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REVIEW ARTICLE

Cognitive dysfunction syndrome: updated behavioral and clinical evaluations as a tool to evaluate the well-being of aging dogs

Síndrome de disfunción cognitiva: actualización del conocimiento como herramienta para el bienestar animal en perros seniles

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RESUMEN

La mejora en la atención veterinaria, la nutrición y el enriquecimiento del entorno doméstico ha facilitado que cada vez haya un mayor número de perros que vivan su etapa senil. Sin embargo, se debe tener en cuenta que una mayor longevidad implica una mayor prevalencia de problemas cognitivos en nuestras mascotas. A lo largo de su vida geriátrica los perros son más vulnerables a padecer síndromes demenciales neurodegenerativos de carácter progresivo. Una de las enfermedades neurodegenerativas que actualmente está siendo objeto de estudio por su elevada prevalencia es el síndrome de disfunción cognitiva. La enfermedad es una entidad neurocomportamental que se presenta en perros ancianos, caracterizado por un déficit en el aprendizaje, en la memoria y en la conciencia espacial del perro, además de ser un trastorno clínico que tiene un impacto significativo en la vida de los perros de edad avanzada y sus propietarios. La escasez de pruebas diagnósticas de alta confiabilidad que garanticen la presencia o no de la enfermedad, otorga a la identificación temprana de los signos clínicos, un papel crucial para el establecimiento de un buen pronóstico en términos de mejoría y extensión en la calidad de vida de los pacientes afectados. El tratamiento debe dirigirse a frenar la progresión de la pérdida de cognición, mediante la instauración de técnicas y programas que mejoren la actividad física, los aspectos nutricionales y el ambiente del animal. Por lo tanto, son necesarias futuras investigaciones en el conocimiento, control y prevención de esta patología, para disminuir el impacto generado por sus efectos sobre la calidad de vida de los perros y sus propietarios.

Palabras clave: síndrome de disfunción cognitiva, perros, proteína β-amiloidea.

SUMMARY

Improvements in veterinary medicine, nutrition and the enrichment of the domestic environment have helped more dogs reach to their senile phase. However, greater longevity leads to a higher prevalence of cognitive problems in our pets. Throughout their geriatric lives, dogs are more vulnerable to suffering progressive neurodegenerative dementia syndromes. Due to its high prevalence, cognitive dysfunction syndrome is one of the most studied neurodegenerative diseases. This disease is a neurobehavioral entity that presents itself in senile dogs, and it is characterized by a deficit in learning, memory and the dog's spatial awareness. It is a clinical disorder that has a significant impact on older dogs and their owners. There is a lack of reliable diagnostic tests that can guarantee the presence of the disease, provide early identification of the clinical signs of this disease, or establish a prognosis in terms of the recovery and expansion of the quality of life of the affected patient. Treatment should be directed at slowing down the progression of cognitive loss through the establishment of techniques and programs that improve physical activity, nutrition and the animal's environment. Therefore, future research on the knowledge, control and prevention of this pathology are necessary to lower the impact generated by its effects on the quality of life of the animal.

Key words: cognitive dysfunction syndrome, dogs, β-amyloid protein.

INTRODUCTION

Interest in the study of the brain's aging process and the behavioral changes linked to the age of senile dogs has increased in the last decade, a consequence of the increase in the prevalence of cognitive dysfunction problems (Landsberg and Ruehl 1997).

Due to improvements in veterinary service, nutrition and the enrichment of the domestic environment, there are more dogs living to their senile phase. In fact, some authors have calculated that 25% to 50% of the dogs in Europe are older than 7 years old, and in the United States, more than 7.3 million dogs reach age 10 (Bonagura 2000). However, greater longevity leads to a higher prevalence of cognitive problems in our pets (Crook and Larrabee 1991). For this reason, it is necessary for the majority of owners to address the aging process and for veterinarians to be more knowledgeable on the physical and mental consequences of aging.

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Throughout their geriatric life, dogs, just like humans, are more vulnerable to suffering progressive neurodegenerative dementia syndromes (Borras *et al* 1999, Head 2001). In humans, one of the most important neurodegenerative disorders is Alzheimer's disease (AD), while in canines, one of the most important neurobehavioral syndromes is cognitive dysfunction syndrome (CDS), or canine counterpart of Alzheimer`s disease (Ruehl *et al* 1995). These types of pathologies are associated with central nervous system disorders during the process of aging (Pérez- Guisado 2007).

Canine cognitive dysfunction, or "canine dementia," is a neurobehavioral syndrome that presents itself in senile dogs; it is characterized by learning, memory and spatial awareness deficits in the dog. Likewise, there are alterations in social interactions and sleeping patterns (Landsberg *et al* 2003). However, some authors state that there is a certain degree of physiological declination in senescence that determines a decrease in the speed of information recompilation, processing, holding and decision-making (Head 2001, Boutet *et al* 2005).

Some authors have outlined the similarities between the changes associated with age in human and canine brains, concluding that dogs may serve as a useful model for the extrapolation of Alzheimer's disease and other human neurodegenerative illnesses in aging studies (Borras *et al* 1999).

