Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado Integrado em Medicina Veterinária

Dissertação

Exploring the impact of canine idiopathic epilepsy on hemogram parameters

Daniela Filipa da Silva Pinto

Orientador(es)| Joana da Costa Reis

Juliana Manuel do Couto Moreira

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A dissertação foi objeto de apreciação e discussão pública pelo seguinte júri nomeado pelo Diretor da Escola de Ciências e Tecnologia:

- Presidente | Rita Payan Carreira (Universidade de Évora)
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ABSTRACT

Epilepsy is one of the most common diseases that affect both dogs and humans and may be potentially threatening when not treated. The cause of idiopathic epilepsy remains unclear, but many factors have been identified as potential triggers for developing epilepsy. Nowadays many drugs are available for the treatment of epilepsy, but the cases of total remission are rare and there is no cure. Moreover, most of anti-epileptic drugs (AEDs) induce side effects.

This dissertation includes a literature review outlining the topic of idiopathic epilepsy, together with a preliminary, exploratory study with the objective of investigating possible alterations on blood parameters that might be induced by repeated seizures. Neuroinflammation may be a consequence of seizures, which possibly induces alterations on blood parameters.

The results suggest that a status of inflammation is likely following seizures, however, further studies regarding the topic are necessary to achieve more clear and precise information.

Keywords**:** Idiopathic Epilepsy; Inflammation, Hemogram, Neurology, Seizure

RESUMO

O IMPACTO DA EPILEPSIA IDIOPÁTICA CANINA NOS PARÂMETROS DE HEMOGRAMA

Epilepsia é uma síndrome neurológica frequente em cães e humanos e que pode ser potencialmente fatal caso não seja devidamente tratada. A causa da epilepsia idiopática permanece desconhecida, porém vários fatores têm sido apontados como desencadeantes desta síndrome. Atualmente, várias drogas estão disponíveis para o tratamento da epilepsia, mas são raros os casos de total remissão e não existe cura. Além disso, estas drogas possuem vários efeitos secundários associados.

A presente dissertação incluí uma revisão bibliográfica sobre a epilepsia idiopática em conjunto com um estudo preliminar e exploratório cujo principal objetivo foi investigar possíveis alterações nos parâmetros de hemograma que poderiam ser induzidos por convulsões recorrentes. A neuroinflamação pode ser um resultado de convulsões recorrentes e um estado inflamatório pode levar a alterações nos parâmetros de hemograma.

Os resultados sugerem que a inflamação é provavelmente desencadeada após episódios convulsivos, porém, mais estudos relacionados com este tópico são necessários.

***Palavras-chave*:** Epilepsia idiopática, Inflamação, Hemograma, Neurologia, Convulsão

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LIST OF SYMBOLS AND ABBREVIATIONS

ABC – Airway – Breathing – Circulation

AED – Antiepileptic Drug

ALP – Alkaline Phosphatase

ALT – Alanine Transaminase

AMPA – 5-methyl-4-Isoxazole Propionate

APP – Acute Phase Proteins

AST – Aspartate Transaminase

BZD – Benzodiazepine

Ca^{2+} – Calcium

Cl^{-} – Chloride

CRI – Constant Rate Infusion

CRP – C-Reactive Protein

CSF – Cerebrospinal Fluid

CT-scan – Computed Tomography scan

CVA – Cerebral Vascular Accident

EMA – European Medicines Agency

EU – European Union

GABA – Gamma-Amino-Butyric Acid

GGT – Ɣ- Glutamyl Transferase

GME – Granulomatous Meningoencephalitis

HCT – Haematocrit

HE – Hepatic Encephalopathy

HGB – Haemoglobin

ICP – Intracranial Pressure

IE - Idiopathic Epilepsy

IE – Interleukin

ILAE – International League Against Epilepsy

IV – Intravenous

IVETF - International Veterinary Epilepsy Task Force

K^+ - Potassium

KBr - Potassium Bromide

KD – Ketogenic Diet

MCH – Mean Corpuscular Haemoglobin

MCHC – Mean Corpuscular Haemoglobin Concentration

MCV – Mean Corpuscular Volume

mGluR – Metabotropic Glutamate Receptor

MPV – Mean Platelet Volume

MRI – Magnetic Resonance Image

Na^+ - Sodium

Na^+ K^+ ATPase – Sodium Potassium Adenosine Triphosphatase Pump

NLR – Neutrophils/Lymphocytes Ratio

NMDA – N-Methyl-D-Aspartate

NME – Necrotising Meningoencephalitis

NSAID – Non-Steroidal Anti-Inflammatory Drugs

PB - Phenobarbital

PCR – Polymerase Chain Reaction

PCT – Plateletcrit

PD – Paroxysmal Dyskinesia

PD/PU – Polydipsia/Polyuria

PDW – Platelet Distribution Width

PLT – Platelets

QoL – Quality of Life

RBC – Red Blood Cells

RDW – Red Cell Distribution Width

SE – Status Epilepticus

StE – Structural Epilepsy

TGF - Transforming Growth Factor

TNF – Tumour Necrosis Factor

UE – Uremic Encephalopathy

WBC – White Blood Cells

PREFACE

This dissertation was written following a five-month externship at Hospital Veterinário Breed Paredes, from 5th November 2018 to 5th April 2019 and under the guidance of a supervisor chosen by the trainee and hospital staff.

This externship offered the opportunity to be in contact with different areas of veterinary medicine that included orthopedics and soft tissue surgery, diagnostics, internal medicine including specific areas such as dermatology, nutrition, oncology, ophthalmology, cardiology, dentistry, emergency and critical care medicine.

Throughout this externship the trainee developed essential skills that would be important for the exercise of her future profession. Also, the required data for this study was collected there and subsequently organized and analyzed, resulting in the dissertation below.

