The Journal of Frailty & Aging 15 (2026) 100145

Contents lists available at ScienceDirect

The Journal of Frailty & Aging

journal homepage: www.elsevier.com/locate/tjfa

Original Research

OLD DOG - Validating the dog as an animal model for human aging studies

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ARTICLE INFO

Keywords:
Aging
Biological age
Dogs
Comparative aging

ABSTRACT

Companion dogs represent a valuable and emerging translational model for human aging, as they share the human environment, receive comparable medical care - yet have much shorter lifespans. Despite their potential, a validated set of canine biomarkers of aging has not yet been established. The OLD-DOG Project, launched in 2023 at the University of Padua's Veterinary Teaching Hospital, is a 30-month prospective study designed to identify and validate biomarkers of aging in companion dogs and to assess their predictive value for healthspan and lifespan, thereby evaluating the suitability of dogs as models for human aging research.

A cohort of 209 privately owned dogs aged ≥5 years was enrolled and underwent comprehensive evaluations every six months, including clinical examinations, physical fitness testing, blood and fecal sampling, and owner questionnaires. Collected data encompass physiological, biochemical, hematological, and behavioral parameters, as well as microbiota profiles, telomere length, and DNA methylation patterns. Surplus biological material is stored to establish a long-term biobank.

Preliminary cross-sectional analyses have identified consistent age-related patterns across multiple domains, including hematological and biochemical indices, inflammatory markers, and measures of physical and cognitive performance. Ongoing longitudinal analyses aim to determine the predictive value of these candidate biomarkers for morbidity and mortality, as well as to assess the influence of environmental and lifestyle factors on aging trajectories. Ultimately, the project seeks to construct an integrative model of biological age in dogs, thereby strengthening their value as a robust translational model for human aging research.

1. Introduction

In human populations, individual aging trajectories differ markedly, indicating that chronological age is an imprecise measure of overall physiological fitness [32]. The considerable variability in functional capacity among individuals of the same chronological age has motivated extensive research into alternative metrics for assessing age-related biological changes, collectively referred to as “biological age” [42]. Biological age is inferred using a suite of biomarkers of aging that assess an individual's fitness independently of their chronological age. These biomarkers are characterized as biological parameters that, whether considered singly or as part of a multivariate composite, more accurately predict late-life functional capability in the absence of disease than the years of life lived [5].

Although most studies on the biology of aging have focused on mice and other simple model organisms, privately owned dogs represent a unique and highly relevant system for investigating how environmental exposures, lifestyle factors, diet, pollution, and other influences shape human biology and disease processes, including cancer and aging [7,20]. Companion dogs not only share environments and, to some extent, lifestyles with their owners, but also receive a level of medical care comparable to that of humans, while experiencing much shorter lifespans. This combination makes them particularly well-suited for aging studies that can be conducted over shorter timeframes.

Evidence from previous research indicates that several physiological characteristics, such as DNA methylation patterns [25,54], telomere length [8,15], clinical laboratory parameters [46], and cognitive performance scores [51], vary with chronological age in dogs. These findings suggest that such traits may serve as candidate biomarkers of aging (see [62] for a review). However, although variation with chronological age is an essential characteristic of a potential biomarker of aging, it is not sufficient, as the ultimate goal is to establish its predictive value for

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https://doi.org/10.1016/j.tjfa.2026.100145
Received 12 December 2025; Received in revised form 12 February 2026; Accepted 19 February 2026
Available online 19 March 2026

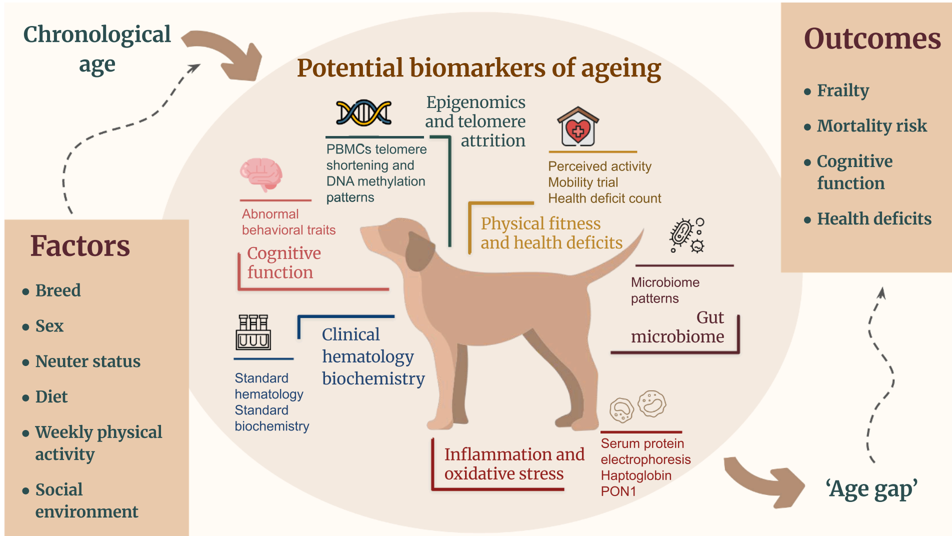

Fig. 1. Adapted from [62]. Categories of potential biomarkers of aging in dogs. The associations between these biomarkers and age-related adverse health outcomes will be investigated. Once a set of biomarkers reliably associated with these outcomes has been identified, the factors capable of modulating them will be assessed. PBMCs - peripheral blood mononuclear cells; PON1 - paraoxonase-1 activity.

adverse health outcomes and mortality. Once validated, such biomarkers can serve as reliable surrogates for studying age-related changes and for evaluating the effects of environmental, lifestyle, and therapeutic interventions. To date, unlike in humans, no integrative models exist for estimating biological age in dogs. The frailty index and frailty phenotype remain the only age-related measures in dogs with demonstrated predictive value for mortality, although previous studies have largely been restricted to single-breed populations, narrow weight ranges, or dogs presented to veterinary hospitals with pre-existing health conditions [6,10,27,36,60].