Due to the similarities between these two dementia syndromes in humans and dogs, studies in senile dogs with CDS are a useful tool for the recognition of some clinical and pathological aspects of AD that have not yet been clarified in humans and may therefore facilitate more efficient management of the disease (Cummings *et al* 1995, Ikeda-Douglas *et al* 2005). However, despite these similarities and the link between many of the predisposing factors for both these pathologies, some differences also exist between these pathologies. Some studies have identified the contribution of other factors that predispose humans, but not canines, to diseases such as AD, including positive family records (Jorm 2000) and genetic susceptibility (positive APOE e4 gene, APP, PSEN1, PSEN2) (Caermelli *et al* 1999, Wang *et al* 2008,). The brains of human AD patients display typical microscopic characteristics, including the presence of neurofibrillary tangles (NFTs), Hirano bodies (HBs), and granulovacuolar degeneration bodies (GVDs) in the parietal cortex and hippocampus. Microscopically, the brains of aged dogs with cognitive dysfunction exhibit neuronal depletion and vascular hypertrophy in the cerebral cortex and hippocampus. However, NFTs, HBs, and GVDs are not found. The reason for the absence of these protein aggregates in dogs with cognitive decline remains poorly understood. One presumption is that the earlier death of dogs precludes this protein accumulation (Yu *et al* 2011). Yu *et al* (2011) detected moderate levels of ubiquitin in the cerebral cortices and hippocampi of humans with Alzheimer´s disease. In the brains of aged dogs with SDC, ubiquitinated bodies were detected in 83% of the cases. Despite the great progress that comes with the use of an experimental model, such as that from dogs that develop a neurodegenerative disease with many similarities to that observed in humans (Cummings *et al* 1995), the results obtained from the use of this model will not always reproduce information that permits complete extrapolation from animals to humans. The techniques employed to evaluate cognitive functions differ in their ability to qualify certain functions, such as the perception, differentiation, storage, and recuperation of cognitive flexibility (Boutet *et al* 2005).

For a dog, reaching the senile stage implies the development of certain cognitive changes. Some dogs grow old without experiences any progress in cognitive dysfunction, while others may exhibit mild or pathological cognitive changes.

Age-related neuropathology in dogs was first observed by Lafora in 1914, when he reportedanomalies in the dendrites of hippocampal pyramidal cells, a finding similar to that observed in humans. In 1997, this syndrome was described by Landsberg and Ruehl as an entity that not only causes behavioral problems but also leads to a deterioration of the organs and sensory functions as well as a decrease in cognitive function (Landsberg and Ruehl 1997). The disease was observed in dogs over 9 years old (Ingram and Williams 2002), and it was established that this disease is caused by chemical and physical changes in the brain that are attributable to normal aging (Godoy 2004).

EPIDEMIOLOGY OF COGINITIVE DYSFUNCTION SYNDROME IN DOGS

Some studies show that the CDS is very prevalent and under-diagnosed, and it exerts a significant impact on the life of elder dogs and their owners (Salvin *et al* 2010). While many studies on the prevalence of CDS have identified CDS as a common problem in old dogs (Neilson *et al* 2001, Osella *et al* 2007, Azkona *et al* 2009), epidemiology studies suggest that the disease is under-diagnosed in up to 85% of potentially affected animals (Salvin *et al* 2010). Its prevalence has not been established in many countries of Europe or America, but a poll taken by 981 owners of dogs older than 7 years in the United Kingdom suggested that approximately one-third of the dogs showed signs of confusion, restlessness and less enjoyment of life, while 1 out of 5 showed an increase in the incidence of habitual hygiene problems (Heath *et al* 2007). A study conducted in Italy that included 124 geriatric dogs showed a CDS prevalence of nearly 50%, in which 75 dogs over 7 years old showed signs that correlated with the disease (Osella *et al* 2007). An American study included 97 bitches and 83 castrated dogs, all between the ages of 11 and 16. The results of this study showed that 28% of dogs 11-12 years

old showed alterations in one or more of the behavioral categories linked to CDS (orientation, interaction with people and other animals, alterations in sleep and guard, inappropriate elimination) (Landsberg *et al* 2003) and that 10% showed alterations in two or more behavioral categories. On the contrary, 68% of the dogs aged 15-16 showed alterations in one or more categories, and 35% showed alterations in two or more categories (Neilson *et al* 2001). Regarding gender, females showed a higher prevalence of behavioral alterations linked to CDS (76%) than males (65%) (Godoy 2004).

These conclusions suggest that, just like in humans, increasing age is a determinant factor of the prevalence of these types of diseases (Adams *et al* 2000, Bain *et al* 2001, Neilson *et al* 2001, Azkona *et al* 2009). Nevertheless, data concerning the global prevalence of this syndrome have not been obtained, partially due to the tendency of a great number of owners to not report the possible behavioral changes in their senile pets to the veterinarian. Additionally, many owners probably assume that these changes are untreatable aspects of age, which has most likely limited the acquisition of precise data that could help estimate the prevalence of CDS on a global level (Osella *et al* 2007).

