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Romane Lemaréchal, Sara Hoummady, Inès Barthélémy, Claude Muller, Julie Hua, et al.. Canine model of human frailty: adaptation of a frailty phenotype in older dogs. Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 2023, 10.1093/gerona/glad006 . hal-03948583

HAL Id: hal-03948583

<https://hal.science/hal-03948583>

Submitted on 8 Mar 2023

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Canine model of human frailty: adaptation of a frailty phenotype in older dogs

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Conflict of interests: none

Accepted Manuscript

Abstract

Frailty is a clinical presentation resulting from age-related cumulative declines in several physiological systems. The aim of this study was to adapt the concept of frailty to the domestic dog, as a model for frailty research, by characterizing a 5-criterion frailty phenotype using objective measurement, and to investigate its independent association with death. A prospective cohort including 80 Labrador and Golden Retriever dogs aged 9 years or older was conducted between March 2015 and July 2020. An adapted frailty phenotype was defined according to the presence of five criteria (weakness, slowness, poor endurance, low physical activity, and shrinking) evaluated at baseline from physical performance tests and items from questionnaire and physical examination. Survival analysis was used to investigate the association between frailty status and time to all-cause death over 5 years of follow-up. Frailty status was significantly associated with all-cause death, with median survival times of 10.5 months, 35.4 months, and 42.5 months, respectively for dogs with three or more criteria (frail dogs), dogs with one or two criteria (prefrail dogs), and nonfrail dogs. Independently of age, sex, breed, sterilization and sex-sterilization interaction, frail dogs died significantly faster than nonfrail dogs at baseline (adjusted hazard ratio = 5.86; 95% confidence interval = 2.45-14.0; $p < 0.01$). This significant association persisted after controlling for other potential confounders. Frailty, assessed by a 5-criterion phenotype, was predictive of all-cause death, in geriatric Labrador and Golden Retriever dogs. The concept of frailty seems adaptable to the dog.

Keywords: Cohort; survival; physical performance; vulnerability

Introduction

With the increase in human life expectancy in developed countries (1), delaying the onset of dependency in older people and promoting a good quality of life during the last years of life become a major public health challenge (2). It is therefore crucial to understand the determinants of a “pathological aging” in order to promote “healthy aging” (3). The definition of “health” by the World Health Organization is “a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity”. In this context, maintaining the functional status of older individuals is as important as the management of age-related diseases, and the assessment of frailty is a key component to evaluate such functional status (4,5).

Frailty is defined as the decrease in reserve and resistance to stress resulting from cumulative declines in several physiological systems and causing vulnerability to adverse events (6). Frail individuals are at risk for long hospitalizations, fractures, falls, surgical complications, institutionalization, disability, and death (7-13). Discriminating frail from nonfrail individuals is an opportunity for geriatricians to provide an adapted medical care (14). The two most widely used tools to identify frail individuals are the frailty index, based on an accumulation of age-related deficits, and a 5-criterion frailty phenotype, a clinical presentation of a definable biological syndrome (5).

Some authors have suggested that frailty should be assessed among non-human animals to better understand the pathophysiology of aging (15). Several frailty assessment tools have already been validated in animal models (16). The domestic dog (*Canis familiaris*) has been considered as a good animal model to study aging (17,18) for several reasons. There are numerous similarities between human and dog genomes and certain genetically related diseases are common between the two species (19). Furthermore, dogs are exposed to an environment similar to humans in terms of pathogens, lifestyle (malnutrition, sedentary lifestyle), and pollutants (20). Finally, they have age-

related diseases similar to humans (21). Two studies assessing frailty in dogs have been published to date. The first study adapted the frailty phenotype to older guide dogs mostly Labrador and Golden Retrievers based on items of a geriatric grid during a geriatric examination (22). In this study, regardless of age, health status, or comorbidities, dogs with two or more frailty criteria died more quickly than dogs with one criterion or none. One limitation of this study was that the adapted frailty phenotype was based on items only, without any performance tests. In the second study, frailty was assessed by using the frailty index, adapted to dogs in a cohort of all-breed dogs aged 2 years or older. In this study, the frailty index was strongly associated with short-term (6 months) mortality (23). These initial findings are promising since they provide some evidence that frailty is a concept which can be adapted to dogs.

In this context, the objective of this study was to adapt the 5-criterion frailty phenotype to geriatric dogs by using physical performance tests derived from human geriatric medicine, and to investigate its independent association with time to all-cause death over 5 years of follow-up, in a prospective cohort study of older Retriever dogs.

