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Putative Cerebral Microbleeds in Dogs Undergoing Magnetic Resonance Imaging of the Head: A Retrospective Study of Demographics, Clinical Associations, and Relationship to Case Outcome

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Background: Cerebral microbleeds (CMBs) are focal intraparenchymal signal voids on gradient-echo magnetic resonance imaging (MRI), corresponding to regions of chronic hemorrhage. In humans, they are associated with systemic disease and shorter survival times. Although similar findings have been identified in dogs, their epidemiology and clinical correlations have not been investigated.

Objective: To determine epidemiological features, clinical associations, and associations with outcome for putative CMB-like foci (putative microbleeds [pMBs]) identified by T2*-weighted MRI in dogs.

Animals: Five hundred and eighty-two dogs undergoing 3T brain MRI between 2011 and 2016.

Methods: Retrospective case–control study. Demographic, diagnostic, and clinicopathological data were obtained from medical records and phone follow-up. Demographic variables were compared between dogs with and without evidence of pMBs. For dogs with such evidence, and a subset of matched controls, associations with clinical presentation, concurrent disease, and survival times were evaluated.

Results: Dogs with pMBs were older ($P < .001$) and smaller ($P = .004$) than unaffected dogs. Compared to matched controls, they presented more frequently for vestibular signs ($P = .030$). Cortical atrophy occurred concurrently with pMBs in 26% (14/54) of dogs. Diagnosed renal disease was not significantly associated with pMBs, but proteinuria was more common in dogs with pMBs than in matched controls (odds ratio = 3.01, $P = .005$). Dogs with pMBs had a shorter median survival time than did matched controls ($P = .011$).

Conclusions and Clinical Importance: Putative microbleeds occurred in 54 of 582 (9.3%) of dogs undergoing brain MRI, but may not be a normal consequence of aging. They were associated with shorter survival time and proteinuria in the study population.

Key words: Arteriopathy; Geriatric; T2*.

Cerebral microbleeds (CMBs) are small, well-defined, rounded, intraparenchymal signal voids within the human brain on T2* gradient-echo or susceptibility-weighted magnetic resonance imaging (MRI) sequences.[1] These signal voids correlate with collections of blood or blood products on histopathology, often in association with vascular disease, in people.[2,3] In dogs, putative CMBs (pMBs) also have been identified, and preliminary results suggest that histopathology of these lesions bears similarities to human CMBs.[4–6]

Abbreviations:

ATIIIA	antithrombin III activity
AUC	area under the curve
CAA	cerebral amyloid angiopathy
CI	confidence interval
CMB	cerebral microbleed
CSF	cerebrospinal fluid
CT	computed tomography
df	degrees of freedom
FLAIR	fluid-attenuated inversion recovery
GRE	gradient-recalled echo
HA	hypertensive arteriopathy
IRIS	International Renal Interest Society
MPRAGE	magnetization-prepared rapid acquisition
MRI	magnetic resonance imaging
OR	odds ratio
pMB	putative microbleed
ROC	receiver operator characteristic
UP/C	urine protein/creatinine ratio
USG	urine specific gravity

In people, CMBs occur at low frequency in the general population,[7] and prevalence increases with age.[8,9] Affected people often are minimally symptomatic, but CMBs have been shown to be reliably associated with small vessel pathology and have proven to be predictive of future clinically relevant disease states. The presence of CMBs is associated with a variety of vascular diseases, with the best established being hypertensive arteriopathy (HA) and cerebral amyloid angiopathy (CAA). Humans

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with HA and CAA are thought to have deep and lobar CMBs, respectively.[10,11] For both etiologies, CMBs predict risk of future intracranial hemorrhage.[12,13] Additionally, the presence of CMBs is considered a risk factor for both intracranial hemorrhage and ischemic stroke in healthy elderly populations[14] and was identified as an independent risk factor in a population of patients with intracranial hemorrhage.[15] In people, risk factors for CMBs include CAA, hypertension,[8] renal disease,[16–18] proteinuria,[19] and pro-inflammatory states.[20]

Pathological changes seen in humans with CAA have been recognized in older dogs,[21,22] although these lesions have not been identified as resulting in neurologic deficits in veterinary medicine. In 1 report of dogs with CAA identified at necropsy, 6 of 9 dogs had evidence of concurrent hemorrhage.[23] Hypertensive encephalopathy has been reported in a small number of dogs,[24,25] 1 of which had a pMB identified on MRI. Other risk factors for CMBs in humans are commonly diagnosed in dogs.

Epidemiological risk factors for the presence of pMBs in dogs are unknown. Likewise, pMB prevalence and distribution, and association between pMBs and concurrent systemic or intracranial disease (eg, additional hemorrhage or ischemic stroke) have not been established in dogs. In 1 study,[4] 5 of 12 dogs with pMBs identified by 0.4 or 1.5T MRI had concurrent systemic medical conditions; none had concurrent intracranial abnormalities. In a separate case series, 4 of 4 dogs imaged at 1.0T had concurrent medical conditions, and 1 of 4 had additional intraparenchymal abnormalities, including larger hemorrhage.[5] In a recent retrospective study of T2*-weighted imaging abnormalities, investigators identified a subset of dogs with lesions consistent with pMBs,[6] 3 of 9 of which had concurrent systemic disease (hypertension).

