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FLUID BIOMARKERS, CLINICAL AND HISTOPATHOLOGICAL FEATURES IN AGED CANINE: THE PROGNOSTIC VALUE FOR THE CANINE COGNITIVE DYSFUNCTION

I. T. STYLIANAKI[1], Z. S. POLIZOPOULOU[1], G. G. KOLIAKOS[2] & N. G. PAPAIOANNOU[1]

[1]Department of Pathology, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University, Thessaloniki, Greece; [2]Department of Biological Chemistry, Faculty of Medicine, School of Health Sciences, Aristotle University, Thessaloniki, Greece

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INTRODUCTION

Cognitive dysfunction (CD) is a common manifestation of several neurological and non-neurological conditions of aged dogs. Almost half of the senescent canine demonstrate for various period times one of the dementia's syndrome symptoms (Osella *et al.*, 2007). Dementia can be defined as an acquired organic disorder that induces dysfunction in cognitive capabilities previously gained. Dementia is not a single nosological entity and many type of dementia exist. It has been improved that aging dogs naturally demonstrate cognitive impairment and neuropathological counterpart that model early Alzheimer's disease (González-Martínez *et al.*, 2011). Furthermore, canine and human CSF has similar biochemical traits (profile). This fact reinforce the dog as a highly appropriate animal model for research in human Alzheimer's disease (Sarasa *et al.*, 2013). So, every study that is conducted in canine model, could be very useful for clarifying the details of the pathogenetic mechanism of senile cognitive dysfunction (Puglieze *et al.*, 2005). This is of the greatest interest because, from any practical point of view, the diagnoses should be improved.

MATERIAL AND METHODS

Four groups of canine are recruited for this study: young, middle aged, cognitively unimpaired and cognitively impaired aged. Classification of cognitive status is carried out using an owner based observational questionnaire. From these patients blood samples are collected into polypropylene vials containing EDTA. They are centrifuged and then plasma is aliquoted and immediately frozen at −80 ^{o}C, measurements of Aβ1-40 levels, Aβ1-42 levels and the Aβ42/40 ratio is realised through ELISA method (González-

Martínez *et al.*, 2011; Olsson *et al.*, 2016). Furthermore, CSF is obtained by lumbar puncture. The samples are centrifuged, aliquoted in 1 mL fractions and then immediately frozen at -80 ^{o}C and stored until assayed. CSF concentrations of Aβ1-40, Aβ1-42 and Aβ42/40 and YKL-40 are also measured (Mulder *et al.*, 2002; Olsson *et al.*, 2016). YKL-40 is a neuroinflammatory protein that is secreted by activated microglia. Saliva samples are also obtained with salivette devices and are frozen in aliquots at −20 ^{o}C, in order to determine the AChE catalytic activity (Sayer *et al.,* 2003). Patients were followed up every 6 months. Canines that died or were euthanised underwent pathological examination. Their brains were formalin-fixed and paraffin sections of several anatomical regions of interest were prepared. They were stained with haematoxylin and eosin (HE), PAS, Congo Red, Kluver-Barrera and modified Bielschowsky. Afterwards, immunohistochemistry methods for the detection of AβPP, β-amyloid, ApoE, tau protein, synuclein, GFAP and YKL-40 were performed.

RESULTS

In the histopathogical examination the brain sections were characterised by slight cortical white matter vacuolation, white matter hypercellularity (gliosis) and evident perivascular accumulation of macrophages containing autofluorescent yellowish ceroid/lipofuscin pigment (Cummings *et al.*, 1996). Senile plaques (SP) and cerebral amyloid angiopathy (CAA) were found. The number of senile plaques appeared to increase with age. However, they were less prominent than was amyloid angiopathy. Furthermore, congophilic material was easily recognised by its orange-red colored fluorescence, and is characterised by its apple green birefringence observed in the walls of cerebral arteries and brain tissue capillaries. Through immunochemistry methods, diffuse plaques and primitive plaques were observed. The first type is the major one (Papaioannou *et al.*, 2001).

DISCUSSION

The cognitive status of the dogs that participate in the current research are classified according to an owner-based questionnaire, after ruling-out other medical causes of dementia-like changes. Blood, CSF and saliva were collected, in order to measure prominent biomarkers. The most important fact, in order to consider a biomarker reliable, is its validation, not only with clinical signs, but also with neuropathological assessment (Olsson *et al.*, 2016). Therefore, these dogs had follow up every 6 months and the patients that die or are euthanised undergo histopathological examination. The aim was to prove the reliability of these biomarkers with a complete and objective way.

CONCLUSION

Although animal models have greatly advanced the understanding of AD pathogenesis, the lack of knowledge concerning its causes makes it difficult to develop a model exhibiting all AD features, which hinders the discovery and characterisation of effective therapeutic measures and drugs (Fabíola *et al.*, 2013). Fluid biomarkers are important because they can provide information regarding the underlying biochemical processes that are occurring in the brain (Mulder *et al.*, 2002). Moreover, the similarities between the canine and human CSF profile reinforce the dog as a highly appropriate animal

model for research in Alzheimer's disease (Sarasa *et al.*, 2013). Consequently, CSF analysis may is able to determine the relationship between each disease stage and modification of cerebral energy metabolism. It becomes more and more evident that the measurement of different biochemical parameters in CSF, could be proved to be an important tool in the effort to make clear the pathogenic mechanism. Blood and saliva are easily accessible, so finding reliable blood and saliva biomarkers for diagnosing and staging dementia is desirable.

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