Method

Study sample

The SeniorDog cohort is a single-center prospective cohort designed to study the association between frailty and mortality in geriatric dogs. It was conducted between March 2015 and July 2020 at the Ecole nationale vétérinaire d'Alfort (EnvA) in the region of Paris, including Labrador or Golden Retriever dogs aged 9 years or older living in a family setting. This cut-off of age was decided since it corresponds to the age at which large breed dogs (including Labrador and Golden Retriever dogs) are classified as “geriatric” dogs (24). Dogs were enrolled on a voluntary basis, at the EnvA, with the help of veterinarians, the French Retriever Club, and by meeting owners in parks and streets. Dogs were not included if they presented clinical signs consistent with an infectious disease within two

weeks prior to enrolment, if they were blind, on a weight loss diet, declared aggressive by their owner, not interested by food, not responding well to indoor recall, and not up to date with their vaccinations. Only one dog was included per household. A total of 80 dogs were enrolled in the cohort between March and July 2015. The data collection at enrolment included a physical examination, physical performance tests, biological analyses and data collected through a questionnaire filled in by the owner. Owners were then contacted by phone or email every 3 months during the first year, then every year until July 2020. The information collected during follow-up included the health status of the dog as well as the date and cause of death or cause of euthanasia when applicable. The study received the approval of the Ethical committee of EnvA for clinical research, with the number #2014-08-29.

Baseline data collection

A physical examination was conducted by a veterinarian who evaluated locomotor difficulties, the presence of localized (moderate or severe) or generalized amyotrophy (moderate or severe) according to a chart proposed by the World Small Animal Veterinary Association (25), body condition score on a 5-point scale, oral health, and cardiovascular, respiratory, visual, and hearing functions.

Through a questionnaire, owners provided the dog's demographic data, past and current environment and diet, medical history (including diabetes, dysplasia, osteoarthritis, renal disease, cancer, and oedema), physical and cognitive functions, and physical activity (number of minutes spent per week of several activities). Weight loss was collected by asking the owner whether her/his dog had lost some weight compared to when the dog was 2-5 years. Such weight loss, when reported, was considered unintentional because dogs enrolled in the SeniorDog cohort were not on a weight loss diet at the time of enrolment. A total metabolic activity index (MAI) was calculated to grade the intensity of the overall physical activity according to the following equation adapted from the Minnesota Leisure Time Activity questionnaire (26): $\text{total MAI} = \sum (\% \text{VO}_2\text{max} * D)$ where D is the duration of the activity in minutes spent per week and $\% \text{VO}_2\text{max}$ is the percentage of VO_2max

required to perform the activity for a dog (20% of the VO_2 max for walking, 30% for playing with other dogs and performing agility/obedience tasks, 40% for running with owner, 45% for playing with objects or following the owner on bicycle, and 50% for hunting (27)). The total MAI was not expressed in kcal per week but nevertheless approximately quantified the energy spent per week, which allows relative comparison between the dogs of the cohort.

A blood count, a biochemical examination of blood (urea, creatinine, albumin, glucose, total cholesterol, bilirubin, total protein, alanine aminotransferase (ALT), alkaline phosphatase (ALP), triglycerides) and the determination of specific biomarkers of interest in the study of frailty and aging (insulin-like growth factor-1 (IGF-1), cortisol, CD4/CD8 ratio, N-terminal prohormone of brain natriuretic peptide (NT-Pro-BNP), C-reactive protein (CRP), interleukine-6 (IL-6)) were performed for each 12-hour fasted dog. Hematologic and biochemistry parameters were analyzed by the Laboratory of EnvA (Biopôle). IL-6 and IGF-1 were analyzed on an enzyme-linked immunoassay (ELISA) plate (Canine IL-6 Kit DuoSet, R&D Systems, USA; Kit IGF-I Elisa, Demeditec Diagnostic GmbH) according to the manufacturer's protocol. CD4/CD8 ratio was determined by the Laboratory of EnvA (Biopôle) and NT-Pro-BNP was analyzed by the IDEXX laboratory.

Physical performance tests were instructed by the veterinarian to quantify the muscular strength, the walking speed, and the endurance of the dog. The muscular strength was assessed by measuring the weight (kg) pulled by the dog using a dynamometer attached to the harness of the dog, with the leash fixed to the wall. After sitting the dog back to the wall, the owner moved 3 meters in front of the dog and called it with a food treat. The peak force was measured by the dynamometer and normalized to body weight (kg) (28). The walking speed was assessed by adapting the Timed Rapid Gait test from human geriatrics (29) to dogs. The time needed for the dog to walk 6 meters was measured and normalized to height of the forelimb from floor to elbow (30). The walking time was recorded over 10 meters in order to exclude a 2-meter acceleration phase and a 2-meter deceleration phase from the final time. The dog was motivated by a food treat proposed by its

owner. The test was performed 3 times and the lowest time was retained. The endurance was assessed by adapting the 6-minute Walk test from human geriatrics to dogs (31) which has already been adapted to dogs (32,33). Dogs were instructed to walk down a corridor at their preferred speed (i.e., walking or trotting) for 6 minutes, next to the veterinarian who adapted her gait to the dog's gait. The walked distance was then normalized to height of the forelimb.