Recently, evidence has emerged linking the concentrations of several plasma metabolites to all-cause mortality in dogs; however, their association with chronological age remains unknown [23]. Interestingly, many of the same metabolites have also been associated with mortality risk in humans, highlighting their potential relevance as conserved biomarkers of aging across species.

Over the past decade several large-scale projects were launched to explore the potential of companion dogs as translational models for human aging. Major initiatives include the Dog Aging Project [34], a large-scale study that also comprises a deeply phenotyped Precision Cohort of nearly 1000 dogs from different breeds, for which multi-omics data have been collected [45]; the Vaika Project [16], which collects extremely detailed longitudinal data on genetic and metabolic profiles together with physical performance in retired sled dogs; and the Golden Retriever Lifetime Study[1] , a prospective longitudinal study with a particular focus on cancer risk factors [22].

Despite their major and invaluable contributions, current aging studies often present limitations, including the use of single-breed cohorts or the limited availability of comprehensive clinical data. The latter may hinder the ability to disentangle age-related physiological changes from alterations in potential aging biomarkers driven by concurrent or subclinical pathology.

To address these gaps, the OLD-DOG Project was initiated in late 2023 at the Veterinary Teaching Hospital of the University of Padua. The project aims to integrate detailed genetic, metabolic, clinical, and cognitive performance data with standardized frailty assessments in a multi-breed canine cohort, thereby enabling a more comprehensive characterization of healthy and pathological aging in companion dogs. In order to minimize interobserver variability, the data were consistently gathered by the same veterinarian (PZ).

The overarching goal of the project is to establish a comprehensive set of validated biomarkers of aging in companion dogs, investigate their association with frailty, healthspan and lifespan, and evaluate the influence of genetic, environmental, and lifestyle factors to develop robust measures of biological age in dogs and assess their value as translational models for human aging research (see Fig. 1).

In this paper, we present the rationale and methodology of the project, introduce the study cohort, highlight the progress achieved so far, and discuss future directions.

2. Study design

The OLD-DOG Project is a 30-month prospective observational study designed to identify biomarkers of aging in companion dogs. A total of 209 privately owned dogs were enrolled and assessed at baseline, with follow-up evaluations every six months. All the dogs recruited are located in the Veneto region of Italy. The inclusion criteria for participation were as follows:

- Dogs aged over five years, with at least the month of birth known
- Absence of acute disease symptoms at the time of each evaluation

Minimal inclusion criteria were established to ensure a cohort that accurately represents the dog population while minimizing selection bias. The criterion of 'absence of acute disease symptoms' was specifically included to reduce confounding between disease-related changes and 'normal aging,' as both can influence potential biomarkers of aging.

The assessment of the dogs was designed to facilitate the gathering of samples and data that earlier studies have highlighted as potential aging biomarkers in dogs [62]. To increase the translational scope of the study, the clinical evaluation was inspired by the comprehensive geriatric assessment - a methodology developed by geriatricians to holistically address the complexities of older adults and design personalized interventions based on patients' needs, priorities, and resources. Additional information on factors that may potentially influence aging was also recorded. These potential biomarkers will be investigated primarily based on their association with chronological age, and subsequently on their ability — individually or in combination — to predict lifespan and healthspan. We will also investigate the relationships occurring between

[1] https://www.morrisanimalfoundation.org/golden-retriever-lifetime-study

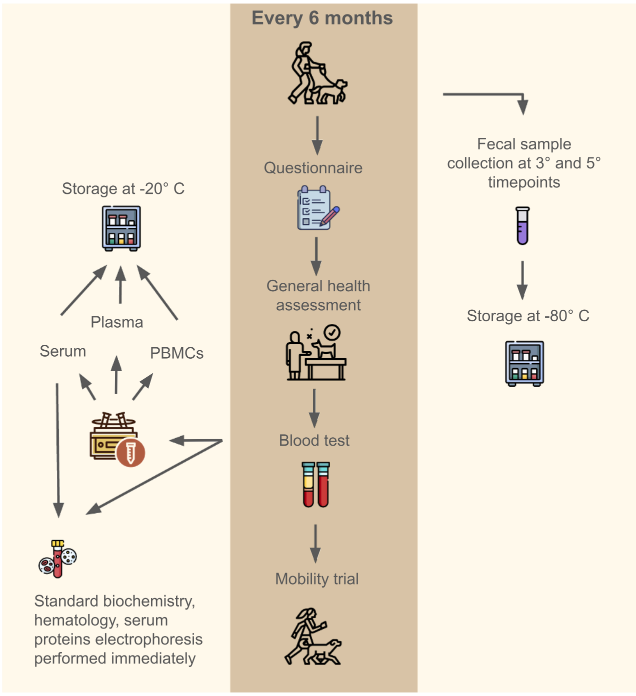

Fig. 2. The workflow of the dog assessment procedures conducted every six months within the OLD-DOG Project.

biomarker-derived biological age and environmental, lifestyle, and social factors (see Fig. 1).

The information collected encompassed:

- A questionnaire completed by the veterinarian based on the owner's responses concerning demographic data, medical treatments, prophylaxis, lifestyle, health issues, and behavioral changes.
- General health assessment performed by the veterinarian.
- Collection of blood specimens.
- Physical fitness assessment.
- Collection of the fecal specimens for the microbiota analysis (at two time points).

Fig. 2 illustrates the schematic workflow of the dog assessment and data collection process.

Details of the collected data and the reasoning behind their selection are presented in the following sections, emphasizing aspects particularly relevant to aging studies in companion dogs. The complete questionnaire used in this project is available in the Supplementary Material's Table 2.1.7.

2.1. Questionnaire

2.1.1. Baseline data

The baseline data collected for each dog during the first assessment included information on congenital problems, breed, morphology (brachymorphic/mesomorphic/dolichomorphic), and height at the withers. Since dog breeds differ considerably in life expectancy - with a notable negative correlation between lifespan and body weight [21, 44,57] - breed information was recorded in detail, including the breeds of both parents when they were purebred but of different breeds. The height was measured to more accurately assess how body size influences aging and to standardize size-dependent parameters (such as the time required to complete the 10-meter mobility test).

