating the growth of a subset of lymphocytes that recognise the 'self' as a stranger. Such nonspecific activation can also lead to immunodeficiency: certain T-cell subpopulations vigorously proliferate and finally die, leaving an open door to opportunistic agents (Howard et al., 1991).

Anomalies, such as the reduction of particular T-cell populations, high interleukin-2 levels and auto-immunity, are commonly described in the human CFIDS literature (Bell, 1994; Johnson, 1996). Surprisingly, a recent *in vitro* study showed that an arsenic drug, sodium arsenite, reduces proliferation of human activated T cells by inhibiting the secretion of interleukin-2 (Vega et al.,

1999). This apparently confirms the results reported here. Staphylococcal superantigens seem to be involved in the pathogenesis of systemic diseases, such as toxic shock syndrome, which is characterised by lymphocytopenia and resistance *in vivo* to antibiotics (Chow et al., 1984).

Similarly, staphylococci found in human CFS patients produced a significant amount of membrane-damaging-toxins, δ - and/or 'horse' haemolysins, whereas those isolated from an ideal musculoskeletal symptom-free control group did not (Butt et al., 1998).

The current medical literature (Waxman and Anderson, 2001) suggests that the mechanisms of action of arsenic derivatives are many and include the induction of apoptosis, partial cytodifferentiation, inhibition of proliferation and of angiogenesis. Some of these mechanisms may also account for the consistent immune enhancement and lasting clinical remission (Table 1) observed in animals diagnosed with CFS and showing cellular and/or humoral immunodeficiencies in 9–22.5% of cases and thrombocytopenia in 20–68% of cases. Superposable anomalies are also observed in a subset of CFS human patients, apparently confirming the similarities between CFS and ICL, and also between CFS in humans and animals.

This work suggests that it may be necessary to remove the retroviral blinkers and look more broadly at the immunodeficiency syndromes in animals and human beings, especially in defining their toxin-producing *Staphylococcus* spp. profile and their possible effective treatments.

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