Assessment of an adapted frailty phenotype

Frailty was assessed by adapting the 5-criterion frailty phenotype (6) using the data collected from the physical performance tests described above, as well as from the questionnaire (for an initial frailty phenotype) or from data collected during the physical examination (for a second frailty phenotype). For the "weakness", "slowness", "poor endurance", and "low physical activity level" criteria assessed through a quantitative measurement, it was necessary to define a threshold for the presence or absence of the criterion. In the absence of established standards in dogs and similarly to previous studies of the frailty phenotype in human medicine (6) and in animal models (34,35), the presence of the criterion was based on a quantile of the distribution of the quantitative measurement within the sample. We chose to use quartiles as in the study of Yamada et al. (35), instead of quintiles as in the original definition of a frailty phenotype (6), because the prevalence of frailty in SeniorDog was expected to be higher than in the Cardiovascular Health Study cohort (6): using human year equivalents (36), the median age of the dogs enrolled in SeniorDog was higher than in the Cardiovascular Health Study cohort (6) and the prevalence of frailty is known to increase with age.

The five criteria of the initial adapted frailty phenotype (FP1) were defined as following (Table 1):

1. Weakness: lowest 25% peak force normalized to body weight.
2. Slowness: slowest 25% walking time normalized to height of the forelimb.
3. Poor endurance: lowest 25% distance normalized to height of the forelimb.

4. Low physical activity level: lowest 25% total MAI.

5. Shrinking: weight loss compared to age of 2-5 years reported by owner.

At baseline, a dog was considered “frail” if it presented three or more of the five criteria, “prefrail” if it presented one or two, and “nonfrail” if it presented none.

Data Analysis

The primary outcome was death due to all causes over the 5 years of follow-up, including euthanasia for any reason. Survival rates by frailty status at baseline were described using univariate Kaplan-Meier survival curves with their 95% confidence interval, and compared using the log-rank test. A Cox proportional hazards model was used to estimate hazards ratios (HR) quantifying the association between the presence of the adapted frailty phenotype at baseline and death occurrence. Multivariate analyses were systematically adjusted for age, breed, sex, and sterilization. A sex-sterilization interaction was also systematically introduced because the association between sterilization and death is likely to differ between males and females (37). Additional adjustments were made for other covariates (named “other potential confounders”) whose association with death was documented in the literature or was associated after adjustment for age with a p-value lower than 0.20 in our data: history of chronic diseases except cardiac and osteoarticular, history of dysplasia, history of osteoarthritis, food quality in the past (supermarket food versus other type of food), living in a house (versus apartment), smoking environment, owners’ income, poor oral health, skin nodule, visual impairment, hearing impairment, lameness, blood concentrations in ALT, urea, creatinine, glucose, triglycerides (38,39). Covariates which were considered potentially more “intermediate factors” than “confounders” included: cardiac history, resting heart rate, heart murmur, hematocrit, CD4/CD8 ratio, blood count of lymphocytes and granulocytes, blood concentrations in NT-pro-BNP, albumin, IGF-1, and CRP (40-43). A careful adjustment was nevertheless made on these last covariates in order to be as conservative as possible in studying the association between frailty and death. The assumption of proportional hazards for each variable

included in the Cox model was verified by the Schoenfeld residuals plot (44) and by the Therneau test (45). The assumption of log-linearity of the association for each quantitative variable included in the Cox model was visually tested by plotting restricted cubic spline functions (46). The absence of interaction between the added covariates and frailty was tested if there was a prior hypothesis for this interaction. The individual adjusted associations between the presence of each criterion and time to all-cause death are also presented.

Because reasons for euthanasia included non-rapidly fatal conditions reported by the owners (such as paralysis/paresis of the hindquarters, inappetence, urinary incontinence, lethargy, or disorientation), our primary outcome of all-cause death did not therefore exactly represent the outcome of all-cause death in frailty studies in humans (47). In a sensitivity analysis, our secondary outcome was natural death (i.e., no euthanasia) or euthanasia for rapidly fatal conditions. Censored dogs were dogs still alive at the end of the follow-up, dogs lost to follow-up, and euthanized dogs for a non-rapidly fatal condition. Dogs who died from an unknown cause were excluded from this sensitivity analysis. The assumption of non-informative censoring inherent when estimating HR from a Cox model is expected to be met in this sensitivity analysis since such conditions leading to a decision of euthanasia are, a priori, not strongly associated with early natural death, independently of the age, breed, sex, sterilization, sex-sterilization interaction and the major risks factors of death.

A post-hoc analysis was also performed to improve the validity of the initial FP1 in terms of predicting time to all-cause death by defining a second adapted frailty phenotype (FP2). The definition of the FP2 was conducted in two steps. First, a new definition of the “shrinking” criterion was considered (to reduce misclassification errors on the frailty status) by replacing “weight loss” reported by owners with “generalized amyotrophy” (moderate or severe) assessed by the veterinarian during physical examination at baseline, if the individual adjusted association between weight loss and time to all-cause death was weaker than the one between generalized amyotrophy and time to all-cause death. Generalized amyotrophy was chosen because, like unintentional weight

loss, it is an indication of loss of muscle mass and is included in the “shrinking” criterion of the frailty phenotype (14). Second, for the four quantitative criteria, quintiles (instead of quartiles) were used (to reduce the potential lack of specificity to identify frail or prefrail dogs) if such use of quintiles increased the strength of the association between the adapted frailty phenotype and time to all-cause death.

All analyses were performed with R version 4.1.2.

