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Diagnosis and long-term outcome in dogs with acute onset intracranial signs

Hanne Gredal1§, Barbara Blicher Thomsen1§, Ulrik Westrup1, Antonio Boza-Serrano2, Tomas Deierborg2, Fintan J. McEvoy1, Simon Platt3, Kate Lykke Lambertsen4,5, Mette Berendt1

1Department of Veterinary Clinical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark, 2Experimental Neuroinflammation Laboratory, Department of Experimental Medical Science, BMC, Lund University, Lund, Sweden; 3Department of Small Animal Medicine and Surgery, University of Georgia, 4Department of Neurobiology Research, Institute of Molecular Medicine, University of Southern Denmark, Denmark, 5Department of Neurology, Odense University Hospital, Denmark

§Shared first authorship

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*Corresponding author
Hanne Gredal, DVM, PhD, Department of Veterinary Clinical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Dyrlægevej 16, 1870 Frederiksberg, Denmark (E-mail: hbg@sund.ku.dk)

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Abstract

Objectives: To investigate dogs with acute onset of intracranial signs suspected of stroke by primary veterinary clinicians, and establish possible differential diagnoses and long-term outcome. Additionally, serum C-reactive protein and plasma cytokines were investigated as potential biomarkers of disease.

Methods: All cases were evaluated by neurologic examination, routine haematology and biochemistry and measurement of serum C-reactive protein, plasma cytokine concentrations (IL-2, IL-6, IL-8, IL-10, TNF) and low-field MRI.

Results: Primary veterinarians contacted the investigators with 85 suspected stroke cases. Only twenty met the inclusion criteria. Of these, two were diagnosed with ischaemic stroke. Other causes were idiopathic vestibular syndrome (n = 6), brain tumour (n = 5), and inflammatory brain disease (n = 2); in five cases a precise diagnosis could not be determined. Median survival times were: brain tumour, 3 days, idiopathic vestibular syndrome, 315 days, ischaemic stroke, 365 days and inflammatory CNS disease, 468 days. The median plasma concentrations of IL-2, IL-6, IL-8, IL-10 or TNF were not significantly increased in any of the diagnosis groups.
compared to healthy controls. Serum CRP was higher in dogs with brain
tumours and inflammatory brain disease but not above the upper bound of
the reference interval.

**Clinical importance:** Dogs that present with acute onset intracranial
disease may have ischaemic stroke but are more likely to have other causes.
Many dogs with such acute onset of neurological dysfunction (brain tumours
excluded) may recover within a couple of weeks despite their initial severe
clinical appearance.

**Keywords:** Canine; Cytokine; Infarct; Interleukin; Stroke

**Introduction**
Dogs with acute onset intracranial signs are often suspected to have stroke,
which to many owners implicates a poor prognosis. In fact, a stroke was the
suspected cause of death in 597 dogs (3.9%) as reported by the ownersto
Adams et al (2010) in a questionnaire-based mortality study from the UK.
However, as evidence of the final diagnosis was not available in that study,
and the work-up of stroke patients is usually comprehensive and costly, the
reported numbers presumably reflect a *suspected* rather than an *established*
diagnosis and thus likely include stroke and its differentials, rather than stroke cases only.

It is true that dogs can suffer from ischaemic as well as haemorrhagic stroke, of which ischaemic stroke appears to be the most common (Garosi et al. 2006, Wessmann et al. 2009, Goncalves et al. 2011, Gredal et al. 2013a, 2013b). Ischaemic stroke is caused by the thrombotic occlusion of a brain artery resulting in infarction of the related vascular territory and acute onset of neurological signs referable to the affected intracranial structures (Garosi et al. 2006, Goncalves et al. 2011, Gredal et al. 2013a, 2013b); these signs last beyond 24 hours, after which they often improve, but they may also lead to death (World Health Organization 2006). However, the clinical presentation of acute onset intracranial disease is not unique for stroke. In fact, a stroke can only be definitively confirmed by post mortem neuropathological examination, although MRI can help to discriminate stroke from other neurological conditions, which clinically may appear very similar (Wessmann et al. 2009). Yet, as MRI is rarely an option in primary care veterinary practices, it is important to increase evidence-based information on the possible aetiologies responsible for acute onset intracranial signs, as this will likely affect treatment and clinical outcome.
To our knowledge, no studies have made an overall assessment of the underlying aetiologies of acute onset of intracranial signs in dogs and the outcome of each of these in a prospective study. We believe such information is valuable to help clinicians guide owners with respect to the expected course of disease and its prognosis.

