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Use of Cognitive Testing, Questionnaires and Plasma Biomarkers to Quantify Cognitive Impairment in an Aging Pet Dog Population

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Abstract

Background: Aging dogs suffer from Cognitive Dysfunction Syndrome (CCDS), a condition in which cognitive decline is associated with amyloid pathology and cortical atrophy. Presumptive diagnosis is made through physical examination, exclusion of systemic/metabolic conditions and completion of screening questionnaires by owners.

Objective: This study aimed to determine whether cognitive function could be quantified in aging pet dogs, and to correlate cognitive testing with validated questionnaires and plasma neurofilament light chain (pNfL) concentration in aging dogs.

Methods: Thirty-nine dogs from fifteen breeds were recruited (9.3 to 15.3 years). Owners completed the Canine Dementia Scale (CADES), and Canine Cognitive Dysfunction Rating scale (CCDR). Executive control and social cues were tested and pNfL was measured with single molecule array assay. Comparisons were made between cognitive testing scores, CADES, CCDR scores, and pNfL.

Results: CADES scoring classified five dogs as severe CCDS, six as moderate, ten as mild and eighteen as normal. CCDR identified seven dogs at risk of CCDS and thirty-two as normal. Cognitive testing was possible in the majority of dogs, although severely affected dogs were unable to learn tasks. CADES score correlated with sustained attention duration (r=−0.47, p=0.002), inhibitory control, (r=−0.51, p=0.002), detour, (r=−0.43, p=0.001), and pNfL (r=0.41,

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CONFLICT OF INTEREST STATEMENT

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p=0.025). Concentration of pNfL correlated with inhibitory control (*r*=−0.7, *p*=<0.001). The CCDR scale correlated with performance on inhibitory control (*r=−0.46, p=0.005*).

Conclusion: Our findings suggest that a multi-dimensional approach using a combination of questionnaires, specific cognitive tests and pNfL concentration can be used to quantify cognitive decline in aging pet dogs.

Keywords

canine cognitive dysfunction syndrome$_1$; CCDS$_2$; cognitive testing$_3$; neurofilament light chain$_4$; NfL$_5$; blood biomarkers$_6$; dementia$_7$

INTRODUCTION

Neural aging disorders encompass a broad range of pathological processes that impact cognitive, sensory, and mobility-related functions vital for quality of life [1,2]. Canine Cognitive Dysfunction Syndrome (CCDS) represents an analogue of human Alzheimer's Disease (AD), an age-related condition in which cognitive decline is associated with amyloid pathology and cortical atrophy [3]. The translational potential of this condition has long been recognized and extensive work has been performed in a laboratory setting using dogs (usually beagles) trained to perform a range of cognitive tasks [4,5]. More recently, there has been increasing focus on studying CCDS in pet dogs to improve canine health and quality of life, identify clinically relevant risk factors, and develop robust therapies pertinent to the aging human population with whom they live[6–11].

The current diagnostic approach to CCDS relies on physical and neurological examination, blood work (serum biochemistry panel and complete blood cell count) to identify systemic and metabolic conditions that may cause clinical signs similar to CCDS, and completion of screening questionnaires for CCDS by owners; unlike in AD, clinical measures of cognition in aging pet dogs are lacking. The neurological examination confirms that there are no focal deficits suggestive of an alternative cause of neurological dysfunction such as stroke or neoplasia. Advanced imaging, such as an MRI, would ideally be performed to rule out these causes of neurological dysfunction. Due to the anesthetic requirements of MRI in dogs and financial burden to owners, veterinary neurologists often must rely on the neurological examination. Two different CCDS questionnaires are used widely to establish a diagnosis of CCDS, the Canine Dementia Scale (CADES) and Canine Cognitive Dysfunction Rating scale (CCDR) [12,13]. These instruments ask owners about their dog's behaviors within domains affected by CCDS including social interaction, house soiling, activity, and sleep-wake cycles. Both CCDS questionnaires have been developed with appropriate psychometric validation, but capture different stages of CCDS [12,13]. Neither instrument has been compared to actual cognitive testing with pet dogs thus far. Indeed, while cognitive testing has been performed and reported on in puppies [14,15] and adult pet dogs [16,17], the potential challenges of performing cognitive testing in elderly, untrained pet dogs have resulted in a dearth of published data on their performance on cognitive testing [18,19].