Results

Sample characteristics

The 80 dogs of the cohort were 9.0 to 16.9 years old with a median of 11.3 years (Table 2), 43 dogs were male (54%), 45 were neutered (56%), and 35 were Golden Retrievers (44%). Weight loss was reported for 12 dogs (15%). A total of 11 dogs (14%) presented three or more criteria of the FP1, and were accordingly considered as “frail”, 40 dogs (50%) presented one or two criteria and were considered as “prefrail”, and 29 dogs (36%) did not present any criterion and were considered as “nonfrail”. Table 2 presents the baseline characteristics of the 80 dogs according to their frailty status.

Follow-up

At the end of the 5 years of follow-up, 75 dogs were dead (94%), 3 were still alive (4%), and 2 were lost to follow-up (2%). Of the 75 deaths, 22 (29%) were secondary to euthanasia due to a non-rapidly fatal condition, 39 (52%) were secondary to euthanasia due to a rapidly fatal condition, 11 (15%) were natural death, and for 3 dogs (4%), the cause of death was unknown.

Main analysis

The FP1 was significantly associated with time to all-cause death (p log-rank < 0.01, Figure 1). The median survival times were 10.5 months, 35.4 months, and 42.5 months, respectively for frail, prefrail and nonfrail dogs (Figure 1). Independently of age, breed, sex, sterilization and sex-

sterilization interaction, the FP1 was significantly associated with death in the multivariate Cox model (overall $p < 0.01$). More specifically, frail dogs died significantly faster than nonfrail dogs (adjusted HR [aHR] = 5.86; 95% confidence interval [CI] = 2.45-14.0; $p < 0.01$; Table 3); prefrail dogs also died faster than nonfrail dogs but this difference was not significant (aHR = 1.40; 95% CI = 0.80-2.45; $p = 0.24$; Table 3). Of note, in this multivariate model, age was significantly associated with death (aHR for a 1-year increase = 1.27; 95% CI = 1.12-1.45; $p < 0.01$). The FP1 remained significantly associated with death after adjustment for other potential confounders as well as after adjustment for variables potentially more “intermediate factors” than “confounders”.

There was a significant interaction between osteoarthritis and the FP1, which means that the association between the FP1 and all-cause death was significantly different between osteoarthritic and non-osteoarthritic dogs. Both in non-osteoarthritic and osteoarthritic dogs, the FP1 was significantly associated with death (overall $p < 0.01$ in the two sub-groups). Among the 29 non-osteoarthritic dogs, frail dogs (aHR = 10.28; 95% CI = 2.13-49.56; $p < 0.01$) and prefrail dogs (aHR = 4.03; 95% CI = 1.61-10.11; $p < 0.01$) died faster than nonfrail dogs. Among the 51 osteoarthritic dogs, frail dogs died faster than nonfrail dogs (aHR = 3.10; 95% CI = 1.07-8.94; $p = 0.04$) but there was no difference in time to death between prefrail and nonfrail dogs (aHR = 0.60; 95% CI = 0.28-1.32; $p = 0.20$).

Sensitivity analysis

In the sensitivity analysis, the FP1 remained significantly associated with natural death or euthanasia for rapidly fatal conditions (overall $p < 0.01$). More specifically, frail (aHR = 3.81; 95% CI = 1.27-11.39; $p = 0.02$) and prefrail (aHR = 1.52; 95% CI = 0.76-3.05, $p = 0.23$) dogs died faster than nonfrail dogs, although the association was not significant when comparing prefrail with nonfrail dogs. The FP1 remained significantly associated with natural death or euthanasia for rapidly fatal conditions after adjustment for other potential confounders as well as after adjustment for variables potentially more “intermediate factors” than “confounders”.

Post-hoc analysis

Individually, the presence of each criterion was associated with time to all-cause death, although not significantly for the “weakness”, “slowness”, and “shrinking” criteria: “weakness” (aHR = 1.45; 95% CI = 0.77-2.72; $p = 0.25$), “slowness” (aHR = 1.31; 95% CI = 0.75-2.31; $p = 0.34$), “poor endurance” (aHR = 2.89; 95% CI = 1.52-5.49; $p < 0.01$), “low physical activity level” (aHR = 2.17; 95% CI = 1.24-3.80; $p < 0.01$), and “shrinking” (aHR = 1.12; 95% CI = 0.58-2.18; $p = 0.74$). By replacing “weight loss” by “generalized amyotrophy” (present at baseline in 21 dogs) to define the “shrinking” criterion, this criterion was more strongly associated with time to all-cause death (aHR = 4.71; 95% CI = 2.48-8.96; $p < 0.01$). By using quintiles for the four quantitative criteria and generalized amyotrophy for the “shrinking” criterion, 14 dogs (17%) presented three or more criteria of the FP2, 27 dogs (34%) presented one or two criteria, and 39 dogs (49%) did not present any criterion. Independently of age, breed, sex, sterilization, and sex-sterilization interaction, the FP2 was significantly associated with time to all-cause death in the multivariate Cox model (overall $p < 0.01$). More specifically, dogs with three or more FP2 criteria died significantly faster than nonfrail dogs (aHR = 12.09; 95% CI = 5.12-28.54; $p < 0.01$); dogs with one or two FP2 criteria died faster than nonfrail dogs and this difference was of borderline significance (aHR = 1.78; 95% CI = 0.96-3.30; $p = 0.07$; Table 3).