The purpose of our study was to report the prevalence, epidemiology, and anatomic distribution of pMBs in dogs undergoing brain MRI. A secondary objective was to relate the number and distribution of pMBs to other clinical findings, including risk factors for arteriopathy and the presence of concurrent intracranial abnormalities.

Materials and Methods

Case Selection

Medical records of all dogs presented to the Texas A&M University Veterinary Teaching Hospital that had brain imaging using a 3.0T magnet and including a T2*-weighted transverse series between September 2011 and March 2016 were reviewed. Although many of these dogs presented to the neurology service, all dogs for which the required magnetic resonance images were available were included in the study, regardless of the indication for MRI. Dogs that had at least 1 pMB (see below) were included in the case sample population. The remainder of dogs made up the control sample population.

Imaging

All brain MRIs were performed on a 3.0T system.[a] Standard sequences run on dogs undergoing brain MRI included T2-weighted spin echo transverse, sagittal and dorsal plane images, T2*-weighted gradient-recalled echo (GRE) transverse images, T2-weighted fluid-attenuated inversion recovery (FLAIR) transverse images, and T1-weighted 3D magnetization-prepared rapid acquisition gradient-recalled echo (MPRAGE) pre- and postgadolinium images reconstructed in the transverse, sagittal, and dorsal planes.

We used the following definition for pMBs, adapted from previous publications[1,5]:

1 Hypointense and blooming on T2*-weighted MRI
2 Round or ovoid shape
3 Surrounded by parenchyma
4 Isointense or hypointense to surrounding tissue on T2-weighted MRI (and T1-weighted postcontrast MRI, if available)
   a Not confined within the bounds of a lesion identified on T2-weighted or T1-weighted postcontrast MRI
5 Maximum diameter ≤5.7 mm diameter[26] on T2*-weighted transverse MRI
6 No known history of head trauma

An example case is shown in Figure 1. Magnetic resonance images were reviewed, and the number and distribution of pMBs were recorded by a board-certified radiologist (JFG) blinded to the dogs' clinical histories. Other abnormalities on MRI were derived from original radiology service reports.

The location of each pMB was recorded with respect to 7 major anatomic canine brain regions (cerebral cortex, deep cerebral structures [comprising basal nuclei and surrounding white matter], thalamus, midbrain, pons, medulla, and cerebellum). Deep cerebral structures, thalamus, midbrain, pons and medulla were considered "deep" structures. The cerebral cortex (gray matter and subcortical white matter) and cerebellum were considered "lobar" structures.

Medical Records Review

The following data were retrospectively retrieved from medical records: age at the time of MRI; breed; weight; presenting neurologic examination findings; concurrent diseases; MRI findings; CBC, serum biochemistry, and urinalysis results; and, cerebrospinal fluid (CSF) analysis (where available). Outcome data (survival times) were determined from medical records from Texas A&M or the referring veterinarian, and a dog was considered lost-to-follow-up on the last date of communication, of either clinic, with the owner confirming the pet was still living.

Presenting complaints were categorized as follows based on history and neurologic evaluation at presentation: vestibular dysfunction, seizures, other intracranial dysfunction, spinal cord disease, or neuromuscular disease, or no neurologic abnormalities. Concurrent systemic diseases were categorized as follows based on the master problem list: Renal disease, systemic hypertension, hypothyroidism, hyperadrenocorticism, diabetes mellitus, other, or no disease. Concurrent intracranial diseases were categorized as follows based on MRI diagnosis (with CSF analysis, where available): extra-axial or intra-axial mass, encephalitis, other vascular event (large cerebrovascular accident [maximum diameter >5.7 mm[26]] or ischemic infarct), cortical atrophy, Chiari-like malformation, other, or otherwise normal MRI.

Evaluation of the relationship of breed to the presence of pMBs was carried out for breeds with at least 3 representatives in the pMB sample population, which were Golden Retriever, Bichon Frise, Chihuahua, Yorkshire Terrier, Boston Terrier, Shih Tzu, and Toy or Miniature Poodle. Breed was taken to be the recorded breed in the medical record summary. Dogs that were identified as mixed breed, and dogs that belonged to breeds with <3