PHYSIOPATHOLOGY OF COGNITIVE DYSFUNCTION SYNDROME IN DOGS

From a physiological point of view, CDS also presents organic disorders that have been widely studied with diagnostic clinical tools. CDS is a complete physiopathological process in which one of the early events is oxidative damage linked to age (Anderson *et al* 2001, Milgram *et al* 2002, Cotman *et al* 2002, Skoumalova *et al* 2003, Rofina *et al* 2004).

The mitochondrial genome participates in the pathogenesis of these diseases, as there is a decrease in the activity of the respiratory chain complex. These defects are associated with an oxidative-antioxidative imbalance, in which underlying alterations in energetic metabolism induce cellular degeneration (Cumsee *et al* 2006). It has been demonstrated that both mitochondrial dysfunction and apoptosis contribute to the development and progression of mitochondrial and degenerative diseases (Liu *et al* 2009). The brain is particularly susceptible to oxidative damage due to its high oxygen consumption ratio, high energy demand, great abundance of polyunsaturated fatty acids and lipids and its relative antioxidant capacity compared to other organs. Generally, 2% of the oxygen consumed by cells during oxidative phosphorylation turns into ROS (reactive oxygen species), suggesting that oxidative damage may be an early factor in this disease's pathogenesis (Cerdá *et al* 2009). Studies show that both the extension of oxidative injury and the amyloid load are related to the severity of the dysfunction in both cats and dogs (Cummings *et al* 1996, Rofina *et al* 2004).

The central nervous system's characteristic senile plaques are injuries of the neuropil, a spheroid structure found in the extraneural space. Neuropil is mostly composed of a protein called β-amyloid (Guimera *et al* 2002), which is a peptide of variable length between 39 to 43 amino acids and a size of 4-6 kDa. This protein is a natural product of the metabolism of the amyloid forerunner protein, which possesses the structural characteristics of a membrane protein (Gra *et al* 2002). It has been reported that an increase in β-amyloid deposits is directly related to the severity of CDS in dogs (Landsberg *et al* 2003). Some years ago, a third type of deposit was discovered called ubiquinated bodies, which are mainly made of ubiquitin, a small protein composed of 76 amino acids; this protein folds itself into a compact globular structure. Its name is based on its abundance and ubiquity. This protein is only found in eukaryotic organisms, where it appears in a free form or covalently linked to other proteins (Briones *et al* 2010).

Studies performed by Briones *et al* (2010) in canine brains older than 10 years showed the presence of the same abnormal protein deposits that are present in human Alzheimer's disease, such as senile plaques, ubiquinated bodies, and amyloid angiopathy, but they did not find any neurofibrillary tangles. These tangles are composed mainly of a protein called tau, which is the main representative of a class of proteins associated to microtubules, usually called MAPs (Microtubule-Associated Proteins) (Morelli and Castaño 2004). This protein facilitates a process of hyperphosphorylation that results in the formation of paired helical filaments, which saturate the cytoplasm and generate the destruction of microtubules and neurofilaments, promoting neuronal dysfunction (Goedert 1993, Pérez 2007[a]). Tau pathology has been studied in animal brains, but neurofibrillary tangles (NFTs) have not been observed in the brains of aged dogs (Uchida *et al* 1992). Neurofibrillary plaques are not common in other species, and they are one of the main differences that exist between SDC and EA, as dogs do not develop these structures. This is partially because the tau protein sequence is different in dogs than in humans, which could affect the formation of double helix filaments and ultimately the formation of plaques. However, recent studies suggest that incipient plaques in the brains of aged dogs most likely do not reach maturity (Head 2001). The phosphorylation sites of tau in the canine brain have been studied, but these have not been fully elucidated. The phosphorylation of tau-1 sites, such as Ser189 and 207 of the amino acid sequence, has been reported in dogs (Wegiel *et al* 1998), but Colle *et al* (2000) demonstrated that the brains of aged dogs were not labeled by an antibody that recognizes human phosphorylated Ser194 and Ser202 residues.

Additionally, Pugliese *et al* (2006) showed a concurrent but spatially independent presence of amyloid diffuse plaques and tau hyperphosphorylation in the bra-

ins of dogs with CDS, along with augmentation of tau hyperphosphorylation, immunoreactivity and an increase in cognitive deficits. In the absence of clinical signs of cognitive dysfunction, discrete tau hyperphosphorylation in animals with slight cognitive deficits may reflect normal aging in dogs (Pugliese *et al* 2005). Because the presence of cognitive dysfunction, whether slight or severe, is also age-dependent, further experiments performed with a large number of animals are needed to properly interpret the relationship between aging, cognitive dysfunction, and tau phosphorylation (Pugliese *et al* 2006).

In the same study, the majority of the canines (70%) showed signs of dysfunction at the time of the survey. The dogs that showed a higher prevalence of dysfunction were those older than 15 years (88%), indicating that higher age is associated with more ubiquitin deposits and greater cognitive deterioration. Therefore, it can be speculated that ubiquitin bodies may be one of the causes of cognitive impairment in geriatric dogs. It has been shown that β-amyloid is a deposit related to normal aging rather than to pathology, which conflicts with the results of other studies, such as that of Mentzel (2005), who argues that cognitive impairment is associated with a more extensive formation of plaques (Briones *et al* 2010).