In people, studies investigating AD and mild cognitive impairment often include a variety of different outcome measures such as questionnaires and biomarkers. Specifically, these

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diseases are characterized by changes in performance in certain cognitive tasks such as executive function, memory, and attention [20]. The terminology, diagnosis, and staging of AD and dementia are a topic of great importance for early and accurate diagnosis, research, and treatment optimization [21–24]. A clinical diagnosis is established using a combination of patient history, often provided with the of help caregivers, testing of various cognitive domains, blood work and urinalysis to rule out other conditions, and brain imaging. Performance on cognitive testing is a critical part of this diagnostic process. Historically, AD was a neuropathological diagnosis, but the recognition and validation of disease specific biomarkers has allowed antemortem diagnoses to become more accurate. Indeed, biomarkers can change prior to the emergence of cognitive impairment. As a result, measurement of CSF and plasma biomarkers as well as specific imaging biomarkers is increasingly important, particularly to define preclinical patients and to facilitate appropriate recruitment to clinical trials [25]. Newly identified CSF and plasma biomarkers are still undergoing validation and standardization.

In dogs, CADES and CCDR questionnaires capture critical (human) owner assessments of their pet's performance in their daily life, comparable to caregiver input on human patients with possible Alzheimer's dementia, however there is a need for specific cognitive testing and non-invasive biomarkers to further validate and complement the CCDS questionnaires and further develop CCDS as a model of AD. We recently presented data on plasma neurofilament light chain (pNfL) and amyloid β 40 and 42 (Aβ40/42) concentrations in dogs. These data demonstrated an age-related increase in pNfL as well as elevation in dogs with neurodegenerative diseases, and age-related increases in Aβ40 and 42, with a subsequent decrease in dogs with CCDS, supporting their use as biomarkers of neuro-aging and neurodegeneration in this species [26,27]. However, there is still a lack of data on cognitive testing in these dogs to complement these biomarkers.

In the current study we sought to determine whether senior and geriatric pet dogs could perform previously published cognitive tests (with no pre-training requirements) and then to identify correlations between cognitive testing outcomes, CCDS questionnaires, and pNfL concentrations to provide a sensitive, multidimensional platform capable of capturing a range of changes over the course of canine CCDS.

MATERIALS AND METHODS

Study population

Dogs in this cross-sectional study came from a population of pet dogs enrolled in a longitudinal study of neuro-aging at the NC State College of Veterinary Medicine (CVM). In order to participate, dogs had to be in the last 25% or beyond their expected lifespan per AKC breed standards[28]. Exclusion criteria included presence of comorbidities that would preclude testing such as blindness, and inability to walk independently. Dogs with neurological conditions such as epilepsy that required use of psychoactive drugs that might alter the results of behavioral testing were also excluded. Dogs with comorbidities commonly seen in advanced age that did not interfere with cognitive testing, such as osteoarthritis or stable chronic kidney disease, were not excluded. Dogs were recruited by contacting owners in the local community and the NC State CVM through emails, word

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of mouth, as well as postings on the NC State CVM clinical trials website. Recruitment occurred from January 2019 through May 2021 and dogs were included into the study consecutively throughout the study period. Prior to enrollment, dogs were screened by researchers (GF, WKP, MZK) to ensure they were amenable to being separated from their owner during cognitive testing; this entailed taking them to the cognitive testing room and making sure they would explore the room and take treats from the researchers. All owners were provided with details of the study, given the opportunity to ask questions and then signed an informed consent before participating. Participation was voluntary and all pet owners were able to withdraw their dog from the study at any time. All procedures were approved by the North Carolina State University Institutional Animal Care and Use Committee.

Clinical assessment

All dogs underwent physical, ophthalmologic, orthopedic and neurological examinations followed by complete blood count, serum biochemistry and urine analysis to exclude neurological disease without clinical signs that may imitate CCDS. Post-mortem, histopathologic examination was performed on the dogs who were euthanized between January 2019 and March 2021.

Questionnaires

Owners completed both the CCDR and CADES questionnaires within a week of cognitive testing. Two veterinarians (GF, WKP) oversaw owner completion of the questionnaires and were available to clarify any questions from owners. Specifically, there was a short discussion with the owners to assess if behaviors generally considered abnormal (i.e. house soiling or petting avoidance) were new behavioral changes that could be associated with neuro-aging or long-established behaviors. The Canine Cognitive Rating scale (CCDR) evaluates a cluster of thirteen behaviors associated with abnormalities in orientation, memory, apathy, olfaction and locomotion. The CCDR sum score was used to classify dogs as non-CCDS (0–39), at risk of CCDS (40–49) or CCDS (≥50). The Canine Dementia Scale (CADES) evaluates seventeen items, distributed into four domains associated with behavioral changes (spatial orientation, social interactions, sleep-wake cycles and house soiling) and grades (normal, mild, moderate and severe) are assigned for each item. The CADES score was used to classify dogs as normal (0–7), mild (8–23), moderate (24–44) or severe (45–95) cognitive impairment.