2.1.2. Neuter status

Spaying or neutering has been associated with an increased risk of certain health conditions, including cruciate ligament rupture, several cancers such as lymphoma and osteosarcoma, obesity, and urinary incontinence in females [24,57]. Conversely, it provides protection against diseases such as pyometra and mammary tumors and has been linked to increased lifespan, particularly in female dogs [24,57]. Recent findings suggest that intact females exhibit lower levels of IL-1β, indicating that estrogens - owing to their antioxidant properties - may play a protective role against inflammaging in dogs as well [33]. Furthermore, the duration of lifetime gonadal exposure may influence frailty [61]. To account for the potential impact of gonadal hormones on aging, neuter status was recorded at each examination, along with the age at the time of surgical neutering or, for pharmacologically neutered animals, the date of implant administration and its expected duration.

2.1.3. Prophylaxis and treatments

Maintaining up-to-date vaccinations and consistent parasite management are fundamental for proper pet care. While neglecting vaccinations doesn't directly relate to aging, it is a valuable measure of an owner's diligence [10]. In accordance with WSAVA guidelines, each dog's core and *Leptospira interrogans/kirschneri* vaccinations were tracked [52] along with prophylaxis against *Dirofilaria immitis* and endoparasites.

All medical treatments reported by the owners within the previous six months were recorded to account for their potential impact on blood analysis results and to provide additional context regarding the dogs' overall health status. When the exact drug type or treatment duration was not available, the corresponding pharmacological class (e.g., NSAIDs, antibiotics) and treatment type (e.g., single course) were recorded instead.

2.1.4. Life-style and social environment

To our knowledge, the effect of diet type on aging in dogs has not yet been studied. However, diet is known to significantly influence canine gut microbiota [28], in particular the microbial diversity, a parameter known to vary with age in dogs [40]. To consider potential dietary influences on age-related parameters, information on each dog's diet was documented.

To account for the physical activity levels and to trace its variation with age owner reported weekly physical activity time, including all types of activity regardless of intensity (e.g., walks, play, training), was recorded.

Additionally, the potential effect of the social environment on aging and cognition was assessed by recording the number of people living in the household, as well as the presence of children and other animals.

2.1.5. Owner-reported health deficits

A comprehensive anamnesis was collected for each dog at every time point. The assessment items (see Supplementary Material Table 2.1.7) were designed to enable replication of the frailty index originally developed in dogs by the project's lead researchers [6].

2.1.6. Symptoms observed in the last six months

A significant number of canine health issues lack adequate investigation, which frequently hinders a conclusive diagnosis. To capture as many health deficits as possible, a set of questions addressing recurrent symptoms was specifically developed. The complete list of the symptoms assessed is presented in Supplement Table 3.

When house soiling was reported, additional clarification was asked to the owner, including the amount of urine produced during each episode, any signs of urgency, and whether the owner believed the dog was confusing indoor and outdoor environments at the time of urination. This clarification is important, as house soiling may also be indicative of cognitive impairment.

2.1.7. Physical fitness

Many physical fitness biomarkers commonly used in human aging research cannot be directly applied in veterinary contexts [31], or they

Table 1
Perceived physical capacity and Cognitive and Behavioral Health assessment.

Sections	Questions	Possible scores
Physical fitness	Ability to sustain usual physical activity Running speed Ease of climbing/walking down the stairs Ease of jumping	from 1 to 5 (the dog's maximal capacity when young)
	Episodes of reluctance to move Episodes of early fatigue Need for assistance to stand up Need for assistance to climb stairs	1 (few times a week), 2 (few times a months), 3 (once a month), 4 (never)
Cognitive status	Capacity of learning new tasks Curiosity	from 1 to 5 (the dog's capacity when young)
	Disorientation/circling Staring blankly Getting lost in the home Change in recognition of owners Change in sleeping cycle	1 (few times a week), 2 (few times a months), 3 (once a month), 4 (never)
Social behavior	Interaction with owners and other people Interaction with other dogs Play behavior	from 1 to 5 (the dog's maximal capacity when young)
	Episodes unusual fear/anxiety Episodes of avoiding contact or petting Episodes of unusual aggression	1 (few times a week), 2 (few times a months), 3 (once a month), 4 (never)

Table 2
Main demographic data of the dogs included in the study. Median values and range are reported for age and weight. Dogs were considered as crossbreeds dogs if both parents were purebred but of different breeds.

Age (years)	9 (5 - 16)
Weight (kg)	21 (2.7 - 80)
Female spayed/intact	108/15
Male castrated/intact	44/42
Purebred	151
Mixed breed	38
Crossbreed	20

require specialized equipment such as treadmills, weighted carts, or leash-mounted dynamometers fixed to a wall [16,36].

In this study, we focused on practical, clinically applicable biomarkers. Physical capability was therefore assessed using a questionnaire on the dog's ability to perform everyday tasks along with a brief physical test, described in detail below. To assess the dog's ability to perform each task, owners were asked to rate performance on a scale from 1 to 5, with 5 representing the dog's peak capacity during its youth (see Table 2.1.7).

Evaluating physical fitness is particularly important in determining the frailty phenotype [13,18,48]. Physical frailty is thought to result from cumulative declines across multiple physiological systems and, in humans, is characterized by five key criteria: weakness, slow walking speed, low physical activity, fatigue or exhaustion, and unintentional weight loss [18]. The data collected in this project were selected, in part, to enable the application of this five-criteria frailty phenotype framework to dogs. A major challenge in the application of frailty measures to multi-breed canine cohorts is the standardization of physical fitness characteristics across different breeds. To date, frailty phenotypes in multi-breed dog cohorts have largely relied on indirect and subjective assessments, primarily based on owner-reported questionnaires [26,49]. In this project we aim to integrate objective physical performance testing (mobility trial) with comprehensive clinical evaluations (variations of weight, muscle mass and body condition score) and owner-reported measures to develop an easily accessible frailty phenotype applicable across dogs of different breeds.