In line with this, biomarkers or tests that could assist in discriminating between these differential diagnoses and possibly contribute to the prediction of outcome in patients with acute onset intracranial signs, would be useful in the clinic. In humans, circulating inflammatory cytokines and C-reactive protein (CRP) have been investigated as possible predictors of survival in relation to different diseases. For instance, in ischaemic stroke acutely elevated interleukin (IL)-6 and CRP have been found to correlate with early deterioration and unfavourable functional outcome (Vila et al. 2000, Marquardt et al. 2005, Waje-Andreassen et al. 2005).

The main aim of the present study was to investigate dogs with acute neurological signs, which on the initial examination by the primary clinicians, were interpreted as a possible stroke, in order to identify the distribution of underlying aetiologies, clinical characteristics and long-term outcome of such cases. Our second aim was to determine concentrations of serum CRP and
the plasma interleukins IL-2, IL-6, IL-8, IL-10, and tumour necrosis factor (TNF) in affected dogs irrespective of diagnosis, investigating these as possible biomarkers of disease.

Material and Methods

Study design
The study was designed as a three-step prospective observational cohort study with follow-up. Dogs were included consecutively between June 2012 and August 2014 (step 1 + 2). A telephone-based follow-up interview was conducted between July and October 2015 (step 3).

Study population and inclusion
Dogs presenting at Danish primary care veterinary clinics with acute onset intracranial signs lasting more than 24 hours, but non-progressive beyond this time frame, were eligible for inclusion in the study.

Dogs were recruited prospectively and consecutively based on the following setup. The study was announced to all primary care veterinary practices (540) in Denmark in a mailed letter with the study guidelines and detailed
information of the possible signs of ischaemic stroke, emphasising the clinical pattern of acute onset intracranial signs, and non-progressive beyond 24h, e.g. first-ever seizures, hemiparesis or unilateral facial paralysis, other acute cranial nerve deficits, mental changes, and/or vestibular signs. Further information is available in Figure S1 (Supplementary Material). To further increase awareness of the study the primary investigators conducted eight informative seminars throughout the country at which 240 veterinarians attended. The veterinarians were given a dedicated telephone number on which they could contact the primary investigators 24/7 for acute referrals.

Dogs were excluded from further investigation if clinical signs had existed for more than 72 hours at the time of presentation. This criterion ensured that they presented at an appropriate time with respect to measuring the acute cytokine response. Dogs were also excluded if they died before appropriate diagnostic work-up could be performed, or if they suffered from concurrent medical conditions, which imposed an increased risk in relation to the MRI-related anaesthesia.

*Three-step investigation*
In each case a three-step investigation protocol was followed. This included; 

*Step 1* - recruitment of the study population through telephone interviews with the referring veterinarians; *Step 2* - diagnostic work-up of included patients; and, *Step 3* - long-term follow-up (telephone interviews with the owners).

**Step 1 - Study population (telephone interviews)**

When contacted with a possible case, the primary investigators, in collaboration with the referring veterinarian, would evaluate the individual dog’s history and clinical status as described by the referring veterinarian, who was asked about the signalment, clinical signs, time of onset and duration of neurological signs, neurological deficits and possible progression or regression of signs. The referring veterinarian’s immediate presumption of the case as possible stroke was important in the overall considerations for inclusion. If the inclusion criteria were met, as assessed by the primary investigators, the dog was included in the study and proceeded to *Step 2* for further diagnostic work-up at our hospital, which at the time of the study offered the only veterinary MRI facility in Denmark. All investigations associated with the study were offered free of charge to the owners.
Step 2 - Diagnostic work-up and short-term outcome

Dogs were examined by an experienced veterinary neurologist and a veterinary-neurologist-in-training, according to a standard protocol including a thorough clinical history, physical and neurological examinations, routine haematology and biochemistry, thyroid profile (thyroid-stimulating hormone, total and free levels of thyroxine), serum CRP and MRI of the brain. Cerebrospinal fluid (CSF) analysis was recommended for all dogs, unless obvious contraindications were present.

MRI scans were obtained using a 0.2 T MRI (Vet-MR, Esaote) with the dogs placed in sternal recumbency under general anaesthesia. MRI sequences included T1-weighted (T1W) images pre- and post-contrast in transverse, sagittal and dorsal planes; T2-weighted (T2W) images in transverse and sagittal planes, and T2W fluid attenuated inversion recovery (FLAIR) images pre- and post-contrast in transverse planes. Gadoteric acid (Dotarem, Guerbet) at a dose of 0.1 mmol/kg IV was used as the paramagnetic contrast medium.