Recent research indicates that brain aging could be related to an alteration of the corticotrophic axis and the production of cortisol (Laughlin and Barret-Connor 2000, Ferrari *et al* 2001). Other studies have evaluated the association between dehydroepiandrosterone levels (DHEA) and the cortisol hormone throughout the determination of blood parameters, with the aim of establishing the impact of this association on the aging of the brain (Villegas *et al* 2011). These authors found that cortisol levels tended to increase with age, and when the influence of gender was evaluated, geriatric females with cognitive impairment showed significantly higher cortisol levels than healthy females of the same age.

On the other hand, it is important to emphasize the presence of abnormal brain neurotransmitter levels in the pathophysiological changes that occur in CDS in dogs, such as the documented decrease in the levels of dopamine and the D2 dopamine receptor. In some primates that have suffered from cognitive dysfunction, low levels of norepinephrine have also been found (Osella *et al* 2005). Both these catecholamines and the serotoninergic system are affected. It has been observed that the brain levels of this neurotransmitter and its metabolites in the cerebrospinal fluid are low in neurodegenerative diseases (Osella *et al* 2005). Monoamine oxidase (MAO) is increased in old animals, so it may influence the observed decrease in levels of these neurotransmitters (Rosaldo 2006). Decreases in the learning capacity as a consequence of aging seem to be related to alterations in the function of the hippocampus and its cortex-directed projections (Manteca 2003).

Other characteristics of CDS in dogs include the dilatation of the brain ventricles, alterations in brain circulation that reduce blood flow, a decreased number of neurons in the brain and cerebellum, meningeal fibrosis, white substance degeneration, atrophy of the central nervous system (CNS), decreased action of neurotransmitters (serotonin, acetylcholine and dopamine), and an increase in the existence of monoamine oxidase B. MAOB controls the catabolism of dopamine, resulting in a greater liberation of free radicals with an affinity for the unsaturated fatty acids present in the cellular membrane, which produces membrane damage and cellular death (Landsberg 1998, Campbell *et al* 2001, Milgram *et al* 2002, Rofina *et al* 2004). It is rare to find atherosclerosis, ischemia and brain hemorrhage in old dogs; however, there can be microhemorrhages and infarctions in blood vessels located around the brain ventricles with a higher frequency. Non-lipidic atherosclerosis is commonly observed in dogs due to the fibrosis of the blood vessel walls, a proliferation of the vascular endothelium, hyalinization and mineralization. These types of angiopathies may compromise blood flow towards the brain and the use of glucose (Landsberg *et al* 2003).

CLINICAL SIGNS OF THE DISEASE

In CDS, the term "cognition" refers to mental processes such as perception, conscience, learning, memory and decision-making, which allow the individual to obtain information from the environment and make decisions, act and function in a normal way (Shettleworth 2001). In this sense, the cognitive deficit observed in patients with CDS refers to a decreased capacity to obtain information, process it, retain it and make decisions that result in behavioral changes (Head 2001, Landsberg 2005).

The most notorious clinical signs are changes in behavior, which tend to be mild during the initial stages of the disease and gradually progress, with the eventual appearance of manifestations of memory loss, such as the inability to recognize family members, forgetting things that were once learned, difficulty in performing simple tasks, alterations in the sleep-wake cycle and inappropriate elimination as a result of the inability to remember formerly established elimination places (Head 2001, Campbell *et al* 2001). Phobic processes that did not originally exist may appear, and the pet's insecurity may limit its social interaction. Likewise, signs of separation anxiety, aggressiveness and compulsive problems may be observed, which may lead to disorders in social relations (Landsberg 1998) and result in the rejection of some owners to assume the care of their pets, increasing their risk of being abandoned or put down (Gallego *et al* 2010).

Signs of behavioral alterations in geriatric dogs are sometimes considered to be normal features of the aging process; however, it is important to differentiate between these mild decreases in psychomotor activity, or "normal

aging," and those that are related to serious damage of the cognitive processes, i.e., "pathological aging" (Rofina *et al* 2001, Head and Zicker 2004, Osella *et al* 2007).

DIAGNOSIS

Normal cognitive aging is complex, and there are many difficulties that lead to the establishment of a correct diagnosis that may allow for the differentiation of the physiological process of aging from a pathological process related to dementia (Lorenzo *et al* 2003). The scarcity of trustworthy diagnostic tests that can guarantee the presence or absence of the disease, allow for the early identification of clinical signs, and help establish the prognosis in terms of improvement and the extension of the affected patients' life span (Gallego *et al* 2010).

In geriatric human beings, as well as in geriatric dogs, several metabolic and functional diseases should be considered before excluding a diagnosis of dementia (AD and CDS, respectively). Therefore, the most useful diagnostic algorithm should encompass a complete physical and neurologic evaluation, a complete blood profile, biochemical assessment, urine analysis, and abdominal ultrasound to evaluate the presence of metabolic diseases (Windsor and Olby 2007) before utilizing advanced diagnostic imaging.

However, it should be noted that diagnosing a medical condition does not exclude the possibility of concurrent unrelated cognitive dysfunction (Frank 2002). The diagnosis of CDS is made by pathological assessment once other medical and behavioral causes are ruled out (Landsberg *et al* 2011).