Table 3
Number of dogs with health issues reported by the owners at the first time point.

Health deficit		Number of dogs
Infectious diseases	Leishmaniasis	1
Inflammatory diseases	Recurrent cystitis	4
	Pemphigus foliaceus	1
Hematopoietic diseases	Chronic neutropenia	2
Endocrine diseases	Hypothyroidism	4
	Diabetes	1
Heart diseases	MMVD B1	11
	MMVD B2	2
	Dilated cardiomyopathy	1
	Unknown	1
	Pulmonary valve stenosis	1*
Renal insufficiency	IRIS 2	2
	IRIS 3	2
Epilepsy	Idiopathic	7
Hepatic diseases	Hepatocellular adenoma	1
Tumors	Mast cell tumor (removed)	6
	Benign mammary gland tumor (removed)	1
	Acanthomatous ameloblastoma (removed)	1
	Cutaneous hemangiosarcoma (removed)	1
	Seminoma (removed)	1
	Melanoma (removed)	1
	Fibroma (removed)	1
	Duodenum mass lesion (removed)	1
	Chronic lymphocytic leukemia	1
	Hepatocellular adenoma	1
	Mammary gland tumor	1
	Thyroid gland tumor	1
Osteoarthritis		32
Respiratory disorders	Laryngeal paralysis	1
	Tracheal collapse	1
Gastrointestinal disorders	Chronic gastritis	4
	Suspected food allergy/intolerance	4
	Canine chronic enteropathy	1
	IBD	1
	Exocrine Pancreatic Insufficiency	1
Neurological deficits	CCD	2
	Congenital paraparesis (mild)	1
	Intervertebral disc disease	9
Dermatological problems	Recurrent otitis externa	17
	Suspected allergic dermatitis	21
	Chronic dermatitis	2
Oral cavity disease	Severe dental calculus	4

MMVD B1 and B2 - Myxomatous Mitral Valve Disease stages B1 and B2 [4]; IRIS 2 and 3 - International Renal Interest Society staging system for the chronic kidney disease stages 2 and 3 https://www.iris-kidney.com/iris-guidelines-1; IBD - Inflammatory Bowel Disease; CCD - Canine Cognitive dysfunction.
* partially treated with balloon valvuloplasty.

Table 4
Comparison of the number of reported health issues and clinical signs.

Cardiological issues	Reported heart disease	16
	Murmurs detected on cardiac auscultation	20
GI issues	Reported GI disorders	10
	Reported episodes of diarrhea at least several times/month	10
	Reported episodes of vomiting at least several times/month	15
Neurological issues	Reported intervertebral disc disease	9
	Proprioception deficit of hind limbs	24
Oral cavity issues	Reported oral cavity disease	4
	Severe dental calculus	28
	Severe gingivitis	20

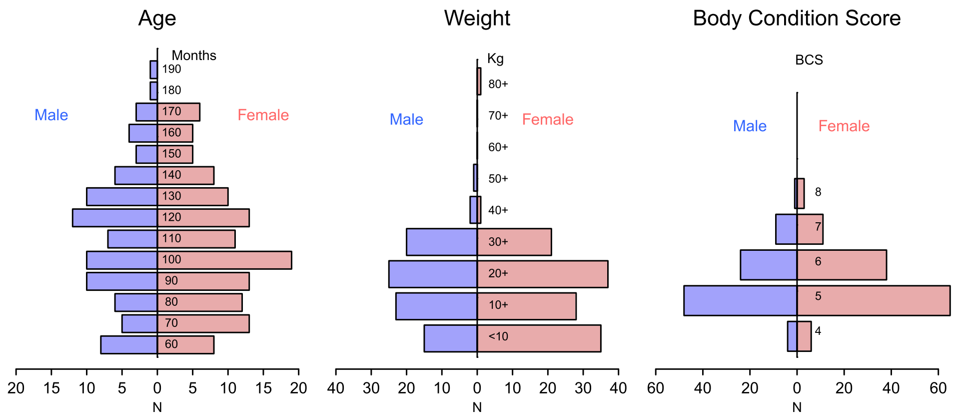

Fig. 3. Distribution of the enrolled dogs by age, sex, weight, and a 9-point scale BCS.

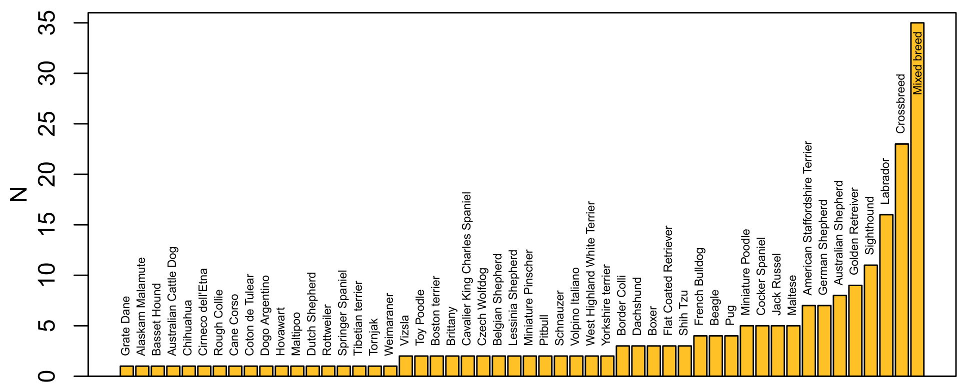

Fig. 4. Distribution the enrolled dogs by breed.