The MRI scans were evaluated by a Diplomat of the European College of Veterinary Diagnostic Imaging (ECVDI) and an ECVDI resident working
under the supervision of an ECVDI Diplomat. A suspected diagnosis was determined according to previously published recommendations (Tress 2003, Garosi et al. 2006, Hecht and Adams 2010, Wisner et al. 2011, Bentley 2015).

For cytokine investigations, whole venous blood (4.5 mL) was collected from the cephalic vein, within 72 hours of onset of neurological signs, into a citrate-stabilised glass tube and centrifuged at 4,400g for 2 minutes. Plasma was subsequently collected and transferred to cryotubes, which were snap-frozen in liquid nitrogen and stored at -80°C until analysis.

The plasma concentrations of the cytokines IL-2, IL-6, IL-8, IL-10, and TNF were measured by means of an electrochemiluminescence detection system using the Meso-Scale Discovery technology applying the Canine Proinflammatory Panel 3 Ultra-Sensitive Kit (IL-2, IL-6, IL-8, TNF) and Canine IL-10 Ultra-Sensitive Kit (Meso-Scale Discovery, Rockville, USA). Analyses were performed according to the protocol provided by the manufacturer but, in order to improve the detection and specificity, samples were run overnight at 4°C, to avoid evaporation. Duplicate determination was performed and the average value included for statistical analysis.
For comparative purposes, the same cytokine and CRP investigations were performed on blood from a group of healthy dogs (n = 9), in which routine haematology and biochemistry were performed for other purposes (e.g. pre-anaesthetic evaluations for simple elective operations, or routine blood monitoring of blood donors).

The control group consisted of five females (three neutered) and four males (one neutered) with a median age of 5 years, 9 months (range 1 year, 6 months - 14 years, 6 months). Breeds included the greyhound (n = 3), Labrador retriever (n = 2), dachshund (n = 2), whippet (n = 1), and Italian greyhound (n = 1). Haematological and biochemical parameters were within normal reference intervals in all control dogs.

Step 3 - Follow-up and long-term outcome

Owners of included dogs could contact the primary investigators on a dedicated telephone number at any time from inclusion to the point of follow-up, and were instructed to do so if any unexpected events or new neurological signs were identified after their dogs were discharged from the hospital.
Standardised telephone follow-up interviews were performed in autumn 2015, allowing for a minimum of 1 year to have elapsed since the acute onset of neurological signs. The owners of the included dogs were interviewed by one of the primary investigators (BBT) using a questionnaire investigating long-term outcome. The main focus of the questionnaire was if the dog was alive or dead at the time of follow-up and, if the dog had died, time and cause of death or, if then dog alive, information about its current clinical status (including information on progression or regression of previously reported neurological signs or the occurrence of new signs).

**Statistical analyses**

Median CRP and cytokine concentrations were statistically compared for each diagnosis group and healthy controls by means of the Mann-Whitney *U* test for non-parametric data using commercially available statistical software (GraphPad Prism 6, GraphPad Software Inc., La Jolla, CA, USA) to identify differences in cytokine concentrations acting as possible biomarkers of disease. Results are given as medians and interquartile ranges (IQR). *P* values ≤ .05 were considered statistically significant.
Survival time (days) after neurological incident was summarised for each dog in a dot plot (Figure 2).

**Ethical approval**

The study was approved by The Local Administrative and Ethics Committee (Permission number 1N/2013). Written consent was obtained from all owners.

**Results**

**Study population (step 1)**

From June 2012 to August 2014 the primary investigators received a total of 85 calls with possible referrals following the given inclusion and exclusion criteria from primary care practices. Following the telephone screening (Step 1) only twenty of 85 dogs (24%) met the inclusion criteria, and 65 were excluded due to the reasons listed in Figure 1. The final study population comprised 13 females (four neutered) and seven males (four neutered) with a median age of 9 years and 11 months (range; 5 years and 1 month - 16 years and 4 months). Breeds included Labrador ertriever (n = 2), wirehaired dachshund (n = 2), and one of each of the following breeds: American
cocker spaniel, American Staffordshire terrier, beagle, Belgian shepherd dog (Tervueren), Boston terrier, cavalier King Charles spaniel, French bulldog, German shepherd dog, golden retriever, Irish setter, Rottweiler, samoyed, and four large-sized mixed breed dogs.