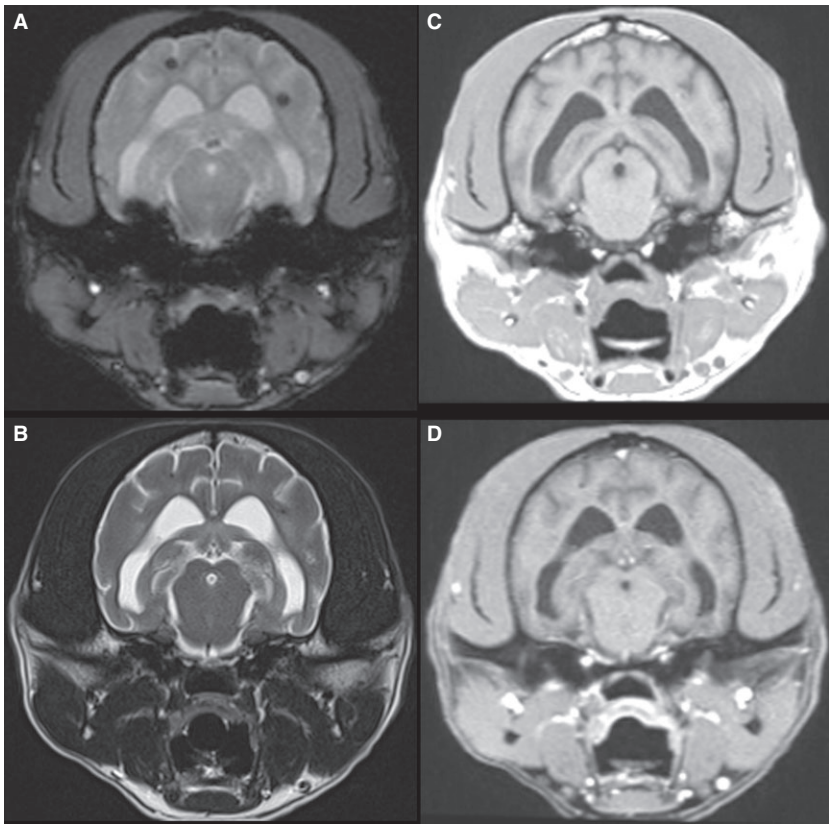

Fig 1. Selected MR images for a representative case with putative microbleeds (pMBs) are shown. (**A**) T2*-weighted transverse image at the level of the tympanic bulla shows 2 circular, blooming areas of T2* signal loss, each ~3 mm in diameter, within the parenchyma of the cerebrum. (**B**) T2-weighted transverse image at the same level. There are smaller foci, slightly hypointense to surrounding parenchyma, in locations corresponding to the pMBs identified in A. (**C**) T1-weighted, pregadolinium transverse image and **D**. T1-weighted, fat-suppressed, postgadolinium transverse image at the same level. The previously identified regions are isointense to surrounding tissue and show no contrast enhancement.

representatives in the pMB sample population, were recorded as a single breed, "other."

For analysis of the relationships among neurologic abnormalities, concurrent disease, and the presence of pMBs, an age- and breed-matched subset of dogs was chosen from the larger group of control dogs (ie, dogs that had undergone brain MRI during the specified time frame, with the required scans available), and matched one-to-one with dogs with evidence of pMBs. Specifically, ages were matched to within 1 year wherever possible, and within 2 years in all cases. When several control dogs of the appropriate age were available, a dog of the same breed was chosen wherever possible. In the absence of a matching breed, a breed of similar size was chosen.

The age- and breed-matched control group also was used for evaluating relationships between clinicopathological variables and the presence of pMBs. Dogs were stratified into categories using criteria adapted from the International Renal Interest Society (www.iris-kidney.com[27–29]) based on (1) a combined criterion for serum creatinine concentration and urine specific gravity (USG), (2) systolic blood pressure, and (3) proteinuria. Table S1 defines the categories for each of these variables. Antithrombin III activity (ATIIIA) was considered low if <114%. This reference value is standard for our clinical pathology laboratory, and represents activity as a percentage of human control ATIIIA. Because normal dog ATIIIA is greater than normal human ATIIIA activity on the functional assay used in our clinical pathology laboratory, the lower end of the reference range for normal dogs in our laboratory is 114%. Platelet counts were considered low if <200,000/μL, normal if 200,000–500,000/μL, and abnormally high if >500,000/μL.

Statistical Analysis

For descriptive statistics of age and weight, as well as variables pertaining to CSF, median and range are reported. For examining the difference between population mean ranks of these variables, a Wilcoxon rank-sum test was used. A P-value < .05 was considered statistically significant.

For determination of the relative effects of age and weight on the finding of pMBs, data initially were assessed by univariable analysis using the Wilcoxon rank-sum test applied to the group of dogs with pMBs compared to the full control group (all dogs without evidence of pMBs undergoing a 3T brain MRI in the specified time frame). For breed association, Fisher's exact test was used to assess significance. Variables with $P < .25$ on univariable analysis then were fit using forward stepwise multiple logistic regression. Model predictive ability was assessed using area under the receiver operator characteristic (ROC) curve (AUC). Goodness-of-fit was assessed using Pearson's generalized χ^2 computed on grouped data (for continuous demographic variables, the nearest year was used for age, and decile for weight), implemented in commercial software[b] as described.[30]

After creation of the age-, weight- and breed-matched control set, a Wilcoxon rank-sum test was performed to confirm that age

and weight did not differ between dogs with pMBs and their matched controls, confirming that our matching procedure had the desired effect. A manual inspection of the data sets confirmed that similar numbers of breeds overrepresented in the pMB group compared to the full control group were present in the matched control group (see Results).