IMAGING DIAGNOSIS

There are several studies that have demonstrated the usefulness of different diagnostic techniques for CDS, including nuclear magnetic resonance (NMR), positron emission tomography (PET), lumbar puncture (LP), computed axial tomography of the cranium (CAT), brain biopsy, biological markers, genetic studies and diagnostic ultrasound (Llibre and Guerra 1999).

Magnetic resonance imaging (MRI) has been used as a noninvasive and useful diagnostic technique for evaluating age-related brain disorders, such as Alzheimer´s disease, Parkinson´s disease, and dementia, indicating the severe atrophy of the brain, hippocampus, amygdala, temporal lobe, and gray matter and the dilatation of the ventricle space and cortical sulci (Drayer *et al* 1988, Murphy *et al* 1992, Cuenod *et al* 1993).

Investigations performed in senile beagle dogs proved that, using nuclear magnetic resonance as a tool, volumetric changes could be found in the brains of aged dogs. These studies found a significant increase in the ventricular volume of the dogs after the age of 11 (Su *et al* 1998); additionally, most of the injuries were observed in the frontal cortex and caudate nucleus. This finding suggests that the frontal lobules could be particularly vulnerable to changes in age (Su *et al* 2005).

Magnetic resonance imaging is also useful in assessing the risk of cerebrospinal fluid collection at the cerebellomedullary cistern. This is the last step in ruling out any neurologic diseases that might mimic CDS, such as inflammatory, infectious, or neoplastic diseases (Golini *et al* 2009).

In recent years, voxel-based morphometry (VBM) has emerged as a technique to examine regional brain changes associated with normal and pathological aging. Despite its popularity in studies of human aging, the application of VBM in animal models of brain aging is rare. VBM techniques were developed to validate earlier region of interest (ROI) measures of brain aging in dogs and to provide a more comprehensive analysis of local changes in a canine model of brain aging. Specifically, aged males exhibited greater decreases in the internal capsula and cranial nerve bundles compared to decreased volumes in the alveus of the hippocampus in old female dogs (Tapp *et al* 2006).

Furthermore, white matter (WM) changes observed *in vivo* in dogs may reflect degenerative changes in myelin sheath integrity or a loss of oligodendrocytes concomitant with aging in humans (Peters 2002[a]) and non-human primates (Peters 2002[b]). The present VBM analysis suggested that morphological brain aging in beagles varies regionally. Further, the present results suggest that, although both sexes show brain atrophy in the frontal and temporal lobes, the extent of brain aging in the frontal lobes is greater in males, while females exhibit greater aging in the temporal lobes (Tapp *et al* 2006).

Positron emission tomography (PET) is a research technique that allows for the quantitative assessment of the rate of glucose utilization and oxygen consumption. Most patients with Alzheimer's disease show cerebral hypometabolism compared with age-matched controls. These changes correlate with disease severity and may be correlated with neuropsychological test performance. The value of PET studies in determining the stage of disease, documenting progression, and assessing the effects of treatment is unknown (McKhann 1984).

The electroencephalogram has been used as a tool that allows for differential diagnosis with epilepsy processes in the temporal lobe, where behavior changes are often observed. Investigations in human medicine show that patients with Alzheimer's disease exhibited significantly lower D2 and L1 values than those for age-approximated healthy controls. These results suggest that brains afflicted by Alzheimer's disease demonstrate behaviors that are less chaotic than those of normal healthy brains. This paper shows that non-linear analysis can serve as a fruitful tool for detecting relative changes in the complexity of brain dynamics, which cannot be detected by conventional linear analysis. The authors propose that

non-linear dynamic analyses of the EEGs from patients with Alzheimer's disease will be a diagnostic modality in the appropriate clinical setting (Jeong *et al* 1998).

BIOMARKERS DIAGNOSIS

Laboratory biomarkers are important milestones for confirming or establishing a diagnosis in many diseases. They are expected to be sensitive tools for guiding evidence-based medical therapy (Herrmann and Obeid 2011). Some biomarkers possess biological activities and are thought to be toxic to the central nervous system. If so, they may actually be a causal agent that may contribute to disease pathogenesis. In AD, variations of the β peptides, tau, phosphorylated tau, lipid peroxides, peroxide products, and toxic sugars are associated with disease. Neuropathological examination of cerebrospinal fluid (CSF) permits a definitive diagnosis of AD (Gendelman 2007). A recent study carried out in healthy beagles (4–16 years old) showed that levels of Aβ1-42, but not Aβ1-40, decreased slightly in the cerebrospinal fluid (CSF) as brain amyloid deposition increased with age (Head *et al* 2010).

CDS in dogs is not only important as a model of AD for research purposes but is also important from a clinical and veterinary point of view. Therefore, there is an urgent need for reliable biomarkers that can detect the onset of brain amyloid pathology before irreversible neurodegeneration occurs.

The availability of biomarkers and more refined neuropsychological tests in veterinary clinics may enable the definition of a canine model for mild cognitive impairment in the near future (Sarasa and Pesini 2009). Recent research has found that the canine model is a suitable model for AD from the molecular point of view because APP and most of the enzymatic machinery for its processing bear extensive homology between dogs and humans (Sarasa and Pesini 2009).