Discussion

A 5-criterion frailty phenotype was adapted to geriatric dogs by using physical performance tests and items from a questionnaire or from a physical examination, derived from the frailty phenotype assessed in humans (6). In 80 geriatric Labrador and Golden Retrievers enrolled in the SeniorDog cohort, this adapted frailty phenotype was significantly associated with time to all-cause death after adjustment for age, breed, sex, sterilization, sex-sterilization interaction and other risk factors for death. To our knowledge, this is the first prospective cohort which characterizes a frailty phenotype in dogs based on physical performance tests. In a previous retrospective cohort study including older guide dogs, mostly Labrador and Golden Retrievers, an adapted 5-criterion frailty phenotype based

only on the most relevant items from a geriatric examination grid was associated with time to all-cause death (22). Our study therefore confirms these findings by assessing 3 of the 5 criteria by using objective measures. In addition, Banzato et al. adapted the Frailty Index to dogs in a cohort of adult dogs of any breed (23). Altogether, these studies provide further evidence that frailty can be adapted to domestic dogs and that the domestic dog seems a good animal model to study frailty.

The information on both weight loss and amyotrophy was collected at baseline in the SeniorDog cohort to represent the “shrinking” criterion, respectively through the questionnaire filled in by the owner and by the veterinarian during the physical examination. Because included dogs were necessarily not on a diet (non-inclusion criterion), weight loss, if present, should therefore represent unintentional weight loss. Unintentional weight loss was chosen for the FP1 because it corresponded more closely to the “shrinking” criterion assessed in the original definition of the frailty phenotype (6), compared to generalized amyotrophy. However, in our data, the adjusted association between the presence of (unintentional) weight loss and time to all-cause death was much weaker compared to the one between the presence of generalized amyotrophy and death. This lack of association with (unintentional) weight loss may be explained by non-differential misclassification errors on the presence of the “shrinking” criterion and subsequently on the frailty status of the dogs. Owners may not be able to correctly report whether their dog had lost some weight compared to when the dog was 2-5 years old. Another misclassification error on this criterion may come from our hypothesis that this weight loss, when reported, was unintentional because the dog was necessarily not on a diet. By using generalized amyotrophy for the “shrinking” criterion and a stricter threshold for each quantitative criterion (20% instead of 25% for the FP1) to define the FP2, the adjusted associations with time to all-cause death were stronger compared to when using the FP1 (Table 3). Furthermore, the use of FP2 detected more frail dogs compared to the use of FP1. Therefore, the PF2 seems more sensitive than the PF1 to detect all the forms of frailty while remaining specific. However, the definition of this FP2 was derived from a post-hoc analysis, which was not driven from an a priori hypothesis. It is therefore necessary to confirm in other studies that using a stricter threshold for the

quantitative criteria (i.e., 20%) and the presence of generalized amyotrophy for the “shrinking” criterion identify frail, prefrail, and nonfrail dogs with good sensitivity and specificity.

At the time of the writing of the SeniorDog cohort protocol, there was no validated performance test to easily quantify the strength of a dog. The use of a dynamometer attached to a leash fixed to a wall was inspired by weight pulling activities where a dog has to pull a maximally weighted wheeled cart (28). It is therefore possible that the value of the peak force measured by the dynamometer normalized to body weight in the SeniorDog cohort does not well represent the strength of the dog as much as the peak force measured by a Jamar hand-held dynamometer in humans does to quantify hand grip strength (48). However, such misclassification errors on the “Weakness” criterion are expected to be non-differential, which should not invalidate the observed significant association between the adapted frailty phenotypes and time to all-cause death in our study.

In our data, there was a significant interaction between frailty and osteoarthritis in the association with time to all-cause death. One hypothesis which can explain such interaction is that osteoarthritis can degrade physical performance without being a consequence of a multisystemic dysregulation leading to the clinical expression of the frailty phenotype. Independently of the frailty status, dogs with osteoarthritis are expected to perform worse on physical performance tests than dogs without osteoarthritis because of the discomfort caused by osteoarthritis. Therefore, for the same physical performance, non-osteoarthritic dogs are likely to be frailer than osteoarthritic dogs, since in the latter case, one cause of a poor physical performance could be the presence of osteoarthritis only. Moreover, the thresholds for the presence of the quantitative criteria were defined by using the quantile of the distribution within the sample of all dogs (25% for the FP1 and 20% for the FP2), without differentiating specific thresholds in osteoarthritic and non-osteoarthritic dogs. Therefore, it is expected that osteoarthritic dogs would be identified as prefrail or frail dogs less severely than non-osteoarthritic dogs, leading to a weaker association between frailty and time to death in osteoarthritic dogs compared to non-osteoarthritic dogs, as it has been observed in our data. It is

worthy to note that there was no clinical examination to confirm the presence of osteoarthritis at baseline when reported by the owner. It is therefore necessary to confirm in further studies in dogs this interaction between frailty and presence of osteoarthritis in the association with mortality.