**Diagnostic work-up and short-term outcome (step 2)**

Physical examination was normal in 15 of the 20 dogs. Abnormal clinical findings in the remaining five included heart murmurs in three (two with mitral valve regurgitation evidenced by echocardiography), mammary tumours in two, a cutaneous tumour in one dog, and otitis externa in one. Neurological signs listed by final diagnosis are shown in Table 1. MRI was unremarkable in 13 dogs and abnormal in seven dogs in which ischaemic stroke was suspected in three dogs, brain tumour in three and inflammatory CNS disease in one. CSF analysis was performed in nine dogs, revealing inflammatory CNS disease in two (one of those with inflammatory changes on MRI), and unremarkable in seven. For the remaining 11 dogs CSF analysis was not performed because the owners declined.

Haematology, serum biochemistry and thyroid profiles revealed no significant variations from the normal reference interval values except in two
dogs. One dog with inflammatory CNS disease had increased alanine aminotransferase and alkaline phosphatase levels; ultrasonography of the liver, repeated blood investigations (including gamma-glutamyl transferase and bile acids) and an adrenocorticotropic hormone (ACTH) stimulation test did not reveal any underlying cause. The owner declined further investigation by liver biopsy. In one dog with ischaemic stroke, thyroid results were compatible with hypothyroidism (T4 << 6.44nmol/L (reference interval (RI) 11.2-40.8); Free T4 << 3.86 pmol/L (RI 7.7 - 47.6), TSH 0.6222 ng/mL (RI 0.000 – 0.500), which was previously undetected in this dog.

Based on all clinical investigations, the diagnoses of the 20 dogs at the time of inclusion were distributed as follows: idiopathic vestibular syndrome (n = 7), suspected ischaemic stroke (n = 3), brain tumour (n = 3), inflammatory CNS disease (n = 2), and undetermined diagnosis (n = 5).

Fifteen out of 20 dogs survived the acute neurological incident and were discharged within 1-3 days of hospitalisation (median = 1 day), while five dogs (brain tumour n = 3, ischaemic stroke n = 1, undetermined n = 1) were euthanised at the owner request within 3 days because of the neurological deficits. For the dog with presumed ischaemic stroke which was
euthanased, the dog’s disease-associated aggression contributed to the decision for euthanasia, as there were small children in the household. *Post mortem* investigation of this dog confirmed the MRI diagnosis of a suspected middle cerebral artery infarct.

**Follow-up and long-term outcome (step 3)**

Of the 15 dogs that were successfully discharged, 11 had died at the time of follow-up while four dogs were still alive (Figure 2). Median survival time after onset of acute neurological signs for the 11 dead dogs was 11 months (range 1-24 months), and median age at death was 12 years and 11 months. The median follow-up time for the four dogs still alive (one with ischaemic stroke and three with undetermined underlying aetiology) was 2 years and 2 months (median age 8 years and 4 months).

Of the 11 dogs that had died during the follow-up period, seven dogs were euthanased due to a non-neurological medical condition, while the remaining four dogs were euthanased due to recurring or progressive neurological signs. In the latter group, two of these dogs were re-evaluated by the investigators within 2 months of discharge due to recurring neurological signs. One, originally presenting with acute vestibular signs and
diagnosed on MRI with a presumed right-sided rostral cerebellar artery infarct, had made significant progress and was, according to the owner, close to normal, when, 2 months later, new vestibular signs suddenly occurred and rapidly progressed to a state of recumbency and reduced mentation. MRI revealed two distinct space-occupying lesions, one in the left cerebral hemisphere and one in the right cerebellar hemisphere. The other dog was originally diagnosed with idiopathic vestibular syndrome, based on a normal MRI and an improved clinical status, although mild vestibular signs persisted. However, 2 months after the initial presentation, the dog suddenly presented obtunded with acute seizures in addition to circling and nystagmus. Computed tomography now indicated a slight mass effect in the right cerebral hemisphere. Both dogs were euthanased: gross necropsy and histopathological examinations were performed confirming neoplastic lesions (the first identified as metastasing gall bladder carcinoma; in the second a final histopathological conclusion could not be reached).

One dog with idiopathic vestibular syndrome was euthanased at the owner’s request 169 days after discharge because of recurrent (three) vestibular episodes. In-between these episodes the dog was normal, according to the owner, and the investigators were not contacted. On
readmission, the dog showed no other signs, but the owner nonetheless requested euthanasia and declined further work-up or necropsy. The age of the dog (13.4 years) contributed to this decision.

Finally, one dog with seizures (with an unremarkable MRI, but no CSF analysis although recommended) was euthanased by the referring veterinarian at the owner’s request 46 days after discharge due to progressive seizures. Unfortunately, the owner did not contact the investigators in-between the time of discharge and euthanasia; accordingly, further diagnostic work-up or necropsy was not performed.