Plasma Aβ1-42 and Aβ1-40 peptides have been proposed as non-invasive peripheral biomarkers to distinguish between cognitively healthy people and patients with mild cognitive impairment (MCI) with a high degree of sensitivity and specificity (Mehta *et al* 2000, Lopez *et al* 2008, Blasco *et al* 2008).

Studies by González-Martinez *et al* (2011) analyzed plasma levels of Aβ1-42 and Aβ1-40 in relation to age and cognitive dysfunction in dogs. Their study hypothesized that plasma Aβ peptide levels would differ among the different groups of animals and would relate to the presentation of cognitive dysfunction in the aged groups (Pesini *et al* 2009).

The concentrations of these two Aβ isoforms were correlated, and Aβ1-40 levels were higher than Aβ1-42 levels in all the study groups. Both of these features agreed with the results reported in the majority of similar studies conducted in humans (Schupf *et al* 2008). Plasma Aβ peptide levels tended to decrease with age in cognitively intact dogs (i.e., in young, middle-aged and cognitively unimpaired age groups). In particular, dogs less than 4 years old showed significantly higher Aβ1-42 and Aβ1-40 levels, as well as higher Aβ42/40 ratio values, than those greater than 9 years old (González-Martinez *et al* 2011). This finding suggests that the observed reduction of plasma Aβ1-42 and Aβ1-40 concentrations in cognitively unimpaired aged dogs might also be a consequence of increasing brain amyloid deposition. Increases in Aβ1-42 plasma levels and the Aβ42/40 ratio were detected in dogs suffering from CDS when compared with cognitively unimpaired dogs (González-Martinez *et al* 2011).

The investigation of brain aging in dogs also deserves attention from a veterinary point of view. As in the case of human medical technologies, improvements in veterinary medicine and husbandry have contributed to the longer lifespans of our domestic animals, with the unwanted side effect of a greater incidence of age-related neurodegenerative diseases (Landsberg 2005). Due to this, pioneer investigations have designed tools that have produced highly specific and sensitive antibodies against the two major isoforms of the Aβ amyloid to aid in this research, and they are currently exploring the use of these Aβ immunogens in the search for premorbid biomarkers and immunotherapeutic strategies (Sarasa and Pesini 2009).

On the other hand, Ray *et al* (2007) showed a molecular test for Alzheimer`s disease could lead to better treatment and therapies. They found 18 signaling proteins in blood plasma that could be used to classify blinded samples from Alzheimer`s and control subjects with close to 90% accuracy and to identify patients who had mild cognitive impairment that progressed to Alzheimer`s disease 2-6 years later. Biological analysis of the 18 proteins points to systemic dysregulation of hematopoiesis, immune response, apoptosis and neuronal support in presymptomatic Alzehimer`s disease. Therefore, this findings in proteomic and metabolomic could open the way for diagnosis and pharmacologic response to a therapeutic agent, that in the future could be used in animal models and veterinary medicine.

BEHAVIOR DIAGNOSIS

The zoopsychiatric evaluation plays a fundamental role in identifying dementia and in its differential diagnosis. The majority of signs are the result of psychiatric alterations that are consequences of pathological processes that have previously altered the organism. In this sense, and according to some authors, the most effective way to detect this condition is through the use of behavioral questionnaires in the routine geriatric clinic (Head 2001, Head and Zicker 2004), obtained from a series of questionnaires that intended to clarify the patients' CDS-affected behavior (Rofina *et al* 2001, Cotman *et al* 2002). The ARCAD scale (evaluation of age-related cognitive and effective disorders) has also been presented (Colle

et al 2000, Pageat 2001), in which the dogs' behavior is analyzed through a "formal questionnaire" that assesses the behavioral categories affected by cognitive and affective disorders, including orientation, recognition of family members and other animals, inappropriate elimination at home, and alterations in sleep-wake patterns.

A large number of theories and scales has been proposed to confirm CDS in dogs (Colle *et al* 2000, Landsberg *et al* 2003, Pugliese *et al* 2005). However, the selection of scoring criteria has not been clear and tends to be subjective. Although these data provide a useful basis, it is important to perfect the identification and classification of such high-prevalence behaviors to evaluate the senile dogs' cognitive profile as an adequate clinical evaluation tool.

Studies conducted by Salvin *et al* (2011) distributed polls to dog owners from Australia, the United States, New Zealand and the United Kingdom, and they found that behavioral signs support the neuropathophysiology of canine patients with CDS with a high level of precision. This poll, named the CCDR (canine cognitive dysfunction rating) evaluation scale, evaluates 13 behavioral items related to problems in orientation (blank stare, disorientation and getting lost at home), memory (lacking in owner recognition and inappropriate elimination at home), apathy (decrease in the amount of time dedicated to activity), smelling inability (difficulty to find food) and locomotion. This study shows a high diagnostic accuracy (99.3%) and correct estimation of the prevalence in senile dogs with this disease, due to its favorable psychometric properties (Salvin *et al* 2011).

A definitive diagnosis of the disease requires histopathologic confirmation. Clinical diagnosis of possible cognitive dysfunction may be made in the presence of other significant diseases (McKann *et al* 1984). The aged dog brain could be a useful model for understanding the pathogenesis of early AD, and these animals represent a valuable resource for the preclinical testing of therapeutic approaches that could be used to diagnose and treat this devastating disorder in man (Yu *et al* 2011).