Clinical cognitive testing (examples of dogs undergoing cognitive tests are provided in Supplementary materials)

Clinical cognitive testing was performed at the NC State CVM in a designated cognitive testing room. Cognitive tests were always performed in this room and were recorded via two digital video cameras for future analyses. A one-hour period was allowed prior to testing for acclimation of the dog to the room. Dogs had free access to fresh water and were provided short play breaks to maintain motivation. A non-slip cognitive testing mat with labelled markings was placed on the floor for testing (Suppl. Figure 1). Tests were performed in the following order for each dog: warm ups, social cues (pointing, marker, and odor control), working memory, cylinder (inhibitory control and detour), and sustained

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gaze. All test methods have been previously described [14,15,17,29]; brief descriptions are provided below (video examples are available in supplementary materials).

Warm-ups and social cues (determines whether dogs used a communicative cue to locate a hidden food treat.)

Prior to the start of testing with social cues, all dogs were required to pass a warm-up phase. In this phase, a piece of food was placed on the mat by the experimenter while the dog was held by the handler at the starting line. After placing the food, the experimenter would say “okay” and the dog would be released to approach and consume the treat. This was performed until the dog completed 4 trials. Next, one red Solo® cup was placed on the mat. The experimenter picked up the cup, placed a food treat underneath (in view of the dog) and then said “okay” and the dog was released by the handler. When the dog approached the cup and either touched it or broke the plane of the cup, the experimenter would lift the cup and the dog would receive the treat. Using alternating sides, this was performed until the dog completed 4 trials. In the final phase of warm-ups, two identical cups were placed down, and the experimenter lifted one cup and placed a food treat underneath (in view of the dog). The experimenter then said “okay” and the dog was released by the handler to approach the cups. If the dog chose the correct cup (by touching or breaking the plane of the cup) then the experimenter lifted the cup and the dog was able to consume the treat. If the dog chose the incorrect cup, the experimenter lifted the cup to display that there was no treat, and the dog was returned to the start line for another trial. Using alternating sides, this phase was conducted until the dog correctly retrieved the treat on 4/5 consecutive trials up to a maximum of 20 trials. If the dog did not succeed within the maximum number of trials, a short play break was taken, and warm-ups repeated. Dogs who completed the warm-ups moved on to the social cues. Dogs who failed to complete the warm-ups did not complete the social cue tasks and moved on to other tasks.

For all social cue tasks, a food treat was hidden under one of two cups, out of view of the dog. The experimenter indicated (by pointing with the finger or placing a square marker) which cup contained the hidden treat and said “okay” at which point the dog was released by the handler and allowed to approach the cups. When the dog selected the correct (indicated) cup, the food reward was given; when incorrect, they were calmly returned to the start line. Ten trials each were performed for the pointing and marker tasks; data were expressed as percentage of correct choices. To control for the potential use of odor to find the treat, both cups had a treat taped to the inside (inaccessible to the dog). As a further measure, an odor control task was performed where the food treat was hidden as before, but no indication was provided by the experimenter prior to releasing the dog. Eight trials were performed for the odor control task.

Working memory task (Determines whether dogs could remember where a food treat was hidden after an increasing delay)

Similar to the social cues tasks, two cups were placed on the mat and a small food treat was hidden under one of the cups (in view of the dog). After placing the treat, the experimenter would stand and start a timer for an incrementally increasing delay (up to 120-seconds). After the delay, the experimenter said “okay” and dogs were allowed to choose one of the

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two cups. When the correct cup was chosen, dogs received the food reward. Six trials were performed at each time delay. All dogs participated in the 3- and 6-second delays; after the 6-second delay, dogs who chose the correct cup on at least 4/6 trials moved to the next delay. There were up to nine delays tested: 3, 6, 10, 20, 40, 60, 80, 100, and 120 seconds. The number correct at each delay and the longest delay with at least 4/6 correct was recorded and dogs were then assigned one of three grades: grade 1: <20 seconds, grade 2: 20–60 seconds, grade 3: >60 seconds. The grades were used in statistical analysis.