The generalization of the association observed between frailty and death in the Retriever dogs in our cohort to other breeds requires further investigations. However, to our knowledge, there is no hypothesis according to which the adapted frailty phenotype defined by using the same 5 criteria for a frailty phenotype (6) is associated with death only in Retrievers and not in dogs of other breeds. In addition, the Frailty Index used in the Banzato et al. study was associated with mortality in an all-breed sample (23), which suggests that selection bias, if any in our study, should not invalidate our findings. Furthermore, the misclassifications errors on the frailty status of the dogs due to misclassification errors on at least one of the five criteria are necessarily nondifferential ones, and should therefore not invalidate our results.

In our study, the primary outcome was all-cause death. Although all-cause death is also a primary outcome in human studies of frailty, this outcome in dogs did not necessarily represent the same health event as in humans, because of euthanasia. Using such primary outcome should however not be an issue to meet our objective because a tool assessing frailty can be validated if it is predictive of adverse health events and deterioration in health status (15). Studies of frailty in humans often have multiple endpoints: long hospitalization, falls, disabilities, surgical complications, institutionalization, or death. In dogs, the cause of euthanasia is most of the time a deteriorated quality of life consecutive to numerous negative health conditions (49), including non-rapidly fatal conditions such as poor quality of life, inappetence, or lethargy. In human medicine, these non-rapidly fatal conditions could have led to institutionalization. Therefore, our primary outcome in dogs would be equivalent to a composite outcome encompassing institutionalization and death in humans, which is still relevant to evaluate the validity of the adapted frailty phenotype in our cohort of older dogs. Moreover, in a sensitivity analysis, frailty remained independently and significantly associated with

time to natural death or euthanasia for rapidly fatal reasons. However, in further studies in dogs, it would be interesting to study the association between frailty and time to several cause-specific death, such as death from neoplasia, organ failure (eg, renal, hepatic, or cardiac failure), or from neurological disorders.

The majority of suspected potential confounders in the association between frailty and all-cause death was controlled for within the multivariate Cox models. However, residual confounding bias probably persists due to body condition score and sterilization. Body condition score (BCS), and in particular obesity, could not have been taken into account in our study for two reasons. First, BCS was collected at baseline only and may be in the causal pathway between occurrence of frailty and death (at that time, BCS may have already been modified by diseases and/or age-related changes). In such situation, it is not recommended to adjust for such “intermediate factor” (50). Second, BCS was highly related to the “shrinking” criterion of the adapted frailty phenotype. However, BCS before the dog becomes frail could be a confounder since overweight seems a risk factor for death in dogs, especially in Retrievers (51) and is likely to be a risk factor for frailty as it is in humans (52). Actually, the study by Banzato et al. included baseline BCS in their model, and found a protective effect of overweight on overall survival after adjustment for frailty (23). This a priori counterintuitive association was probably the consequence of a reverse causality bias or a collider bias and is named the “obesity paradox” (53). Given the strength of the association between frailty and death in our cohort (adjusted hazard ratios greater than 5 when comparing frail to nonfrail dogs), it seems unlikely that obesity in adulthood would fully explain it. Furthermore, the main mediators between obesity and death were controlled for (comorbidities, inflammation, and immune and hormonal changes). Regarding residual confounding bias due to sterilization, the data collection on neutered status in the SeniorDog cohort did not differentiate early (i.e., when the dog was about 1 year old or younger) versus late sterilizations, whereas late sterilizations usually follow a medical indication (pyometra or neoplasia, for instance). Therefore, the sterilization variable was not properly defined, and potential residual confounding bias may persist after adjustment for it. In future studies of

frailty in dogs, it would be appropriate to define the sterilization variable by the number of years under the influence of sex hormones (54), which would better define the window of hormone exposure and allow to investigate the association between sex hormone exposure and frailty (55).

Despite the limitations cited above, our study has strengths which lend confidence in the findings. First, this prospective cohort study was set up with a data collection at baseline of objective measurements deriving from geriatric human medicine in order to identify frail and prefrail dogs. The data collection at baseline also included many exposures potentially playing a role of confounders. The recruitment was focused on geriatric dogs, as it is the case in most of the frailty studies in humans, and it was restricted to two very similar breeds (Labrador and Golden Retrievers) which is recommended to study aging in dogs (56). The total length of follow-up was 5 years, which is equivalent to roughly 35 human years of follow-up (36), with very few lost to follow-up dogs. This length of follow-up allowed us to study short, middle, and long-term mortality.

Provided that selection and residual confounding biases, if any, do not invalidate our findings, frailty, as assessed by a 5-criterion frailty phenotype, seems predictive of all-cause death in geriatric Labrador and Golden Retriever dogs. The concept of frailty seems adaptable to the domestic dog. The domestic dog therefore seems a good model for the study of human frailty as it was suggested previously (22,23), including identification of factors which would delay the onset of frailty (57). Frailty also seems a geriatric syndrome of interest in the management of functional aging in dogs in the same way as it is in humans. The exposures associated with frailty in dogs remain to be studied, including inflammatory, immune, and hormonal profiles, as well as comorbidities, cognitive decline, and exposome.