TREATMENT

Treatment must be focused on slowing the progression of cognitive loss through the use of the following techniques and improvement programs, with emphasis on physical activity, nutrition, environmental enrichment and psychopharmacology. The ideal therapy should include all of the indicated techniques because they possess a synergic effect that translates into better results.

PHYSICAL ACTIVITY

Recent research has shown that physical activity contributes to healthier brain aging, and this research has increased the interest in understanding the influence of physical activity as a potential protection factor against deterioration and dementia (Lautenschlager *et al* 2011). Physical activity intervenes in different ways in cognitive efficiency, visual, verbal and working memory, execution functions, and visual and auditory attention.

Physical activity improves blood flow to the brain, decreasing infarct risk, dementia and cognitive decline. Activity could stimulate the growth of nervous cells in the hippocampus, the brain region that participates in memory functions. According to experts, this would help the brain build a sort of reserve to prevent future mental impairment (Landsberg 2005, Pérez-Guisado 2007). On the other hand, physical activity has been proven to exert effects over angiogenesis, synaptogenesis, increases in the brain's blood flow, decreases in inflammation and changes in neurotransmitter balance, which results in an improvement in the syndrome (Lista *et al* 2010).

Some studies have shown the impact of physical exercise as a brain protection factor against the negative effects of chronic stress, stating that one of the main subjacent mechanisms of the way in which chronic stress damages the brain is an increase in oxidative stress with an increase in lipid peroxidation, which later exerts effects on neurogenesis in the hippocampus (Nakajima *et al* 2010).

Some researchers consider physical exercise to be a possible behavioral intervention to improve health and cerebral plasticity (Cotman *et al* 2002). However, in aged dogs, there are painful pathological processes that could interfere with the practice of therapeutic physical activities, which include coordination exercises, strengthening exercises, hydrotherapy, massages, thermo- and cryotherapy and passive range of motion (Mlacnik *et al* 2006, Rivera 2007, Crook *et al* 2007, Holler *et al* 2010).

NUTRITION

Due to the disease's development and its large impact on an organic level, diet is one of the most important aspects in the CDS disease process in dogs. Multiple researchers have proven that a variety of nutritional strategies, such as the combination of antioxidants with certain fatty acids, essential minerals, vitamins, mitochondrial factors and other nutrients, as well as caloric restriction, may be effective in reducing the neuropathology associated with cognitive dysfunction in dogs and in decreasing neuronal degeneration and favoring memory preservation.

Antioxidants. Some studies suggest that antioxidants may be beneficial in the aging of the brain and may play an important role in preventive intervention. Thus, numerous studies have demonstrated that diets supplemented with vitamins B, E and C and other antioxidants, such as β-carotene, selenium, β-lipoic acid, and a series of flavonoids and carotenoids (e.g., spinach stem, celery, blueberries, onion, black chocolate, broccoli, apple, tea, parsley, soya, eggplant, tomato pulp, grape extract, carrot, grano-

la and citrus pulp) can reduce the risk of suffering age-related neurodegenerative diseases (Hagen *et al* 2002).

A study by Shukkit (1999) compared the effects of certain red fruit components (blackberries, strawberries, raspberries, blueberries, currants, etc.) on cognitive results. The study examined the results obtained from older rats in neurocognitive tests as well as the possible action mechanisms and their relation to biochemical changes in the brain. The results concluded that the components of red fruits had a beneficial effect on the rats' performance during tilted screen tests (a standard and valid test that evaluates neurocognitive function and rodent behavior), improving their psychomotor functions. Additionally, blueberry extracts significantly increased the release of striatal dopamine relative to the control group. On the other hand, there was a greater neurocognitive performance in rats when measuring their frequency of falls from the tilted screen. The release of dopamine and the obtained results suggest that there is an effect associated with the wide range of red fruit extracts; however, the anthocyanin present in red fruits is not the only component responsible for these improvements. Other studies have found that supplements with blueberry extract significantly increased the level of insulin-like growth factor (IGF-1) in the hippocampus. These biomarkers are significantly associated with lower levels of errors in cognitive tests and improvements in the rodents' working memory performance (Papandreou *et al* 2009).

The use of ginkgo biloba, acetyl-L-carnitine, S-adenosylmetionine and phosphatidylserine, which have been used as mental dexterity and memory enhancers, is now being studied in animals. Certain authors suggest that, in addition to its antioxidant effects, the gingko biloba vegetable presents with anti-inflammatory, brain vasodilating, mitochondrial function-enhancing and MAO enzyme-inhibiting properties (Launer *et al* 1999, Landsberg 2005, Osella *et al* 2007). In this way, it causes a decrease in the severity of clinical signs observed in patients with CDS. Additionally, natural compounds of animal origin, such as propolis, are now the objects of research due to their antioxidant and neuroprotective properties (Shimazawa *et al* 2005). However, there are still no studies that describe the positive effects of this compound in CDS.