Cylinder tasks (inhibitory control and detour) (Assesses executive function through inhibitory control and adaptation to altered spatial contingency)

In this task, dogs are asked to retrieve a food treat from the inside of a horizontal cylinder (open on both ends, cylinder height: 25.4cm, cylinder width: 27.9cm, cylinder diameter: 25.4cm). First, familiarization trials were performed to ensure that dogs consistently retrieved the treat from the cylinder. The dog's name was called, and a small food treat was placed in the opaque cylinder. Dogs were allowed to retrieve the hidden food and this procedure was repeated until participants retrieved the food four out of five times. Once this threshold had been reached, the cover over the cylinder was removed (making it transparent) and food was placed in the cylinder as in the familiarization trials. Dogs were released to approach the cylinder and retrieve the food. The dependent measure was whether the dog retrieved the food without touching the exterior of the cylinder over eight trials; the side they approached was also recorded. After eight trials, the side most often used by the dog was blocked with a plexiglass circle once the food treat was placed inside; over eight trials, dogs were allowed to approach the cylinder but had to retrieve the treat from their non-preferred side (detour). Data were expressed as a percentage of correct choices.

Sustained attention/eye gaze (Determines the length of time dogs would sustain their attention toward a human face.)

Dogs were invited to make eye contact with the experimenter who called their name and held a food treat up near their eyes. The duration of sustained eye contact was recorded. This was repeated three times for each dog and an average duration used for analysis.

Measurement of plasma NfL

Blood samples were collected into purple top (EDTA) tubes and centrifuged at 2000 × g at 4 °C for 8 min within 2 h of collection. Plasma supernatant was collected, divided into aliquots, and frozen at − 80 °C until further use. Samples were thawed on ice before analysis and the concentration of neurofilament light chain (NfL) using a Single Molecule Array Assay kit (NF-light, Quanterix, Lexington, MA), following the manufacturer's instructions.

Statistical analysis

Statistical analyses were performed using JMP 15 (SAS Institute, Cary, NC). Dogs were grouped according to CADES-designated disease status (normal versus not-normal [mild, moderate, and severe]) as well as CCDR-designated disease status (normal versus at risk); summary data were prepared for each group on age, weight, sex and breed as well as cognitive testing results and NfL and the groups were compared using Wilcoxon Rank Sum

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tests. The correlations between plasma NfL, CADES scores, CCDR cores and outcomes from cognitive testing were established using simple linear regression and continuous data. All reported p values were considered to be statistically significant at $p < 0.05$.

RESULTS

Clinical characteristics

Thirty-nine dogs (25 females and 14 males) of various breeds participated in the study (American Staffordshire Terrier *n=2*, Beagle *n=4,* Bernese Mountain Dog, *n=1*, Border Collie *n=2,* Brittany Spaniel *n=1*, Cairn Terrier *n=1,* Dachshund *n=1*, German Shorthaired Pointer *n=1*, Golden Retriever *n=2*, German Shepherd Dog *n=1*, Jack Russell Terrier *n=1,* Labrador Retriever *n=5,* Mix Breed *n=15,* Pembroke Welsh Corgi *n=1*, Siberian Husky *n=1*). The median age and body weight were 12.2 years (range 9.3–15.3) and 20.7 kg (range 7.2–34.2), respectively. Demographic features of study participants and CADES and CCDR scores are presented (Supplementary Table 1). All dogs were systemically healthy, and met the inclusion criteria regarding mobility, vision and absence of focal neurological deficits.

Cognitive evaluation by CCDS questionnaires

CADES scoring classified five dogs as severe CCDS, six as moderate, ten as mild and eighteen as normal. CCDR identified seven dogs at risk of CCDS and thirty-two as normal (Supplementary Table1). None of the dogs in the study reached the threshold score for CCDS on CCDR. One dog in the severe CCDS group on CADES was identified as normal on CCDR. The remaining four dogs in the severe CCDS group on CADES were identified as at risk by CCDR. Two dogs classified as moderate CCDS and one dog classified as mild CCDS by CADES scoring were considered at risk by CCDR. We found it important to discuss the intent of the CCDS questionnaires to capture aging changes with owners, to differentiate behaviors such as house soiling that had always been present versus those associated with more recent changes potentially attributable to the neuro-aging processes.