Funding

This work was supported by MP Labo and by two research grants from the Ecole nationale vétérinaire d'Alfort.

Acknowledgments

We thank all the owners of the dogs of the SeniorDog cohort who accepted to participate in the study. We also thank all the members of the Biopôle laboratory of the EnvA for the biological and biochemical analyses from the blood samples collected from the dogs of the cohort.

Author contribution

Romane Lemaréchal: statistical analysis and interpretation of the data, drafting the manuscript.

Sara Hoummady: study conception and design, recruitment of the dogs, data collection, critical revision of the manuscript for important intellectual content.

Inès Barthélémy: critical revision of the manuscript for important intellectual content.

Claude Muller: critical revision of the manuscript for important intellectual content.

Julie Hua: critical revision of the manuscript for important intellectual content.

Caroline Gilbert: critical revision of the manuscript for important intellectual content.

Loïc Desquilbet: study supervision, full access to all the data in the study, responsibility for the integrity of the data and the accuracy of the data analysis, critical revision of the manuscript for important intellectual content.

All authors approved the final submitted version.

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Captions for Tables and Illustrations

Table 1: Definition of the initial adapted frailty phenotype (FP1) and from a post-hoc analysis (PF2).

Table 2: Baseline characteristics of the 80 dogs enrolled in the SeniorDog cohort.

Table 3: Association between time to all-cause death and the initial adapted frailty phenotype (FP1) or the adapted frailty phenotype from a post-hoc analysis (FP2) over 5 years of follow-up, by using Cox proportional hazard models.

Figure1: Kaplan-Meier survival curves estimated over 5 years of follow up according to frailty status at baseline by using the initial adapted frailty phenotype (FP1).

Table 1: Definition of the initial adapted frailty phenotype (FP1) and from a post-hoc analysis (PF2).

	Presence of the criterion	
Criteria	FP1	FP2
Weakness	Lowest 25% peak force normalized to body weight.	Lowest 20% peak force normalized to body weight
Slowness	Slowest 25% walking time normalized to height of the forelimb	Slowest 20% walking time normalized to height of the forelimb
Poor endurance	Lowest 25% distance normalized to height of the forelimb	Lowest 20% distance normalized to height of the forelimb
Low physical activity level	Lowest 25% total MAI	Lowest 20% total MAI
Shrinking	Self-reported weight loss	Moderate or severe generalized amyotrophy
<i>Note:</i> MAI = metabolic activity index (see text for details).		

Table 2: Baseline characteristics of the 80 dogs enrolled in the SeniorDog cohort.

Characteristic	Frailty status at baseline using the initial adapted frailty phenotype (FP1)			Overall, N = 80 ¹
	Nonfrail, N = 29 ¹	Prefrail, N = 40 ¹	Frail, N = 11 ¹	
Golden (vs Labrador)	7 (24%)	21 (52%)	7 (64%)	35 (44%)
Male (vs Female)	18 (62%)	19 (48%)	6 (55%)	43 (54%)
Neutered (vs Intact)	15 (52%)	24 (60%)	6 (55%)	45 (56%)
Age at baseline (Years)	11.06 (9.81, 11.59)	11.51 (10.51, 12.72)	13.33 (10.99, 14.71)	11.30 (10.38, 12.31)
Body weight (Kg)	34.8 (31.0, 38.0)	32.8 (30.0, 36.0)	32.0 (29.9, 33.9)	33.0 (30.0, 36.2)
BCS (Scale from 1 to 5)	3.5 (3.5, 4.0)	3.5 (3.0, 4.0)	3.5 (3.0, 3.5)	3.5 (3.0, 4.0)
Smoking environment (vs No smoking environment)	15 (52%)	19 (48%)	1 (9.1%)	35 (44%)
Supermarket food (vs other type of food)	7 (24%)	8 (21%)	1 (9.1%)	16 (20%)
Living in an apartment (vs Living in an house)	9 (32%)	12 (30%)	2 (18%)	23 (29%)

Sleeping outside (vs Sleeping inside)	2 (7.1%)	3 (7.5%)	2 (18%)	7 (8.9%)
No access to outdoor (vs Access to outdoor)	3 (11%)	7 (18%)	0 (0%)	10 (13%)
Income per month < 1000 euros (vs ≥ 1000 euros)	4 (14%)	4 (10%)	2 (18%)	10 (12%)
History of chronic diseases except cardiac and osteoarticular (Yes vs No)	6 (21%)	11 (28%)	6 (55%)	23 (29%)
Cardiac history (Yes vs No)	4 (14%)	3 (7.5%)	4 (36%)	11 (14%)
History of dysplasia (Yes vs No)	5 (17%)	4 (10%)	1 (9.1%)	10 (12%)
History of osteoarthritis (Yes vs No)	12 (41%)	30 (75%)	9 (82%)	51 (64%)
Owner-assessed health (Bad vs Good)	2 (7.1%)	7 (18%)	5 (45%)	14 (18%)
Medications (Yes vs No)	8 (28%)	16 (40%)	5 (45%)	29 (36%)