Likewise, the use of melatonin is being studied with the aim of determining whether this hormone inhibits the production of free radicals (Kline 2002). Certain levels of omega-3 fatty acids play an important role in the health of the cell membranes and offer a high anti-inflammatory potential (Youdim *et al* 2000).

Caloric restriction. It is also important to note that caloric restriction is the only known measure that increases the average life span of a variety of animals, from rats to primates (Beckman and Ames 1998, Kealy *et al* 2002). It also promotes the maintenance of youth in terms of general health, memory and learning. Caloric restriction maintains mitochondrial function while controlling oxidant production (Hart *et al* 2009). Thus, in one study, rats were administered a mitochondrial metabolite (carnitine) and an antioxidant (lipoic acid), which reverted the age-related mitochondrial degenerative changes and decreased oxidative damage in hepatic cells. It also increased the joining affinity of enzymes related to the brain and memory (Hagen *et al* 1998, Liu *et al* 2002). This work with rats led other authors to study the performance of senile dogs under laboratory conditions with tests that involved discrimination tasks. One-year short-term studies revealed that supplementing the diet with carnitine and lipoic acid, as well as vitamins E and C, significantly reduced the impairment in the performance of old dogs in those discrimination tasks (Millgram *et al* 2002).

Medium chain triglycerides. It has been recently suggested that there is a large decrease in brain glucose metabolism in dogs and that these decreases contribute to the increase in age-related cognitive damage. One way to counteract this metabolic deficit is based on the use of nutritional supplements, such as medium chain triglycerides (MCTs), which increase the organism's production of ketone bodies and allow these components to be used as an alternative form of energy (Henderson 2004). Dietary supplements such as medium chain triglycerides increase ketone body levels in the brain, and neurons could potentially use these substances to make up for the glucose deficit produced in neurodegenerative diseases (Pan *et al* 2010).

ENVIRONMENTAL ENRICHMENT

It has been demonstrated that an enriched environment increases the mental stimulation of dogs with dementia, which favors the installment of behavior intervention techniques by the owners in relearning common tasks, such as domestic habits. Any type of mental or cognitive stimulation must be implemented in a gradual way, as dogs with CDS have a limited capacity for concentration (Cline 2011). It is important to establish brain training and mental stimulation programs during walks and even at home, using cognitive toys and search and exploration games. Social enrichment is also important, dedicating more time to walks, interaction games and obedience training. Contact with humans and other dogs in different places and circumstances are helpful (Milgram *et al* 2004). As age increases, the animal's capacities for sensation decrease, so it is important to implement a certain degree of stimulation to maintain brain activity in its best condition. Stimulating sensory pathways (sight, smell, taste, etc.) is necessary, as the deterioration produced in senile animals allows these animals to increase their state of security and to decrease anxiety. It is advisable to increase contact with new smells, tastes and sounds.

The ability to observe exterior activity through a window and the opportunity to view the television when left alone should be allowed (Head and Zicker 2004).

PHARMACOLOGICAL AGENTS

Pharmacological treatment should aim to palliate associated behavior problems and cognitive disorders present in senile patients. Considering that physiological changes may present themselves in these animals, a complete physio-clinical evaluation should be performed before medicating, with the aim of following-up with the patient and detecting the development of any side effects.

In the canine brain, selegiline increases 2-phenylethylemine, which works as a neuromodulator and enhances the function of dopamine and catecholamines (Landsberg 2005). It also shows a mild antidepressant effect derived from the same action mechanism, which simultaneously confers a neuroprotector effect. Studies of selegiline showed a decrease in the progression of the degenerative changes of patients with AD and a significant improvement in dogs with CDS. Likewise, following selegiline treatment, short-term memory improves, signs associated with cognitive dysfunction decrease, and the life span of old dogs increases (Neilson *et al* 2001). Therefore, altering neurotransmitter concentrations through the use of selegiline seems to be the most effective pharmacological therapy against the progression of clinical signs in dogs with CDS. In dogs that show alterations in their sleep/wake cycle, it is recommended to administer selegiline in the mornings. Response to therapy may be seen in a few days, although improvement is properly seen in the first two weeks. It must be considered that in some cases, a relapse of 15% may appear in the treated dogs.

Other pharmacological agents with notable efficiency are nicergoline and propentofylline. Both drugs produce peripheral vasodilatation with an increase in brain risk, which produces an improvement in brain blood flow. The result is the stimulation of psychological functions of memorization and learning, thereby producing a moderate improvement in the cognitive functions of senile dogs. The use of vitamin complexes that include vitamins E, B and C, as well as antioxidant agents, is equally interesting, as there are a great variety of these products on the market (Landsberg 2006).

CONCLUSIONS

Given the veterinarian's role as a guarantor of the quality of life of geriatric dogs, training in the knowledge and detection of the clinical signs associated with CDS is very important, as is the scientific criteria with which the therapeutic management will be approached. On the other hand, considering the existing relationship between dementia syndrome and aging, there are many dogs that are at risk of suffering this disease, and even more dogs will be at risk if the continuous growth of the geriatric canine population is taken into account. Therefore, it is necessary to continue and further the relevant research, with the goal of acquiring deeper knowledge on the most important aspects of this highly prevalent pathology, such as its diagnosis, treatment and prevention, which could help decrease the impact generated by its effects.

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