To evaluate potential risk factors (eg, clinical signs of neurologic dysfunction, concurrent intracranial or systemic abnormalities, clinicopathological abnormalities) in the matched case–control sets, either a chi-squared or Fisher's exact test was used initially to examine each variable independently, and those with a P-value < .25 were included in the multivariable model. A forward stepwise multiple logistic regression then was performed using all variables thus identified. Model predictive ability was assessed using the ROC curve AUC. Goodness-of-fit was assessed using Pearson's generalized χ^2 computed on data grouped by category according to predictor values (see above).

A Kaplan–Meier curve was used to visually evaluate the effect of pMB status on survival in dogs with pMBs and the age- and breed-matched subset. A Cox proportional hazards best-fit model was used to determine significance of the difference in survival between these 2 sample populations.

All statistical tests were performed using built-in function or bespoke scripts based on built-in functions using commercial software[b].

Results

A total of 582 dogs underwent brain MRI in the study time frame. The median age was 7 years (range, 0–19 years). The median weight was 12.7 kg (range, 0.8–78 kg). There were 215 neutered males, 258 spayed females, 63 intact males and 46 intact females. Many breeds were represented; those in the highest numbers were Labrador Retrievers (48), Chihuahuas (35), mixed-breed dogs (34), Yorkshire Terriers (32), Boston Terriers (27), Cavalier King Charles Spaniels (22), Golden Retrievers (20) and Boxers (20). Of these, 54 (9.3%) were found to have ≥1 pMBs in the cerebrum, brainstem, or cerebellum.

Number and Distribution of pMBs

A total of 43 of 54 (80%) dogs with pMBs had >1 pMB identified, with a median of 6 (range, 1–115; Fig 2A). The majority of pMBs (463/598; 77%) were identified in the cerebral cortex of dogs. Only 15/54 (28%) dogs had exclusively "lobar" pMB, limited to the cerebral cortex and cerebellum (Table 1). Figure 2B shows the distribution of pMBs as a function of major intracranial anatomic regions.

Cerebrospinal Fluid Analysis in Dogs with pMBs

A total of 39 of 54 dogs had cisternal CSF analysis performed concurrently with the brain MRI on which pMBs were diagnosed. The median RBC count was 1/μL (range, 0–5,760/μL). The median total nucleated cell count was 0/μL (range, 0–134/μL) and the median protein concentration was 35 μg/dL (range, 10–94 μg/dL). Of the 5 dogs with total nucleated cell counts >5/μL, 2 had mixed pleocytosis, 1 had lymphocytic pleocytosis, and 2 had neutrophilic pleocytosis.

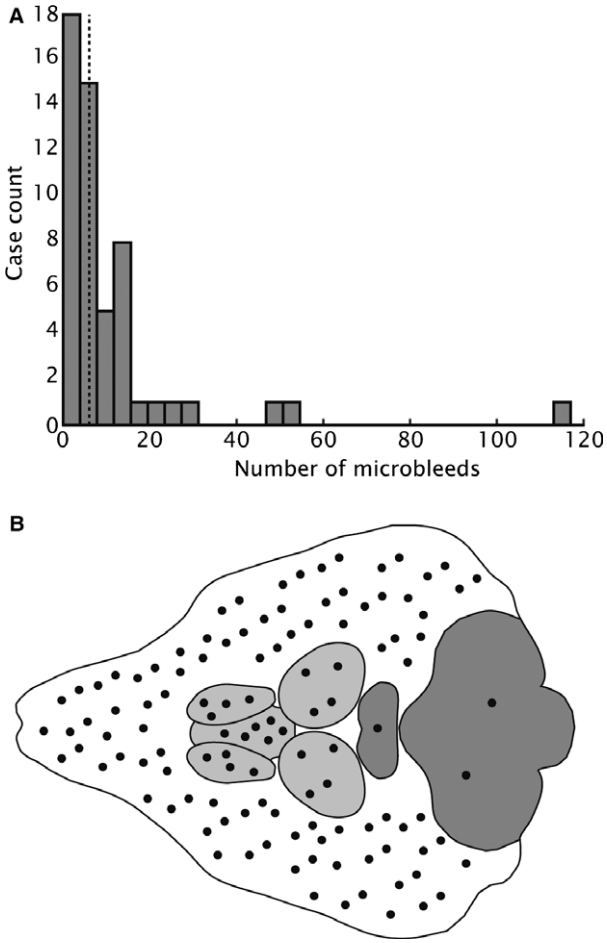

Fig 2. (**A**) Histogram of number of putative microbleeds (pMBs) identified per individual. Dotted line indicates median number of pMBs per dog in which at least 1 pMB was found. (**B**) Schematic of the overall distribution of pMBs within the canine brain. Each black circle represents 5 pMBs in a given brain region (rounded down), and are not meant to represent the exact lesion location within that region. Light gray—basal nuclei, thalamus and deep cerebral white matter; dark gray—midbrain, pons, medulla and cerebellum; white—cerebral cortex and subcortical white matter.