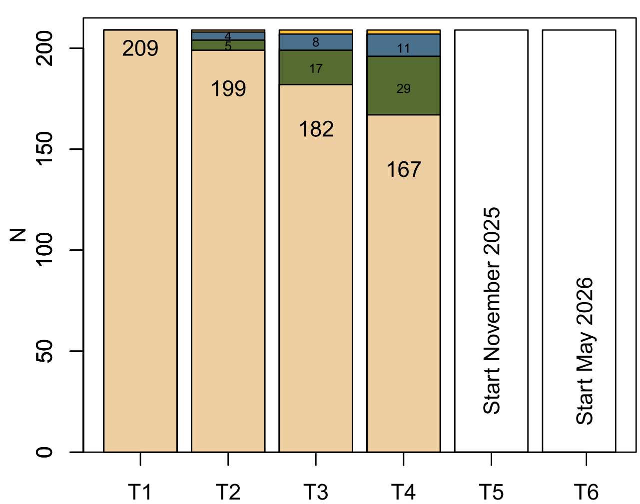

Fig. 5. Distribution of available data across six time points. Beige: Number of dogs for which a complete set of data was obtained, including compiled questionnaire, blood samples, physical examination results, and mobility trial. Green: Dogs that died between time points. Blue: Dogs for which only the alive/deceased status is available. Yellow: Dogs lost to follow-up, with no data available.

2.1.8. *Cognitive and behavioral health*

Assessing the cognitive abilities of dogs is intricate and presents distinct challenges. In this project we have decided to measure canine cognitive function using an expanded version of the Canine Cognitive Dysfunction Scale (CCDS) [50]. This questionnaire focuses specifically on identifying atypical behaviors in dogs. In addition to the CCDS, a few questions assessing the perceived interest of the dog in interacting with people, dogs, or inanimate objects were added based on the items described by [58] (see Table 2.1.7).

2.2. *General physical examination*

Once all the items in the questionnaire were filled, a complete clinical examination of the dog, aiming at evaluating general health, was performed (see Fig. 2). The parameters assessed are listed in Table. 4 of Supplementary Material.

2.3. *Blood samples*

After the general health assessment a blood sampling is performed primarily using a closed vacuum system with a butterfly needle and vacutainer tubes, drawing from either the saphenous or cephalic veins. In smaller subjects, however, samples were collected from the jugular vein using a syringe. For each dog we collected two blood samples: one in a plain tube to obtain serum samples and one in a K3-EDTA tube for hematological testing, plasma collection, and peripheral blood mononuclear cells (PBMCs) DNA extraction (see Fig. 2).

Biochemical, standard hematological testing and serum proteins electrophoresis are performed immediately after the blood collection. The biochemical testing includes classical clinical biochemistry parameters, serum protein electrophoresis, and serum paraoxonase-1 activity (PON1). Haptoglobin was measured for two time points. For each blood test, a board-certified clinical pathologist performed a visual evaluation of the blood smear.

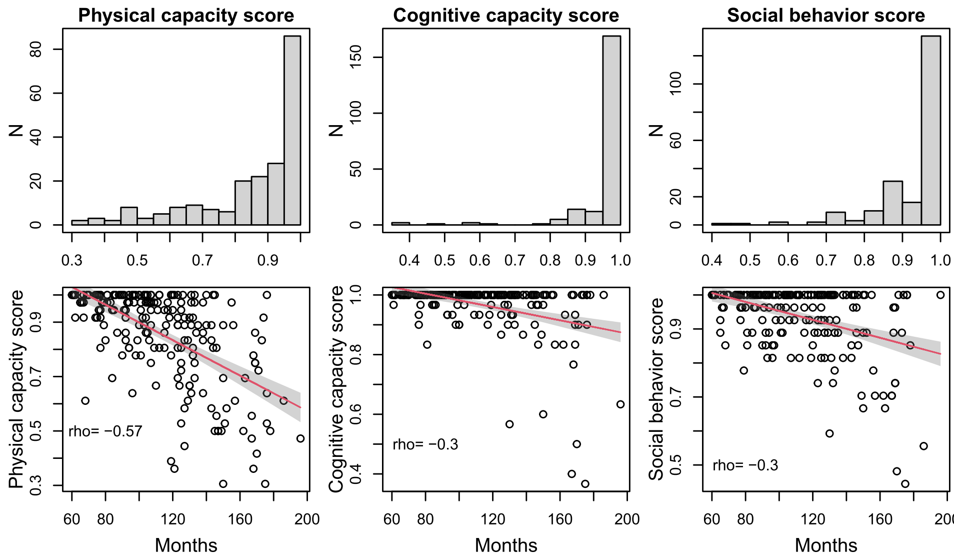

Fig. 6. Distributions of physical capacity, cognitive, and social behavior scores — each normalized to its maximum possible value — and their associations with chronological age (regression line with the 95% confidence interval and the Spearman's rank correlation coefficient are reported at each panel).

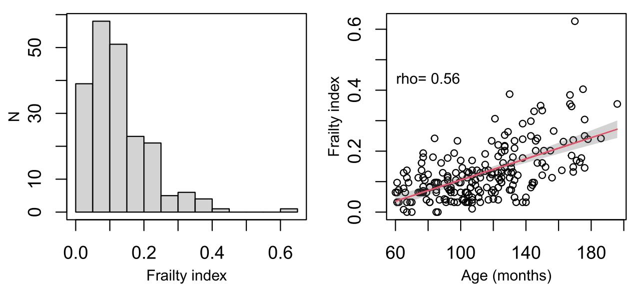

Fig. 7. Distribution of the frailty index and its relationship with the age of the dogs. The solid red line represents the linear regression model, and the shaded area indicates the 90% confidence interval. The Spearman correlation coefficient (ρ) between the frailty index and chronological age is reported in the right panel. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.4. Mobility trial

Following [43] after the physical exam and blood sampling, we performed a mobility test consisting in a 10-meter-long trail with the investigator holding the leash and the owner waiting at the final point of the distance. Dogs were free to choose the speed and, except for some cases of fear and severe vision impairment, tended to run.