Cognitive test results

Of the thirty-nine dogs, 9 dogs were unable to pass warm-up criteria for the social cues tasks. Three of these dogs were unable to complete any tasks, while the remaining 6 dogs were able to complete all of the ‘non’ cup tasks. Of the 3 dogs who could not complete any of the tasks, one was classified as severe CCDS (183 months of age), one as moderate CCDS (156 months of age), one as normal on CADES classification (142 months of age). The normal dog was extremely anxious. The remaining thirty dogs successfully participated in all of the tasks. Total duration of cognitive testing was 2–5 hours depending on the participant and testing was performed early in the morning for most dogs. Longer testing duration was associated with longer acclimation periods (some dogs needed more time to adjust to the new environment prior to testing) or a higher number of breaks (necessary to maintain motivation or to provide rest for dogs with osteoarthritis).

Performance on cognitive tasks is summarized for dogs classified as with or without dementia (CADES) or normal or at risk for dementia (CCDR). (Table 1) There were no significant differences in performance between normal, dementia (CADES) and at

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risk (CCDR) dogs for social cues, working memory or odor control tasks (Table 1). However, there were differences in other executive control tasks. Dogs classified as normal had a higher percentage correct than dogs not classified as normal in both cylinder executive control tests; performance on the detour task was significantly worse (p=0.01) in dogs classified as dementia using CADES. While many dogs performed well on the inhibitory control task, the more challenging nature of the detour component distributed the performance of dogs across a much wider range, particularly evident when dogs were grouped as normal, mild, moderate or severe CCD using CADES scores (Fig. 1A and B). Sustained gaze duration was significantly different between normal and dementia (CADES) or at risk (CCDR) groups of dogs (p=0.007 and 0.005 respectively) with dogs classified as normal having a significantly longer duration of sustained gaze than those not classified as normal. Similar to the cylinder tasks, there was a significant deterioration in performance across dogs grouped as normal, mild, moderate, or severe CCDS using CADES scores (Fig. 1C).

Correlations between CCDS questionnaires, cognitive testing, and plasma NfL

CADES and CCDR scoring were positively correlated with each other (r=0.6, p=<0.001) Plasma samples were available to measure NfL in thirty of thirty-nine dogs; the other nine dogs did not have plasma samples available to measure. Across all dogs, plasma NfL concentrations ranged from 27.93 pg/ml to 327.3 pg/ml with a median of 59.42 pg/ml (Table 1). A summary of the relationships between each CCDS questionnaire score, cognitive testing, and plasma neurofilament light chain concentrations are presented in Table 2. The social cues and working memory tasks did not have significant relationships with the CCDS questionnaires or plasma NfL concentration. As before, both cylinder tasks were more discriminating. Higher CADES scores were negatively associated with performance on the inhibitory control task (r=−0.51, p=0.002) and the detour task (r=−0.43, p=0.001)as well as sustained gaze (r=−0.47, p=0.002) (Table 2). A significant relationship was also found between CCDR score and the inhibitory control task (r=−0.46, p=0.005), but not sustained gaze or the detour task (Table 2). Elevated concentration of pNfL was associated with poor performance on the inhibitory control task (r=−0.7, p<0.001) (Fig. 2A) but not the detour task or sustained gaze (Fig. 2B and 2C, respectively). It was also associated with a higher CADES score (r=0.41, p=0.003) (Fig. 2D).

DISCUSSION

Our work demonstrated the feasibility of performing cognitive testing in aged pet dogs, including those with signs of CCDS. A high percentage of study dogs were able to complete the battery of cognitive tests, designed to assess different cognitive domains important in aging. Additionally, we identified correlations between cognitive testing in the realm of executive function and two commonly used CCDS questionnaires as well as plasma neurofilament light (pNfL) concentrations. These results demonstrate the utility of complementary assessments of cognition that capture owner assessments of behaviors at home, cognitive performance across different domains and a plasma biomarker that measures neuronal health.

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The battery of cognitive tests performed in this study have been previously performed to assess various cognitive domains [19,29–35]. However, there are few data showing this feasibility in pet dogs with CCDS, or without extensive training [13]. Of all the cognitive tasks performed, the marker task was the most challenging for our dogs, with the majority of dogs performing at chance (regardless of CCDS status via questionnaires). Though not systematically evaluated, several aspects of testing may have contributed to successful completion: dogs were given ample time to acclimate to the handlers and the testing room, which was set up to be as calm and comfortable a space as possible. The duration of testing varied between individuals, and it was important to take breaks to minimize the effects of exhaustion and to keep dogs energized and attentive. Dogs who were considered more anxious by the investigators also required more time for testing; this should be systematically evaluated in future work. Even with this difficulty, dogs were able to complete the entire battery in one session. However, this is unlikely to be feasible in a clinical setting and is more applicable in a research setting. Future work will evaluate clinically expedient tasks that can be performed with minimal time and equipment.