Association of pMBs with Demographic Variables

Compared to dogs without pMBs undergoing brain MRI, dogs with pMBs were older (median, 13 years; range, 2–19 versus median, 6 years; range, 0–17; P < .001; Fig 3A). The proportion of dogs with pMBs increased with age (Fig 3B). Based on the relative risk ratio calculated from the coefficients of the best-fit model of the association of demographic variables with pMBs, the chance of having pMBs increased approximately 76% with every year of life. There was no difference in the proportion of dogs with pMBs as a function of sex (P = .73) or neuter status (P = .15).

Dogs were smaller in the sample population with pMBs (Fig 3C), with a median weight of 8.7 kg (range, 1.3–36) compared to 14.5 kg (range, 0.83–78; P = .004) for dogs without pMBs. Based on the relative risk ratio from the coefficients of the same model, the chance of

Table 1. Anatomic distribution of putative microbleeds (pMBs). Deep cerebral structures (basal nuclei and surrounding white matter), the thalamus, midbrain, pons and medulla were considered "deep" structures. Dogs with pMBs restricted to cerebral cortex (gray matter and subcortical white matter), with or without cerebellar hemispheric involvement, were considered to have strictly "lobar" lesions.

	Deep Cerebrum	Thalamus	Other Brainstem	Any "Deep" Structure	Cerebellum	Cerebral Cortex	Strictly "lobar"
Dogs (n = 54)	34	21	4	39	7	49	15
pMBs (n = 598)	74	46	6		13	463	

having pMBs decreased approximately 3.7% with every kg of weight. There was no significant interaction between age and weight in the final model, which had AUC = 0.92 and Pearson's $\chi^2 = 106.56$ ($df = 156$, $P = .999$).

Certain breeds were found in higher numbers in the population with pMBs than expected from their representation in the population of dogs undergoing brain MRI that did not have pMBs. Small (toy and miniature considered as 1 category) poodles ($P = .007$) and Shih Tzus ($P = .02$) were found with significantly higher frequency in the pMB-positive sample population. However, breed was not retained in the final multivariable model.

The age- and breed-matched subset described in the Materials and Methods section showed no significant difference in the distribution of age or weight, when compared to the dogs with pMBs ($P = .16$, $P = .24$, respectively, by Kruskal–Wallis test). There were 4 small poodles and 4 Shih Tzus in the pMB subset, and 5 small poodles and 2 Shih Tzus in the matched control subset.

Association of pMBs with Neurologic Clinical Signs and Concurrent Disease

Using the age- and breed-matched subset, we found that dogs with pMBs were more likely to have presented with abnormalities of vestibular function in both univariable (Table 2) and multivariable (Table 3) analysis. There was no difference in the frequency of seizures as a presenting complaint (Table 2).

In the same subset of dogs, the presence of pMBs was not significantly associated with intracranial mass lesions, inflammatory disease, other vascular events or Chiari-like malformation in univariable analysis (Table 2). However, there was an association between pMBs and cortical atrophy in dogs upon univariable assessment (Table 2), which remained significant in the multivariable model (Table 3).

Using the age- and breed-matched subset, we found that there was no higher frequency of concurrently diagnosed systemic disease previously suggested to co-occur with pMBs in dogs (hyperadrenocorticism, renal disease, hypertension, hypothyroidism or diabetes mellitus) considered as a group in dogs with pMBs than in those without pMBs (28/54 versus 20/54, $P = .17$). When these conditions were considered individually (Table 2), only renal disease had a P-value of <.25 ($P = .04$) when considered in univariable analysis, but it was not retained in the multivariable model of the association between clinical findings and the presence of pMBs (Table 3; AUC = 0.67, Pearson $\chi^2 = 2.18$, $df = 5$, $P = .14$).

Association of pMBs with Clinicopathological Variables

Because of the retrospective nature of our study, clinicopathological variables could only be assessed in some of the affected and matched control dogs (Table 4). In univariable analysis of clinicopathological variables, only proteinuria was significantly associated with the presence of pMBs (Table 4). In multivariable analysis that included clinicopathological variables, proteinuria (defined as a urine protein/creatinine [UP/C] ratio >0.5 with acellular sediment and negative urine culture; Level 3; Table S1) was highly associated with the presence of pMBs (odds ratio, OR, 3.01; 95% confidence interval [CI], 1.25–7.24; $P = .005$). The final model retained only the single variable of proteinuria, and had AUC 0.69 (Pearson's $\chi^2 = 1.19$, $df = 9$, $P = .996$).