2.5. Fecal samples

Fecal samples were collected at the third time point and will be collected again at the end of the study. Owners were instructed to adhere to standard procedures both prior to and during sampling: to avoid administering antibiotics within 30 days before collection; to refrain from giving probiotics or fermented foods during the four days preceding collection; and to collect approximately 0.1 g of feces immediately after defecation using a sterile swab, transferring it into a pre-supplied DNA stabilization tube. Following collection, samples were stored at room temperature for no longer than two weeks, and then transferred to -80° C until processing for analysis. Cell lysis, DNA extraction and the 16S rRNA gene amplification sequencing were performed using the procedure described in Innocente et al. [30] and Berlanda et al. [9].

3. Current progress of the OLD-DOG project

3.1. Study population

Table 2.1.7 shows the main characteristics of the dogs included in the study. The distributions by age, weight, a Body Condition Score (BCS), and breed are presented in Fig. 3 and Fig. 4. Fig. 5 shows the number of dogs with complete data, the number that died between time points, and those lost to follow-up. The dropout rate was very low, with only 2 dogs completely lost to follow-up by the fourth time point, for which no data were available. For 11 dogs at the fourth time point, only survival status (alive/deceased) was recorded.

Table 3 presents the chronic or recurrent health issues confirmed or suspected at the first time point. Dermatological problems were the most frequently reported, including 17 cases of recurrent otitis externa and 21 cases of suspected allergic dermatitis. These were followed by 32 cases of osteoarthritis and 16 cases of cardiac disorders. 100 dogs were treated with any kind of medications in the past 6 months and 39 of them follow chronic or long-lasting therapies. The most frequent therapies are: 5 cases of use of phenobarbital, 5 of oclacitinib, 4 of pimobendan, 4 of levotiroxina, 4 of gabapentin and 4 of phenylpropanolamine hydrochloride for urinary incontinence.

3.2. Comparison of reported health issues and clinical signs.

Table 2.1.7 compares the number of dogs with owner-reported health issues to the number of dogs exhibiting clinical signs typically associated with those conditions. Across all categories, reported cases were consistently fewer than clinically observed cases, indicating that many health issues in dogs remain undiagnosed. The most striking discrepancy was found in oral cavity problems: only 4 owners reported such issues, whereas 28 dogs presented with severe dental calculus and 20 with severe gingivitis; an additional 51 dogs had moderate dental calculus and 46 had moderate gingivitis. Neurological problems were also frequently underestimated: 24 dogs showed proprioceptive deficits of the hind limbs, while only 9 owners reported intervertebral disc disease. Vomiting episodes are frequently overlooked by owners, especially when characterized by bilious vomiting or by vomiting following grass ingestion.

Table 5
Available data on the causes of death for dogs that died during the follow-up period.

	Age at death (yr)	Cause of death	Symptoms prior to death	Euthanasia (yes/no)
Died within 6 months	13.3	Tumor (suspected)		No
	16.5	Unknown	Disorientation, restlessness, vocalization	Yes
	14.7	Thyroid tumor		Unknown
	6.8	Tumor		Unknown
	13	Unknown	Epilepsy crisis, acute tetraplegia	Yes
Died within 12 months	9.5	Tumor		Unknown
	12.9	Sudden death		No
	11.5	Vertebral tumor or spinal disc extrusion	Acute paraplegia	Yes
	11.1	Osteosarcoma		Yes
	13.3	Unknown	Acute diarrhea	No
	13.8	Splenic mass lesion		Yes
	15.1	Unknown		Unknown
	11.1	Melanoma		Unknown
	6.3	Osteosarcoma		Yes
	15.4	Osteolytic mass lesion (C1 vertebra)		Unknown
	13.6	Metastatic tumor	Multiple hepatic and spleen mass lesions	Yes
	12.1	Pericardial effusion		Yes
Died within 18 months	15.1	Sudden death		No
	16.7	Unknown		Unknown
	15.6	Suspected hepatic sarcoma	Hematoperitoneum	Yes
	12.6	Hemangiosarcoma		No
	15.3	Renal failure or diabetic ketoacidosis		No
	7.4	Renal failure		Yes
	15.4	Hepatic adenoma	Hematoperitoneum	Yes
	15.9	Trauma		No
	9.8	Metastatic hepatic mass	Hematoperitoneum	Yes
	12.4	Cardiac failure, pulmonary edema		Yes
	15.8	Unknown	Lethargy	Yes
	15.4	Sudden death		No

3.3. Mortality rate and mortality cause

Of the 206 dogs for which survival status was available, 29 had died by the fourth time point. The available data on causes of mortality are summarized in Table 2.1.7. Among the 24 dogs with know cause of death, 15 had confirmed or suspected tumors as the cause of death or reason for euthanasia, accounting for 62.5% of deaths. A moderate negative correlation was observed between body weight and longevity ($\rho = -0.59$, $p = .001$).

3.4. Frailty index

We applied the frailty index (FI) developed by [6] to our cohort. The items included in the FI calculation and their corresponding scores are provided in the Supplementary Material. We excluded the items “Assistance for eating” and “Acute vascular problems” from the original FI, as no dogs in our population presented with these conditions.

Considering the potential discordance between reported and observed health deficits discussed above, FI items were categorized as present or absent based not only on owner-reported information but also on the clinical examination. For instance, if moderate dental calculus was detected during the medical examination despite no oral cavity problems being reported, the oral cavity deficit was considered present.

The distribution of the FI calculated in this manner, as well as its variation with age, are shown in Fig. 7. The index exhibits a right-skewed distribution and a moderate correlation with chronological age ($\rho = 0.56$, $p < .0001$). Figure 7 also shows the linear regression model with FI as the dependent variable and age as the independent variable.