The results of the cognitive testing demonstrate that cognitive abilities in certain domains decline in old dogs. In order to understand how these test results complement owners' assessment of their dogs' cognitive performance at home, and whether changes are associated with fluctuations in biomarkers, we investigated relationships between these different types of assessments. A number of questionnaires have been developed to detect CCDS in dogs [12,13,36–40]. Among them, the CCDR and CADES underwent extensive psychometric validation and are often used to quantify signs of dementia reported by owners. Unlike in humans, neither CCDS questionnaire has been correlated with biomarkers or changes in observer-based testing of cognitive function within specific domains. Numerous scales have been validated for dementia screening in people, some of which test performance in specific cognitive domains (e.g. Alzheimer Disease Assessment Scale – Cognitive Subscale (ADAS-cog), Mini-Mental State Examination (MMSE)), while others evaluate performance more widely in areas such as self-care, social orientation (e.g. Clinical Dementia Rating (CDR)). The ADAS-cog, MMSE, Geriatric Mental State Schedule (GMS), and CDR have been correlated with biomarkers including Aβ, tau proteins, and neurofilament light chain [41–45].

We evaluated both CADES and CCDR scales, giving us the opportunity to assess the potential difficulties experienced by owners when completing the assessments. Our findings mirrored those of Madari et al. (2015) in the development of the CADES assessment; scores from the two CCDS questionnaires are highly correlated with each other, but the classification of the scores as 'dementia' is rather different. In our study, the CADES questionnaire identified dogs in mild and moderate stages of CCDS who were classified as normal by the CCDR questionnaire, while no dogs identified with cognitive impairment on the CCDR were identified as normal on the CADES. When performing a study using these questionnaires, animals with pre-existing behavioral abnormalities (such as house soiling, anxiety or aggression) might obtain high scores on the CCDS questionnaires for reasons other than age-related dementia and may need to be excluded. It is also clear that answers to questions might change over time due to changes other than cognitive decline, such as pain, or sensory loss. As a result, it is critical both to understand that these scales do not

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diagnose CCDS specifically, and that there is a need to couple the CCDS questionnaires results with hands-on physical, orthopedic, ophthalmological, neurological examinations, and blood work.

We identified a strong relationship between CADES category and sustained attention. Although neural mechanisms underlying eye gaze in dogs remain unexplored, functional magnetic resonance (fMRI) data generated in human studies suggest that eye contact involves not only visual cortical areas, but also other brain regions related to intentionality processing that might be affected in the dementia processes (temporoparietal junction, posterior superior temporal sulcus, medial prefrontal cortex and the dorsolateral prefrontal cortex) [46]. In-home sustained attention tasks in pet dogs have been shown to be significantly associated with CADES scores and show promise as a simple, reliable test for future works[29]. Executive control testing, specifically performance on the inhibitory control task, was the only other cognitive task strongly associated with CCDR category, CADES category, and plasma NfL concentrations. Therefore, executive control may be the most sensitive domain in interpreting progression of CCDS in aging dogs. The association with aging and executive function in dogs has been previously described in a cohort of pre-trained beagle dogs tested in a laboratory setting [47]. Furthermore, executive function performance in beagles was significantly worse with aging and was associated with decreased frontal lobe volume on MRI [48,49]. It was noticeable that adding the detour task to the inhibitory control cylinder task separated out dogs who performed well on inhibitory control. Evaluation of longitudinal data will allow us to determine the accuracy with which the detour predicts future cognitive decline. Furthermore, longitudinal data will elucidate whether executive function testing remains the best cognitive domain to correlate with other markers of CCDS and perhaps predict progression of CDS.

Our recent work demonstrated that plasma concentration of neurofilament light chain (NfL) serves as a biomarker of gradual, age-dependent neuronal loss in healthy pet dogs, and in dogs with neurodegenerative disease [27]. In our current study we have built on these observations by demonstrating that pNfL concentration correlates with performance on the inhibitory control task. Furthermore, increased pNfL concentration was significantly associated with higher CADES scores. This work mirrors recent scientific reports in humans on pNfL concentration as a non-invasive, blood-based biomarker, that reflects both AD dementia associated pathological changes within the central nervous system (CNS) and poor cognitive testing results [50–52]. However, because pNfL is a non-specific marker of neuronal death, the rate of change is more important than absolute concentrations in reflecting changes associated with CCDS. Thus, in a cross-sectional study, we are unable to assess the rate of change in our dogs and a longitudinal study is warranted for further evaluation of pNfL as a biomarker for CCDS.