Association of pMBs with Outcome

A Kaplan–Meier survival curve for dogs with pMBs and the age-, weight- and breed-matched control group is shown in Figure 4. A best-fit Cox proportional hazard function indicated an increased hazard ratio for dogs with pMBs of 2.23 (95% CI, 1.21–4.18).

Discussion

Putative microbleeds were seen in 9.3% of dogs undergoing brain MRI, with the prevalence of pMBs increasing markedly in those >10 years of age. They also seem to be more common in small dogs, with small poodles and Shih Tzus being overrepresented. Weight and breed are not independent; in our sample, few breeds were represented well enough to allow adequate statistical analysis of individual breeds. Although the effect of weight may be a manifestation of breed predisposition, our data set does not allow separation of these variables. Some component of the previously reported high frequency of systemic metabolic disease in dogs with pMBs may be due to the fact that these diseases occur more commonly in older dogs.[27,31–34]

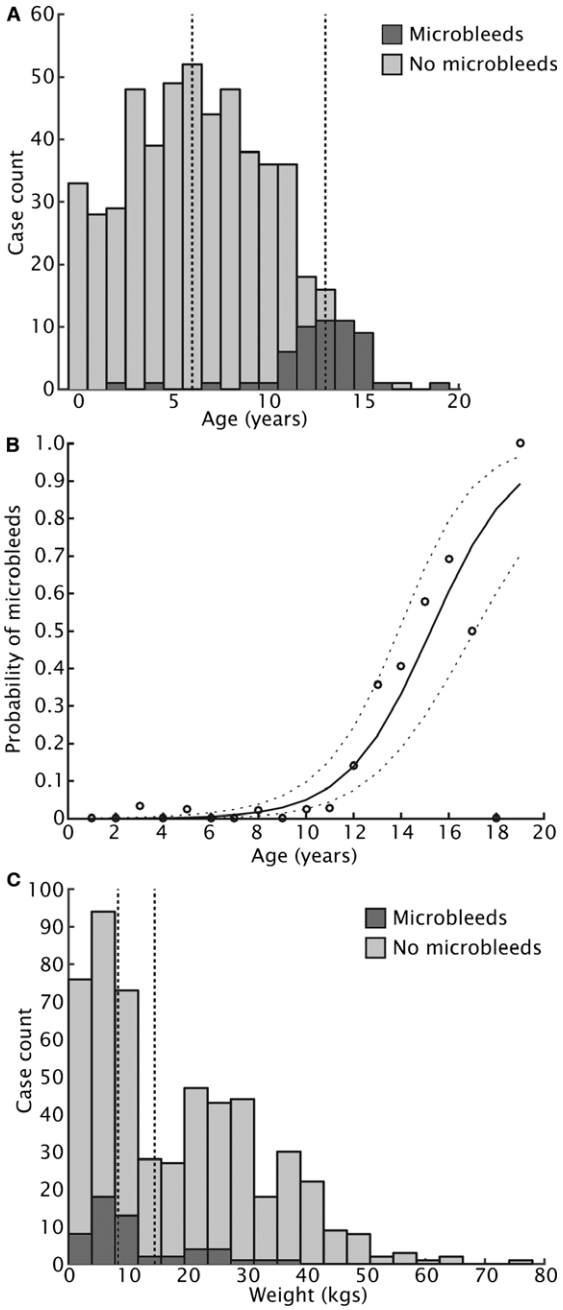

Fig 3. Age and weight demographics for the population of dogs presenting for head magnetic resonance imaging (MRI). (**A**) The histogram shows the distribution of ages in our sample population. Light gray bars represent dogs without putative microbleeds (pMBs), and dark gray bars represent dogs with pMBs. Dotted lines identify the population medians for dogs without and with pMBs as indicated by the legend. (**B**) Probability of pMBs as a function of age. Circles indicate the proportion of dogs in each year of life in our sample population that had pMBs. The solid line shows the relationship between age and pMBs predicted by a logistic regression model including age and weight variables (beta = 0.56, $P < 10^{-14}$); dotted lines are 95% confidence intervals for the fit. (**C**) The histogram shows the distribution of weights in our sample population. Light gray bars represent dogs without pMBs, and dark gray bars represent dogs with pMBs. Dotted lines identify the population medians for dogs without and with pMBs as indicated by the legend.

Table 2. Association of clinical findings with putative microbleeds (pMBs) in an age- and breed-matched subset of dogs having 3T brain/head magnetic resonance imaging (MRI). All dogs having a brain/head MRI in the specified time frame for which T2* transverse images were available were included in our dataset, regardless of presenting complaint or findings on neurologic evaluation. For all variables, 54 dogs with pMBs and 54 dogs without pMBs were evaluated.