The final diagnoses at the end of the study, which were used in further analyses, were based on the total diagnostic work-up and the clinical course of the disease as investigated by the follow-up (step 3), and included: Ischaemic stroke, (n = 2; one middle cerebral artery infarct and one caudate nucleus infarct), idiopathic vestibular syndrome (n = 6), brain tumour (n = 5), and inflammatory CNS disease (n = 2) (Table 1). In five dogs, all with an unremarkable MRI and a normal CSF analysis in two (declined by the owner in three), the diagnosis remained unknown because no cause for the neurological event could be identified during the study period (three of those dogs were still alive at the end of study). Distribution of the final diagnoses
and survival time of included patients by the end of the study is illustrated in figure 2. Median survival time and IQR according to the final diagnosis groups was: ischaemic stroke: >365 days (IQR 3;728*), brain tumour: 3 days (IQR 0;42), inflammatory CNS disease: >468 days (IQR 200;737*), idiopathic vestibular syndrome >315 (IQR 184;509) (asterix indicating that one or more dogs were still alive by the end of the study).

**Owners’ evaluation of the entire course of disease**

In six of the 15 dogs that were successfully discharged the owners reported that the dogs returned to their previous normal function. Two owners declined to participate in the follow-up interview, but the date of death was recorded and used for survival analysis. In 13 dogs, time to maximal recovery occurred within two weeks (median = 5 days) irrespective of diagnosis. For some dogs, owners reported minor changes in activity level and decreased level of interaction.

In dogs surviving the initial neurological event, the majority of owners (77%) had considered euthanasia before the diagnostic work-up. However, at the time of follow-up, these owners were pleased that diagnostic
investigations were completed and in most cases led to a diagnosis and thus an informed basis for decision-making.

**Serum CRP and cytokine analyses**

Serum CRP (mg/L) was investigated for 19 dogs and plasma cytokines for 18 dogs, (suspected ischaemic stroke n = 2; brain tumour n = 5; inflammatory CNS disease n = 2; idiopathic vestibular syndrome n = 4; undetermined cause of neurological signs n = 5). For two dogs, the owners declined extra blood sampling for these analyses. Results appear in table 2. Serum CRP concentrations were significantly higher in dogs with brain tumours (median 8.3; IQR 4.85-19.5; \( P = .002 \)) and inflammatory CNS disease (median 6.84, IQR 3.6-10.08; \( P = .018 \)) compared to controls (median 1.3; IQR 1.2-1.8). However, none of these values were above the laboratory’s normal upper bound of the reference interval (0-25 mg/L).

Median plasma concentrations of the investigated cytokines IL-2, IL-6, IL-8, IL-10, and TNF of the different diagnosis groups were compared to controls but no statistically significant differences were found. In general, IL-10 proved difficult to measure in the available plasma samples because concentrations were below the detection limit in 10/18. Furthermore, median
plasma concentrations of each cytokine were compared pairwise with the groups of dogs diagnosed with ischaemic stroke, brain tumour, inflammation, and idiopathic vestibular syndrome, with no statistically significant differences identified (data not shown).

Discussion

This study is the first to prospectively investigate a cohort of dogs with an acute onset of intracranial signs which, on initial examination by the referring veterinarians, were suspected to be stroke.

Our study demonstrated that, although acute onset of intracranial signs can be due to stroke, the majority of dogs had other suspected diagnoses, which supports the impression of stroke as a condition that is certainly less common in dogs than in humans (Wessmann et al. 2009). The challenge of distinguishing stroke from its clinical mimics is also recognised in humans in whom approximately 25% of suspected ischaemic stroke patients admitted by primary care physicians are found to have non-stroke conditions, such as infections and cerebral neoplasms (Harbison et al. 2003).
No specific clinical neurological signs distinguished the dogs with ischaemic stroke from the other diagnostic groups (as illustrated in Table 1), thus underlining that MRI and other neuro-diagnostic tests should be strongly advocated to owners when trying to establish a diagnosis in dogs presenting with acute intracranial signs.

An important conclusion of this study is that despite severe neurological deficits on presentation, outcome was favourable in the majority of dogs, as 75% were successfully discharged from the hospital and had a median survival time of 11 months, irrespective of aetiology (except brain tumours). Dogs were only hospitalised for 1-3 days and, in most cases (except brain tumours), recovery occurred within 2 weeks. Although 25% of the 20 dogs included in this study were euthanased at the owners’ request immediately following the MRI diagnosis, the median survival time for the eleven dogs that died before the time of follow-up (including four dogs which were euthanased due to persistent or recurrent neurological signs) was 11 months; additionally, the four dogs that were still alive at follow-up, survived more than 2 years.