Limitations of our study include the relatively small sample size, particularly within the more severe dementia categories, the cross-sectional nature of the study, and the lack of advanced imaging for exclusion of other brain disease as a cause of clinical signs and histopathology to confirm the diagnosis of CCDS. Although advanced imaging such as MRI would be ideal to rule out intracranial pathology, older dogs face a significant anesthetic risk to undergo MRI. Therefore, even for the dogs in our study with evidence of CCDS,

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many owners did not wish to pursue advanced imaging. As such, other underlying, diffuse brain pathologies could not be excluded unless a necropsy was performed. Necropsies confirmed the lack of other pathology in six dogs who were euthanized, of which five were identified as having CCDS according to the CADES questionnaire and three were identified as at risk by CCDS questionnaire. Due to the complete absence of data on the relationship between aging, cognition, and biomarkers in companion dogs, this study provides important preliminary data for future work. As such, a power analysis could not be performed at the outset and the strength of our findings is limited by this. Furthermore, a larger sample size of dogs in the severe dementia categories in future studies would help bolster results of the current study. We are collecting longitudinal data in these dogs which will allow critical evaluation of the temporal changes in the different cognitive domains and how such changes manifest as owner reported behaviors on CCDS questionnaires within individual dogs.

In conclusion, we demonstrated that cognitive testing of different domains is feasible in aging pet dogs. Cognitive testing can be coupled with the CADES questionnaire and pNfL concentrations to capture a wide range of cognitive function in aging dogs. Furthermore, executive control, assessed here using the inhibitory control and detour tasks as well as the sustained gaze test, may be the most significant cognitive domain affected in dogs as they age. We propose that combining owner completed CCDS questionnaires with specific, simple cognitive testing and measurement of plasma biomarkers of neuronal integrity provides a holistic means of tracking cognitive decline in pet dogs, though currently only in a research setting. This toolkit provides a powerful opportunity both to discover underlying pathophysiology and to test novel therapies for cognitive decline in pet dogs. In future work we hope to quantify grey and white matter volume and pathology in this population of pet dogs using MRI and correlate these findings to cognitive testing results.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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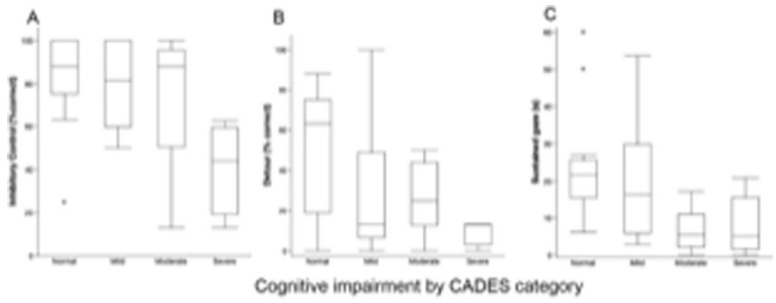

Figure 1:

Severity of CCDS when categorized using CADES is associated with worse performance on executive control tasks: cylinder tasks (A: inhibitory control, p=0.04, B: detour, (p=0.04), C: sustained gaze (p=0.006)).

CCDS: canine cognitive dysfunction syndrome; CADES: Canine Dementia Scale; avg: average; s: second.

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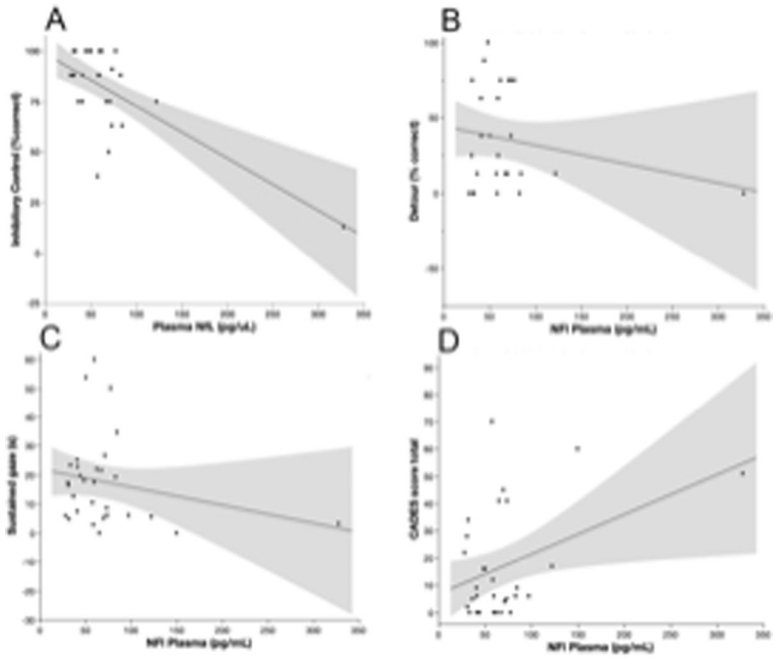