	No pMBs	pMBs Present	*P*-Value
Neurologic dysfunction			
Seizures	21	20	.84
Vestibular dysfunction	*13*	*24*	*.04**
Other intracranial signs	18	13	.29
Spinal cord/neuromuscular disease	7	8	.78
No neurologic abnormalities	0	1	1.0
MRI abnormalities			
Large intracranial hemorrhage	1	4	.36
Ischemic infarct	3	4	1.0
Diffuse atrophy	*4*	*14*	*.02**
Intracranial mass	*22*	*14*	*.15*
Caudal occipital malformation syndrome	8	4	.36
Meningoencephalitis	5	4	1.0
Other	1	1	1.0
Otherwise normal brain MRI	13	18	.29
Diagnosed systemic disease			
Renal disease	*5*	*14*	*.04**
Hypertension	9	13	.34
Hypothyroidism	6	10	.28
Hyperadrenocorticism	9	8	.80
Diabetes mellitus	1	4	.36
None	*22*	*14*	*.15*

Italics indicate variables with $P < 0.25$ on univariable analysis with Chi-squared or Fisher exact test; these were included in the multivariable logistic regression model.

*Indicates univariable $P < 0.05$. Large intracranial hemorrhage defined as max. diameter >5.7 mm.

Table 3. Clinical variables retained in multivariable analysis on the association with putative microbleeds in an age- and breed-matched subset of dogs having 3T brain/head magnetic resonance imaging.

	OR (95% CI)	*P*-Value
Vestibular dysfunction	2.6 (1.7–3.9)	.030
Generalized cortical atrophy	4.4 (2.4–8.2)	.016

OR, odds ratio, CI, 95% confidence interval.

The pMBs in our sample population were located primarily in the telencephalon, as has been previously reported.[4,5] However, most dogs with cerebral pMBs had multiple foci that were located in both the cerebral cortex and deeper structures (gray and white matter) of the cerebrum, which is more consistent with the pattern of microbleeds associated with HA in humans.

Despite this, there was a slightly higher likelihood for dogs with pMBs to present with vestibular signs,

Table 4. Association of clinicopathological variables with putative microbleeds (pMBs). See Materials and Methods section for more details on subject stratification, including definition of creatinine category. For urine protein/creatinine ratios (UP/C) >0.2, an acellular urine sediment and negative urine culture were required for inclusion.

	No pMBs	pMBs Present	No. Evaluated	*P*-Value
Systolic BP (mmHg)				
<150	40	33	103	.38
150–179	7	9		
>=180	5	9		
Creatinine category				
1	*44*	*35*	*100*	*.11*
2	*8*	*6*		
3	*1*	*6*		
UP/C ratio				
<0.2	*15*	*12*	*45*	*.01**
0.2–0.5	*3*	*3*		
>0.5	*1*	*11*		
Antithrombin IIIA				
<114%	5	8	36	.83
>=114%	8	15		
Platelets				
>500 K/μL	*6*	*42*	*97*	*.12*
<=500 K/μL	*13*	*36*		

Italics indicate variables with $P < 0.25$ on univariable analysis with Chi-squared or Fisher exact test; these were included in the multivariable logistic regression model.

*Indicates univariable $P < 0.05$.

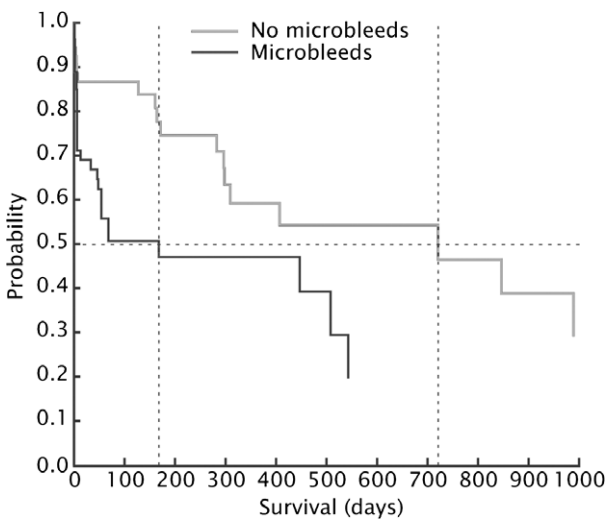

Fig 4. Kaplan–Meier survival curves with censoring for dogs with putative microbleeds (dark gray line; initial n = 54) and their age- and breed-matched controls (light gray line; initial n = 54). The dashed horizontal line marks 50% survival, and dashed vertical lines identify the intercept of this point on the time axis for each group.

although reflex vestibular function does not require cortical processing. Although vestibular dysfunction is commonly reported in geriatric dogs (eg, "old dog vestibular disease"), we do not believe this finding is a consequence of the age of pMB group alone, because the association of vestibular signs with pMBs was found within our age- and breed-matched subset. Alternatively, it may indicate that the multifocal process resulting in pMBs also is occurring to an undetectably small extent in vestibular structures in some dogs.

Putative microbleeds do not seem to be more common in dogs with space-occupying intracranial disease or congenital malformations such as Chiari-like malformation. Many dogs with pMBs had no other abnormalities evident on brain MRI. However, there was an association in our sample population of dogs between the presence of generalized cortical atrophy (determined by decreased interthalamic adhesion diameter on sagittal T2W images,[35] sulcal widening, and generalized hydrocephalus ex vacuo[36]) and the presence of pMBs. In people, cortical atrophy also may co-occur with CMBs.[37,38] Because of the exploratory nature of our data analysis, the clinical relevance of these relatively weak statistical associations ($P < .05$ but >0.01) is not clear and may represent Type I statistical error.