The median age of dogs at inclusion was 9 years and 11 months, which is in accordance with previously reported median and mean ages in
dogs affected by ischaemic stroke (median ages = 8-9 years) (Garosi et al. 2006, Goncalves et al. 2011, Gredal et al. 2013b, Kent et al. 2014), brain tumours (median age = 9 years) (Bagley et al., 1999), and idiopathic vestibular syndrome (mean age = 9.4 years), respectively (Schunk and Averill Jr, 1983). Bearing in mind the relatively high age at presentation, an overall median survival of 11 months amongst dogs surviving the acute incident is considered good, as is a median age at death in this study of nearly 13 years, which is comparable with the median age at death in a general dog population (Proschowsky et al. 2003).

The relatively high number of dogs with vestibular signs in this study, might seem inappropriate, especially as most were diagnosed with idiopathic rather than central vestibular disease. However, these clearly reflect a proportion of acute neurological patients, which may, at first sight, be interpreted as having a stroke. In fact, many dogs with cerebellar ischaemic stroke display vestibular signs, which in our opinion justifies the assessment and inclusion of vestibular patients as possible stroke in the present study (Garosi et al. 2006, Thomsen et al. 2016).

Another important point raised in this study is that misdiagnosis of acute onset intracranial dysfunction can occur even following MRI
investigation, not least with low-field MRI as in the present study. Previous studies investigating the MRI characteristics of ischaemic stroke compared to brain tumours and inflammation have reported difficulties in distinguishing between these diagnoses, furthermore some brain tumours may even go undetected on MRI (Cervera et al. 2011, Wolff et al. 2012, Young et al. 2014).

Two of the cases in the present study were initially diagnosed with ischaemic stroke and idiopathic vestibular syndrome, respectively, based on MRI. Both made spontaneous improvement without treatment and were successfully discharged. However, the clinical signs recurred and progressed 2 months later, when they were euthanased. Repeat investigations with diagnostic imaging and post mortem revealed brain tumours in both dogs. In one dog initially suspected of ischaemic stroke both repeated MRI and subsequent histopathology revealed multiple brain tumours identified as metastatic gallbladder carcinoma. One could consider that a tumour embolus initially caused a cerebellar stroke in this dog and then grew into a distinct tumour in that same area, and that metastases to distant brain locations were coincidental findings at necropsy. In the second dog, which was initially suspected to have idiopathic vestibular syndrome, in the absence of
abnormal findings on the initial MRI, necropsy revealed two brain tumours. Unfortunately, a fully detailed histopathological diagnosis could not be obtained.

In five dogs with miscellaneous neurological signs, MRI was unremarkable, and a diagnosis was never reached. An unremarkable MRI was also found in six dogs with acute vestibular signs, leading to the classic diagnosis of exclusion: idiopathic vestibular syndrome. However, with the limitations of low-field MRI, such as lack of diffusion-weighted imaging (DWI) and apparent diffusion coefficient (ADC) map allowing for the detection of peracute and smaller lesions, we acknowledge that early (<24 h) or lacunar infarcts or small tumours may have gone undetected (Hecht and Adams 2010).

We investigated selected cytokines and serum CRP as potential biomarkers of different causes of acute intracranial signs but no apparent differences in cytokine levels between the different diagnosis groups were identified. Obviously, this lack of statistical difference might be ascribed to the low sample size of our study. On the other hand, it may illustrate the role of the investigated cytokines as general mediators of inflammation rather than biomarkers of specific brain diseases. Based upon human and
experimental research, cytokines are recognised as principal mediators of the inflammatory response following acute brain injury as well as inflammation in relation to encephalitis or brain tumors (Allan and Rothwell 2001, Iwami et al. 2011, Kothur et al. 2016). Depending on the pathological microenvironment and the specific cytokine properties, these may play a pro-inflammatory role, thus potentiating inflammatory reactions, or an anti-inflammatory role (Spitzbarth et al. 2012). IL-6, for instance, appears to be elevated in CSF or serum in several pathological CNS conditions in dogs and humans, including ischaemic stroke and inflammatory diseases as well as in humans with brain tumors such as gliomas (Gredal et al. 2017, Iwami et al. 2011, Kothur et al. 2016; Ormstad et al. 2011, Spitzbarth et al. 2012). In human ischaemic stroke, IL-6 has been shown to correlate with infarct size and to predict a poor outcome (Ormstad et al. 2011, Tarkowski et al. 1995).