Figure 2:

Plasma NfL concentration was negatively associated with poor performance on the inhibitory control task (r^2=0.48, p<0.001) (A) but not with the detour task (r^2=0.05, p=0.28) (B) or the sustained gaze (r^2=0.05, p=0.23)(C). There was a positive relationship between pNfL and CADES score (r^2=0.17, p=0.03) (D).

CADES: Canine Dementia Scale; pNfL: plasma neurofilament light chain.

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Table 1

Summary of the data. Mean (SD) and Median (range) have been used to present plasma concentrations of NfL.

		Dementia dogs via CADES	Non-dementia dogs via CADES	Dogs at risk of dementia via CCDR	Non-dementia dogs via CCDR
Number of dogs (n)		n=16	n=17	n=6	n=27
Number of males and females (n)		m=6, f=10	m=8, f=9	m=0, f=6	m=14, f=13
Plasma NfL concentration (pg/ml)	(mean, SD)	82.5± 75.55	58.4±19.95	103.5±110.84	62.2±29.65
	(median, range)	58.4 (27.9–327.3)	59.5 (31.2 – 97.1)	67.4 (27.9 – 327.3)	58.9 (30.6–149.6)
Pointing Cue (avg % of correct choices) (range)		83 (42–100)	79 (58–100)	67 (42–83)	83 (58–100)
Marker Cue (avg % of correct choices) (range)		50 (8–75)	53 (33–83)	42 (25–58)	50 (8–83)
Odor Control (avg % of correct choices) (range)		44.5 (13–63)	51.2 (25–75)	55.4 (38–63)	46.5 (13–75)
Working Memory (number of dogs classified using three grade scale, n) (Grade1, 0–20s), (Grade 2, 20–60s), (Grade 3, >60s)		Grade 1, n = 6 Grade 2, n= 4 Grade 3, n=3	Grade 1, n=6 Grade 2, n=8 Grade 3, n=2	Grade 1, n=3 Grade 2, n=2 Grade 3, n=0	Grade 1, n=9 Grade 2, n=10 Grade 3, n=5
Cylinder task (inhibitory control) (avg % of correct trials) (range)		88 (13–100)	88 (25–100)	50 (13–91)	88 (25–100)
Cylinder task (detour) (avg % of correct trials) (range)		13 (0–100)	69 (0–88)	13 (0–38)	38 (0–100)
Sustained Attention/Eye Gaze (avg from three trials)(range)(s)		6 (0–53.8)	21.62 (6.13–60)	5.55 (0–10.5)	19.69 (0–60)

Acronyms: CADES – Canine Dementia Scale; plasma NfL– plasma neurofilament light chain; SD - standard deviation; avg – average; (s)- seconds.

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Table 2

Summary of correlations between clinical metrology tools, clinical cognitive testing outcome and plasma neurofilament light chain concentrations. Simple linear regression, continuous data

	SUSTAINED GAZE	WORKING MEMORY	CYLINDER (Inhibitory Ctrl)	CYLINDER (Detour)	POINTING CUE	MARKER CUE
CCDR	*r=−0.16 p=0.32*	*r=−0.19 p=0.31*	*r=−0.46 p=0.005*	*r=−0.27 p=0.12*	*r=−0.2 p=0.28*	*r=−0.12 p=0.57*
CADES	*r=−0.47 p=0.002*	*r=−0.22 p=0.23*	*r=−0.51 p=0.002*	*r=−0.43 p=0.001*	*r=−0.15 p=0.42*	*r=−0.19 p=0.35*
pNfL	*r=−0.23 p=0.23*	*r=−0.22 p=0.29*	*r=−0.7 P<0.001*	*r=−0.22 p=0.28*	*r=−0.37 p=0.07*	*r=−0.25 p=0.27*