3.5. Physical, cognitive function, and social behavior scores

The physical fitness score, derived by summing the points from the first section of Table 2.1.7 and normalizing by the maximum possible score, showed a left-skewed distribution (median = 0.92; range = 0.30-1.00) and a moderate negative correlation with age (ρ = -0.56, $p < 0.0001$; see Fig. 6). Cognitive capacity and social behavior scores, calculated analogously from the second and third sections of Table 2.1.7, followed similar patterns but showed weaker associations with chronological age (ρ = -0.29, $p < 0.0001$ and ρ = -0.31, $p < 0.0001$, respectively). Interestingly, both cognitive capacity and social behavior were more strongly correlated with physical fitness than with chronological age (ρ = 0.37, $p < 0.0001$ and ρ = 0.41, $p < 0.0001$, respectively). Low cognitive capacity score was found to always associated with low physical capacity score: all the dogs with cognitive capacity score <0.7 had physical capacity score <0.7. On the other hand, low physical capacity score was associated with low cognitive capacity scores only in 16% of cases (6 out of 36 dogs).

3.6. Hematology and clinical biochemistry analysis.

Hematological, biochemical, and serum protein electrophoresis data were analyzed first with a focus on parameters showing significant age-related variation, even within laboratory reference intervals. This approach aimed to identify subtle, age-dependent trends while minimizing the influence of pathological outliers. In most cases, inclusion of values outside the reference range strengthened these trends. Creatinine represented an exception, showing a more robust correlation with age after

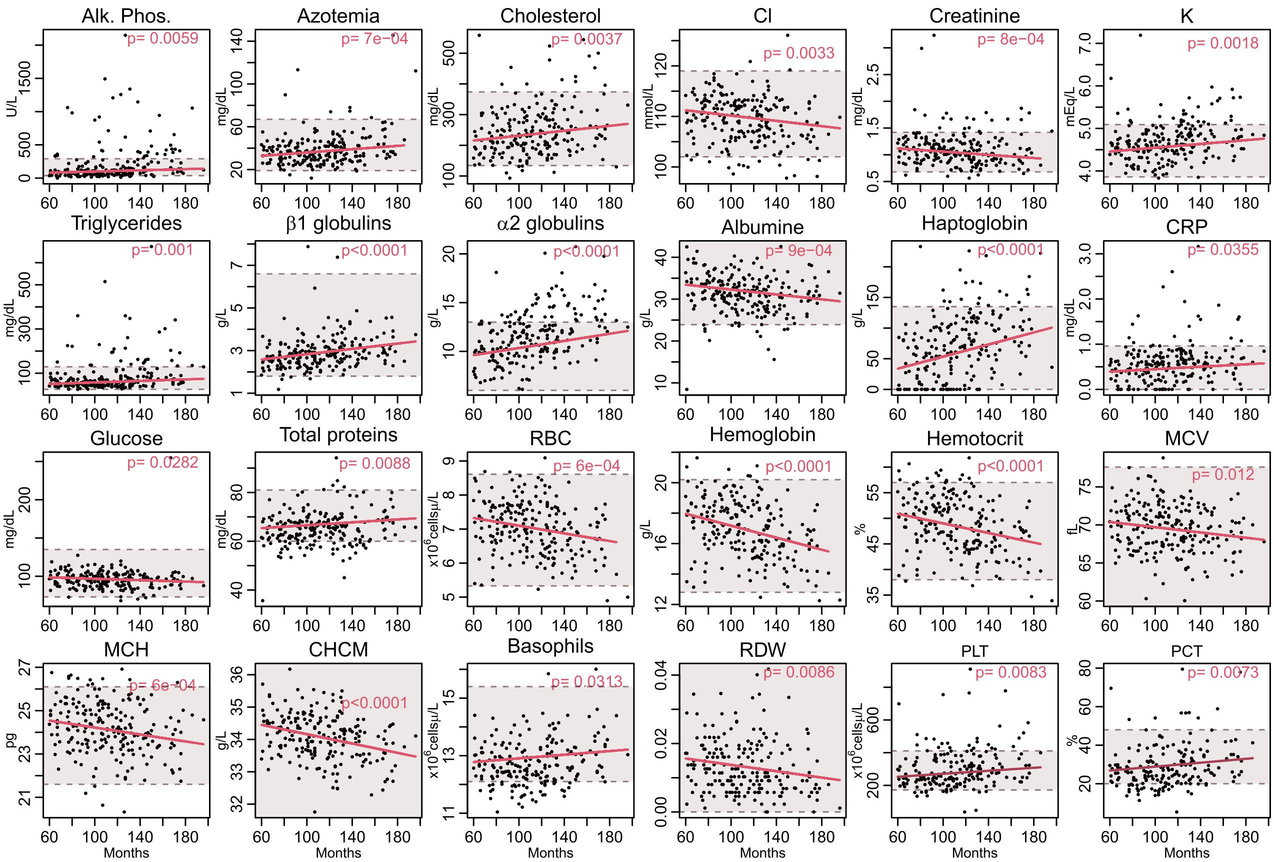

Fig. 8. Parameters of standard hematology, biochemistry and serum protein electrophoresis that were found to show linear variation with age with the p-value ≤0.05 within the laboratory reference intervals. Alk. Phos. - Alkaline Phosphatase, Cl - Chlorine, K - potassium, CRP - C-Reactive Protein, RBC - Red Blood Cells, MCV - Mean Corpuscular Volume, MCH - Mean Corpuscular Hemoglobin, CHCM - Cellular Hemoglobin Concentration Mean, RDW - Red blood cell Distribution Width, PLT - Platelet concentration, PCT - platelecrit.

excluding pathologically high data. Fig. 8 presents the parameters exhibiting statistically significant linear associations with age ($p \leq 0.05$).

3.7. Biobank and future analysis

All surplus biological material - including serum remaining after standard clinical biochemical analyses, as well as plasma and blood cells left after hematological testing - is stored at -20 °C (see Fig. 2). Surplus fecal samples material is stored at -80°. In this way, a dedicated biobank is being created, providing a valuable resource for future analyses and exploratory studies.