We found slightly higher levels of serum CRP in both dogs with brain tumours and inflammatory CNS disease compared to our healthy control dogs, although with little clinical impact, since serum levels did not rise above the laboratory’s upper bound of the reference intervals in any of these, and would likely go undetected in the clinical work-up. CRP is produced in the liver and is a sensitive marker of a systemic inflammatory
response in dogs (Eckersall and Bell 2010). Hence, serum CRP may not increase with brain conditions, unless the blood brain barrier is compromised and a systemic inflammatory response is induced, as for instance in steroid-responsive meningitis-arteritis (Lowrie et al. 2009). CRP may also be increased in neoplastic conditions in dogs (Ryu et al. 2019), but reports on CRP and brain tumours in dogs remain sparse. Our findings of slightly elevated CRP in dogs with brain tumours or inflammatory brain disease only indicate a systemic inflammatory response, but the specific cause was not further clarified.

Overall, the present study was subject to several limitations. Although the primary investigators had advertised the study extensively, the number of patients that could be included after evaluating the 85 phone calls from veterinarians during the 2-year patient inclusion period was limited to 20. This might in part be explained by the strict inclusion criteria with a narrow time frame of 24-72 hours after onset of neurological signs. It is also possible that a substantial number of owners did not consent to further diagnostics, which require anaesthesia in older dogs displaying a sudden and grave symptomatology, and thus they might never be referred or are euthanased in primary care practice.
In addition, several owners declined CSF collection in their dogs, which is usually considered an important tool in the diagnostic work-up of acute neurological patients, especially those with suspected inflammatory disease. However, as most dogs improved within a few days of admittance (except brain tumours) a CSF tap in these did not seem pivotal.

Furthermore, we do acknowledge the risk of misclassification, which is also documented in other studies (Cervera et al. 2011, Wolff et al. 2012, Young et al. 2014), as a final confirmative post mortem diagnosis was only obtained in three dogs. Fortunately, most dogs recovered from the acute neurological event and, as a consequence, owners were not motivated for post mortem investigation in dogs that were later euthanased due to unrelated causes.

In conclusion, in most dogs with acute non-progressive neurological signs suggestive of intracranial disease, ischaemic stroke is not the most probable aetiology. In the present study, idiopathic vestibular syndrome and brain tumours were the most common diagnoses. Dogs with brain tumours had a poor prognosis whereas other aetiologies had a more favourable outcome. Our study results support that clinicians presented with dogs with acute neurological signs, should encourage owners to pursue further
diagnostics and await possible regression with supportive veterinary care only. Brain tumours excluded, the chance for recovery within a couple of weeks is good despite an initial severe clinical appearance.

Conflict of Interest Declaration: The authors disclose no conflict of interest.
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Organization.


Figure legends

Figure 1. Schematic overview of the flow of inclusion (n=20) and exclusion (n=65) (Step 1) of dogs presenting with acute intracranial signs suspected of stroke. The final diagnoses, based on the three-step investigation of the 20 included dogs, are shown.

† Neurological signs not compatible with ischaemic stroke, as assessed by the primary investigors included, for example, bilateral spinal signs with no concomitant signs from the brain; recurrent head bobbing in an English bulldog; tremors following the dog running away; facial paralysis related to middle ear disease.

Figure 2. Dot plot of the survival and outcome of 20 dogs included for the
investigations of acute intracranial signs initially suspected of stroke.
Asterisk indicating dogs that were still alive by the time of follow-up.

Supporting information
The following information is available for this article;

**Figure S1.** Letter of information to referring veterinarians.
Phone calls from referring veterinarians (n = 65)

Patients included (n = 20)

Diagnoses of included patients
- Idiopathic vestibular syndrome (n = 6)
- Neoplasia (n = 5)
- Ischaemic stroke (n = 2)
- Inflammatory CNS disease (n = 2)
- Diagnosis undetermined (n = 5)

Patients excluded (n = 65)

Reasons for exclusion
- Neurological signs not compatible with ischaemic stroke (n = 24)†
- Neurological signs not within required time frame (n = 18)
- Owner declining further diagnostic work-up (n = 11)
- Comorbidities increasing risks of anaesthesia (n = 3)
- Death before completion of diagnostic work-up (n = 1)
- Technical/practical problems with MRI (n = 8)
Table 1. Neurological signs displayed by the 20 dogs at study inclusion