We found no higher frequency of diagnosed systemic metabolic disease in dogs with pMBs compared to age- and breed-matched controls. Because our sample size is relatively small and the frequency of occurrence of these diseases within our population was relatively low, the power of our study may not have been sufficient to detect small increases in risk associated with individual metabolic diseases. Also, because we examined each of the metabolic diseases individually, we cannot make any judgments about the relative odds of the presence of pMBs in dogs among the various conditions examined.

Because some of the dogs in our sample set might have had undiagnosed systemic metabolic diseases, we examined the association between clinicopathological variables associated with these disease states and the presence of pMBs, independent of the diagnosis of a given condition. We found that proteinuria, defined as a UP/C ratio >0.5, appeared to be associated with the presence of pMBs in our age- and breed-matched cohort of dogs. Although we could only evaluate UPC ratio in a relatively small number of dogs, the strength of the association suggests it is not coincidental. Proteinuria may be an early marker for disease states associated with pMBs in dogs, or it may be less labile in a hospital setting than some other clinicopathological variables (eg, systemic blood pressure). Also, because proteinuria can be a feature of many systemic diseases suggested to be associated with pMBs in dogs, it may reflect the combined impact of these diseases on the development of pMBs.

Despite the absence of a clear causative relationship between pMBs and concurrent systemic metabolic or intracranial diseases that might be expected to shorten survival time in dogs, dogs with pMBs in our sample population had shorter median survival time than age- and breed-matched controls. This finding suggests that pMBs may be a negative prognostic indicator for survival in dogs.

Putative microbleeds are becoming more widely recognized in dogs with the advent of standard inclusion of T2* sequences on brain MRI in animals, and with increasing magnet strength. Putative microbleeds must be differentiated from intravascular mineralization, because both appear similar on standard T2* sequences. Indeed, 1 of the weaknesses of our study is the inability to determine with certainty that the pattern we recognized as pMBs is not, in fact, due to a similar distribution of calcifications. In cases in which concurrent computed tomography (CT) studies were available (10 dogs), we did not identify hyperattenuation in the region of the T2* signal voids seen on MRI. Additionally, in the future, susceptibility-weighted imaging may be used to distinguish between hemosiderin (which is paramagnetic), and calcification (which is diamagnetic) in the brains of dogs by taking advantage of differences in signal phase associated with these 2 substances.[39] Optimization of imaging techniques to definitively identify CMBs in veterinary imaging will help establish their true clinical relevance.

In cases for which a similar MRI appearance has been described in dogs and histopathologic examination of the affected areas was possible, pMBs seen on MRI were documented to be accumulations of hemosiderin.[5] Although several dogs with pMBs in our study underwent necropsy, histopathologic examination of areas corresponding to focal T2* areas of signal loss matching the definition of pMBs was rarely performed, because the corresponding lesions were inconspicuous on gross examination of the brain. In the cases from our sample population for which such lesions were examined (2 dogs), evidence of both chronic (Gitter cells containing dark pigment and extracellular hematoidin) and acute hemorrhage (extravasated red blood cells) was found on histopathologic examination. We did not observe increased RBC counts in CSF of dogs with pMBs. This may reflect the intraparenchymal nature of the lesions or the time elapsed between the event and imaging.

Other limitations of our study include its retrospective nature, specifically as it bears on the limitations of diagnosis of concurrent diseases and the large proportion of dogs lost to follow-up soon after diagnosis. Additionally, small sample sizes and low frequency of occurrence of many variables of interest predisposed this data set to Type II statistical error. Nonetheless, these data show several interesting patterns that may help further our understanding of the clinical relevance of pMBs in dogs.

Overall, pMBs are seen on T2* images in dogs, most often those >10 years of age, undergoing brain MRI, as has been reported in humans. This finding however does not mean that pMBs are a normal consequence of aging. Although they previously have been reported to occur concurrently with systemic metabolic diseases and other intracranial abnormalities, once age and breed are taken into account, only proteinuria and cortical atrophy were significantly associated with the presence of pMBs in our population of dogs. Putative microbleeds in dogs most commonly involve both cortical and deep structures, which is more similar to what is reported for microbleeds secondary to HA in humans, although clinical hypertension was not more frequent in dogs with pMBs than in their age-matched controls. Independent of a specific concurrent disease state, the presence of pMBs may be a negative prognostic indicator for long-term survival in dogs.

Footnotes

[a] MAGNETOM Verio, Siemens Medical Solutions USA, Inc., Malvern, PA

[b] MATLAB R2015b academic version with Statistics and Machine Learning Toolbox, Mathworks, Natick, MA

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Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Criteria for stratification of dogs based on clinicopathological data.