Locomotor pain (Yes vs No)	13 (45%)	21 (52%)	7 (64%)	41 (51%)
Sight impairment (Yes vs No)	9 (31%)	18 (45%)	8 (73%)	35 (44%)
Hearing impairment (Yes vs No)	2 (6.9%)	6 (15%)	7 (64%)	15 (19%)
Lameness (Yes vs No)	8 (28%)	21 (52%)	7 (64%)	36 (45%)
Heart murmur (Yes vs No)	8 (29%)	13 (33%)	8 (73%)	29 (37%)
Resting heart rate (Beats per minute)	96 (90, 114)	99 (84, 116)	120 (96, 126)	96 (88, 120)
Skin nodule (Yes vs No)	6 (21%)	22 (55%)	4 (36%)	32 (40%)
Gingivitis (Yes vs No)	8 (28%)	10 (25%)	2 (18%)	20 (25%)
Tartar (Yes vs No)	17 (59%)	28 (70%)	9 (82%)	54 (68%)
Tooth loss (Yes vs No)	2 (6.9%)	6 (15%)	2 (18%)	10 (13%)
Poor oral health (Yes vs No)	20 (69%)	31 (79%)	9 (82%)	60 (76%)
Hematocrit (%)	47 (45, 50)	45 (40, 50)	40 (36, 47)	46 (41, 50)
Leukocyte (/mm ³)	8,440 (6,510, 9,480)	8,490 (7,750, 10,370)	7,210 (6,902, 11,548)	8,460 (7,080, 10,465)
Granulocyte (/mm ³)	5,180	5,495	5,330	5,460

	(4,260, 6,280)	(5,010, 6,495)	(4,282, 6,828)	(4,620, 6,515)
Eosinophil (/mm ³)	460 (310, 730)	482 (300, 795)	590 (418, 927)	473 (310, 800)
Basophil (/mm ³)	10 (0, 10)	10 (0, 10)	10 (0, 10)	10 (0, 10)
Lymphocyte (/mm ³)	2,040 (1,580, 2,350)	2,245 (1,498, 2,892)	1,990 (1,548, 2,520)	2,070 (1,520, 2,600)
Monocyte (/mm ³)	320 (230, 470)	256 (200, 372)	335 (232, 430)	310 (213, 400)
Urea (g/L)	0.30 (0.23, 0.33)	0.30 (0.26, 0.47)	0.44 (0.27, 0.66)	0.30 (0.26, 0.46)
Creatinine (g/L)	9 (8, 11)	9 (8, 11)	8 (8, 12)	9 (8, 11)
Protein (g/L)	68 (65, 71)	68 (65, 74)	67 (64, 72)	68 (65, 74)
Albumin (g/L)	34 (33, 35)	34 (33, 36)	32 (31, 34)	34 (33, 36)
ALT (UI/L)	63 (50, 78)	54 (40, 72)	67 (53, 94)	61 (47, 78)
ALP (UI/L)	148 (80, 211)	116 (84, 207)	182 (120, 301)	135 (83, 242)
Glucose (g/L)	0.91 (0.88, 1.00)	0.91 (0.83, 1.00)	0.84 (0.82, 1.04)	0.91 (0.83, 1.00)
Cholesterol (g/L)	2.37 (1.98, 2.97)	2.66 (1.96, 3.21)	2.94 (2.01, 3.33)	2.50 (1.96, 3.18)
Triglycerides (g/L)	0.74 (0.52, 1.00)	0.67 (0.54, 1.00)	0.72 (0.63, 1.04)	0.71 (0.52, 1.00)

	1.01)	1.07)	1.41)	1.06)
Bilirubin (mg/L)	2.90 (2.30, 3.40)	2.70 (2.15, 3.05)	2.45 (2.10, 2.80)	2.70 (2.25, 3.30)
IGF1 (ng/mL)	378 (319, 426)	376 (326, 410)	445 (384, 460)	382 (323, 437)
Cortisolemia (nmol/L)	82 (51, 155)	85 (52, 152)	93 (73, 109)	90 (55, 151)
NT-pro-BNP (pmol/L)	620 (470, 775)	761 (562, 990)	1,018 (832, 1,909)	759 (524, 982)
C-reactive protein (mg/L)	11.9 (5.0, 15.7)	10.6 (5.0, 13.0)	8.0 (5.0, 21.2)	10.9 (5.0, 14.5)
CD4/CD8 ratio	1.04 (0.70, 1.17)	0.85 (0.64, 1.08)	0.73 (0.66, 1.38)	0.86 (0.65, 1.17)
<p><i>Note: ALT = alanine aminotransferase; ALP = alkaline phosphatase; IGF-1 = Insulin-like growth factor; BCS = body condition score; NT-pro-BNP = N-terminal prohormone of brain natriuretic peptide; ¹ n (%) or median (interquartile range)</i></p>				

Figure1: Kaplan-Meier survival curves estimated over 5 years of follow up according to frailty status at baseline by using the initial adapted frailty phenotype (FP1).

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Figure 1