<table>
<thead>
<tr>
<th></th>
<th>Idiopathic vestibular syndrome</th>
<th>Brain tumour</th>
<th>Inflammatory CNS disease</th>
<th>Ischaemic stroke</th>
<th>Undetermined diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of included dogs</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Subdued</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mental changes</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemineglect</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seizures</td>
<td>4</td>
<td>1</td>
<td></td>
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<td>3</td>
</tr>
<tr>
<td>Proprioceptive deficits</td>
<td></td>
<td>2</td>
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<td>1</td>
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</tr>
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<td>6</td>
<td>2</td>
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<td>Other, e.g. cranial nerve deficits</td>
<td>Transient opisthotonus n=1</td>
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<td>Anisocoria n = 1, Decreased PLR n=1</td>
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</tbody>
</table>

PLR; pupillary light reflex
Table 2 Cytokine plasma concentrations in dogs with acute neurological signs initially suspected of stroke (n=20) and healthy control dogs (n=9), stratified according to diagnosis

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>CRP (mg/L)</th>
<th>IL-2 (pg/mL)</th>
<th>IL-6 (pg/mL)</th>
<th>IL-8 (pg/mL)</th>
<th>IL-10 (pg/mL)</th>
<th>TNF (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischaemic stroke (n=2)</td>
<td>2.5 (0.6-4.3)</td>
<td>12.0 (7.3-16.8)</td>
<td>6.5 (6.1-6.9)</td>
<td>541.3 (432.9-649.7)</td>
<td>0.6 (0-1.3)</td>
<td>1.2 (1.0-1.5)</td>
</tr>
<tr>
<td>Brain tumour (n=5)</td>
<td>8.3 (4.9-19.5)</td>
<td>5.8 (3.1-8.4)</td>
<td>5.4 (4.1-9.0)</td>
<td>706.0 (342.7-1684.0)</td>
<td>0 (0-2.5)</td>
<td>1.1 (1.0-2.1)</td>
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<tr>
<td>Inflammatory CNS disease (n=2)</td>
<td>6.8 (3.6-10.1)</td>
<td>2.6 (1.9-3.3)</td>
<td>17.2 (0.7-33.6)</td>
<td>669.1 (229.2-1109.0)</td>
<td>0 (0)</td>
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<td>Idiopathic vestibular syndrome (for cytokines n=4, for CRP n=5))</td>
<td>4.5 (0.4-13.7)</td>
<td>4.5 (1.8-7.6)</td>
<td>6.0 (3.3-11.6)</td>
<td>531.0 (78.0-4683.0)</td>
<td>0.2 (0-0.6)</td>
<td>1.1 (0.9-3.6)</td>
</tr>
<tr>
<td>Undetermined (n=5)</td>
<td>3.0 (1.3-22.6)</td>
<td>3.9 (2.7-5.6)</td>
<td>4.1 (0.7-5.2)</td>
<td>113.9 (89.1-870.1)</td>
<td>0.1 (0-0.1)</td>
<td>1.0 (1.0-1.6)</td>
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<tr>
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<td>1.3 (1.2-1.8)</td>
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<td>2.7 (1.1-5.3)</td>
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</tr>
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Results are given as medians with interquartile ranges (IQR) in parentheses. Data missing for CRP in 1 dog and cytokine results in 2 dogs.

Abbreviations: CRP = C-reactive protein; IL-2 = Interleukin-2; IL-6 = Interleukin-6; IL-8 = Interleukin-8; TNF = Tumour necrosis factor
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Diagnosis and long-term outcome in dogs with acute onset intracranial signs

Hanne Gredal1§, Barbara Blicher Thomsen1§, Ulrik Westrup1, Antonio Boza-Serrano2, Tomas Deierborg2, Fintan J. McEvoy1, Simon Platt3, Kate Lykke Lambertsen4,5, Mette Berendt1

1Department of Veterinary Clinical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark, 2Experimental Neuroinflammation Laboratory, Department of Experimental Medical Science, BMC, Lund University, Lund, Sweden; 3Department of Small Animal Medicine and Surgery, University of Georgia, 4Department of Neurobiology Research, Institute of Molecular Medicine, University of Southern Denmark, Denmark, 5Department of Neurology, Odense University Hospital, Denmark

§Shared first authorship

Running Title: Acute intracranial signs in dogs

*Corresponding author
Hanne Gredal, DVM, PhD, Department of Veterinary Clinical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Dyrlægevej 16, 1870 Frederiksberg, Denmark (E-mail: hbg@sund.ku.dk)

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