4. Discussion

The cohort enrolled in the present study provides good coverage of dogs, ranging from miniature breeds to those weighing up to 40 kg, with only five individuals exceeding this weight. Females were approximately 30% more represented than males. The proportions of neutered and intact males were nearly equal; however, intact females accounted for only 12% of all female dogs. Although the higher proportion of neutered females compared to neutered males reflects the general distribution of the dog population in Italy [12], the overall percentage of intact dogs participating in this project was lower than that reported in demographic studies [12,47]. Several factors may explain the observed discrepancy. In our cohort, only 39% of dogs were neutered at a young age, meaning that the proportion of intact animals decreases with advancing age, leading to differences between cohorts with different median ages. Another possible explanation is that owners who participate in research projects may not fully represent typical pet ownership practices. The low number of intact female dogs may pose certain limitations when investigating the influence of estrogens on age-related inflammation [33].

The most prevalent disorders observed in our sample are consistent with findings from other population-based studies [3,53]. Although it is difficult to determine the exact prevalence of specific pathologies in dogs aged five years and older, we attempted to compare the frequencies observed in our sample with previously reported data. Approximately 15% of dogs in the present study had a previously diagnosed or clinically suspected case of osteoarthritis, which aligns with the 20% prevalence reported in post-mortem studies [55][14]. reported prevalence of 11%, 24%, and 38% for any kind of cardiac problems in dogs aged 5-8, 9-13, and over 13 years, respectively, with suspected presence of cardiac abnormalities, representing the upper limit for prevalence in the general population. In our study, heart murmurs were detected in 2.9% (3/102), 9.4% (8/85), and 41% (9/22) of dogs in these same age groups. The observed mismatch between reported health issues and clinically detected signs emphasizes the need for thorough medical evaluations in aging dogs to avoid underestimating their true health condition and risks.

The lifespan of dogs participating in the project showed a moderate but expected inverse relationship with body weight [21,41,44,56]. Neoplastic disease was the most frequent cause of death in our cohort, followed by degenerative conditions, consistent with findings from large-scale population studies [56].

The cross-sectional results presented here indicate that numerous parameters potentially associated with aging show clear relationships with chronological age and will therefore be further examined for their ability to predict mortality risk. Physical and cognitive capacity scores display a moderate age-related decline. A consistent association was observed between low cognitive and low physical capacity, with the latter likely reflecting a symptom of cognitive decline. This association has also been reported in previous studies on dogs [11]. Physical activity is a well-established protective factor against cognitive decline in humans [1], and the possibility that the observed association represents not only a symptom but also a potential risk factor is particularly intriguing. Future studies within the current project will investigate the relationship between baseline activity levels and longitudinal changes in cognitive function.

The FI developed by [6] was also applied to our cohort and yielded comparable results, despite substantial differences between the two study populations. In [6], dogs were presented to a veterinary hospital for existing health conditions, whereas the cohort in the present study consisted exclusively of dogs that, at the time of first assessment, showed no clinical indications requiring hospital referral. Despite these differences, the FI showed a similar correlation with chronological age in both studies ($\rho = 0.51$ in [6] and $\rho = 0.56$ in the present work), as well as comparable linear relationships between FI and age (slope $\beta = 0.017$ in [6] and $\beta = 0.020$ in the present study).

Significant age-related trends were also identified in many standard biochemical and hematological parameters. While most patterns align with previously reported findings in dogs [62], our data also reveal, to our knowledge for the first time, a marked age-associated increase in haptoglobin as well as in the α2- and β1-globulin fractions. In contrast to earlier reports, no decline in total lymphocyte count was observed. Interestingly, C-reactive protein (CRP) - typically regarded as age-invariant - showed a borderline-significant trend toward increase [2,29,35,38].

The collective pattern of age-related changes points to several biological processes underlying canine aging. Decreases in hemoglobin-related indices (MCH, CHCM, RBC, HCT) and albumin, together with increases in platelet indices (PLT, PCT), haptoglobin, and possibly CRP, are compatible with low-grade chronic inflammation, reminiscent of the “inflammaging” phenomenon described in humans [17,19,37]. Increases in triglycerides, cholesterol, and globulin fractions containing lipoproteins (α2 and β1) potentially indicate an age-related dysregulation of lipid metabolism, previously found to be associated with increased frailty in dogs [39]. The observed decrease in creatinine likely reflects loss of muscle mass with aging [59]. Longitudinal analyses of these indices and clinical parameters - and their associations with mortality - will therefore be a key focus of future investigations.

4.1. Perspectives

The next phase of the laboratory analyses will include measurement of PBMC telomere length at all time points, as well as the characterization of age-associated epigenetic patterns and their relationship with mortality. In parallel, microbiota profiling - particularly focusing on microbial diversity - will be conducted at two time points.

The application of the frailty phenotype to the described cohort, as well as the evaluation of the predictive performance of the FI and the frailty phenotype for mortality, will be addressed in subsequent publications.

The ultimate goal of these investigations is to develop a cumulative predictive model for age-associated adverse health outcomes in dogs, thereby enabling the design and evaluation of anti-aging interventions in companion animals.

Ethical statement

The study was approved by the ethical committee of the University of Padua. Protocol number: 215906 released on 31/10/2023.

CRediT authorship contribution statement

Polina Zemko: Writing - original draft, Investigation, Formal analysis, Data curation; **Federico Bonsembiante:** Writing - review & editing, Project administration, Methodology; **Marco Canevelli:** Writing - review & editing, Methodology, Funding acquisition; **Simona Buscarnera:** Writing - review & editing; **Matteo Cesari:** Writing - review & editing, Supervision; **Tommaso Banzato:** Writing - review & editing, Project administration, Methodology, Funding acquisition, Conceptualization.

Data availability

Data available on request due to privacy/ethical restrictions.

Funding

The following study has been funded by the Italian ministry of university, and research (MUR) with a grant PROGETTO DI RICERCA DI RILEVANTE INTERESSE NAZIONALE (PRIN) 20228NKPNH OLD DOG - Validating the dog as animal model for human ageing studies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors would like to thank Silvia Bedin and Luana Mellia for their valuable contributions to the laboratory analyses conducted in this study.

Supplementary material

Supplementary material associated with this article can be found in the online version at 10.1016/j.ces.2025.121880.

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