1. LITERATURE REVIEW

1.1 INTRODUCTION

Epilepsy is a neurological syndrome and known to be one of the most common among the canine and feline population [1], as well as in humans [2]. An epileptic seizure can be defined as abrupt, involuntary electrical discharges happening within a population of neurons, leading to sudden and involuntary muscle movements and behaviour changes [3–5]. As said by Moore, epileptic seizures are a clinical manifestation of abnormal hyper-synchronisation of neurons within the forebrain [6]. The effect of multiple and continued anomalous discharges will affect the edge neurons and these discharges will further progress to other neurons [7]. As stated by Berendt, an epileptic seizure is characterised for consciousness impairment in most of the cases, paroxysmal events and identical clinical manifestations in all episodes [8]. The formation of these hypersynchronous discharges is called epileptogenesis [7]. According to De Risio *et al*., inflammation, infection, metabolic disorders, trauma, tumours and oxygen deprivation can lead to abnormal neuronal activity [9]. Regardless of the mechanism, the epileptic seizure is defined as a brain disorder [8]. Given the ideal conditions, an animal may experience a seizure and many factors may be involved in its progression [4].

The term epilepsy is only considered when two or more seizures occur within more than 24 hours between each event [1,10]. Therefore, it is crucial to explore underlying causes that may lead to seizures, being an imminent emergency if the epileptic episode is prolonged in time [10]. A single seizure may be a result of cerebral overload after brain injury due to multiple causes and, even though epilepsy should be considered as a differential diagnosis, it is unlikely to be diagnosed when direct damage is inflicted to the brain [8]. Regarding epilepsy, the term idiopathic refers to the epilepsy of unknown cause when in the presence of unprovoked seizures and absence of brain abnormalities, thus with a possible hereditary predisposition [4,11,12]. Idiopathic epilepsy (IE) is known to be the most common type of epilepsy in dogs [13–16].

The presence of seizures is not pathognomonic for IE. When undertaking diagnosis, it is essential to keep in mind that many different disorders may induce seizures [17]. Nevertheless, there may be cases where the aetiological cause is unknown, being IE the most likely diagnosis [9]. Usually, these abnormal electrical signals can be propagated throughout different structures in the brain and depending upon the structure that has been affected, the clinical manifestations may be

different [3]. Nevertheless, the observable signs will be related to the affected area of the cerebral cortex [10].

1.2 EPIDEMIOLOGY

According to Heske *et al.*, the estimated prevalence of epilepsy within the canine population is 0.75% [18]. In humans, the prevalence of epilepsy is around 1% [19]. In other studies, a prevalence of 0.62% - 0.75% of epilepsy in dogs was also reported [20,21]. The prevalence of epilepsy within a specific group of dogs included in a study in Japan was 1.9%, having a prevalence of 0.9% of IE compared with the 0.4% of structural epilepsy (StE) [22].

Male dogs seemed more likely to develop epilepsy compared with female dogs [15,23,24]. When considering dogs diagnosed with IE, males have a shorter life span when compared with females [25].

As described in many studies, some breeds may have a higher likelihood for IE, including the Labrador Retriever [18,23,26], Boxer [18,27], Belgian Shepherds [18,28,29], German Shepherds [23], Border Collies [23,30], Chihuahua [15], Bernese Mountain Dogs [24], Lagotto Romagnolo [31] and Toy Poodles [15]. Pure breeds have a higher incidence of IE compared with cross-breeds [32].

Breeds which have a higher incidence rate of IE can be genetically predisposed to this disease [18]. IE is likely to be diagnosed in dogs whose seizure onset occurred between six months to six years [33], with a clinical history of repeated seizures and normal consciousness after episodes [23,34,35]. According to Smith *et al.,* a 97% confidence diagnosis of IE was found in such presentations [34]. As stated by Coates and O'Brien, only one-third of the dogs aged between one to five years are diagnosed with StE [36]. It is possible that some of these dogs that experienced seizures may not have a definitive cause for their seizures [16,37]. It is unlikely that structural brain alterations are detected during magnetic resonance imaging (MRI) in epileptic dogs younger than six years [34]. However, it is likely to find such alterations in epileptic dogs older than six years [34].

Many studies found a higher prevalence of IE in many purebred and this strongly suggests a genetic predisposition [4,30]. In fact, in human medicine, it is said that 40% of epileptic patients have a genetic disorder underlying their epilepsy [38]. Only one type of epilepsy, related to a genetic disorder was described in Lagotto Romagnolo dogs and it is associated with a mutation in the *LGI2* gene [31]. This mutation leads to a defect on the regular conformation of proteins that constitute the neuronal membrane, therefore it is recognised as a channelopathy [3,12,31]. This finding emphasises the correlation between IE and genetic predisposition [30].

1.2.1 PRECIPITATING FACTORS

Many precipitating factors for seizures are known from human medicine and many studies have been conducted in veterinary medicine with the same purpose of finding precipitating factors associated to seizures in dogs [20,39,40]. The real mechanism by which precipitating factors may lead to seizures is still unknown. However, it is believed that these factors may change the normal homeostasis within the brain and therefore they may lower the seizure threshold, increasing the likelihood of seizures [41].

Some studies suggested a relationship between oestrus cycle and epilepsy, stating that estrogens may reduce the seizure threshold, whereas progesterone may have an inhibition effect [28,42]. Thus, there is an increase in seizure frequency both in periods when estrogens are mainly present or when progesterone is significantly decreased [28,42]. In a study by Van Meervenne *et al.,* an association was found between seizures and specific stages of the reproductive cycle [42]. According to Van Meervenne *et al.,* dioestrus and oestrus have been identified as important stages regarding epileptic seizures and this could be explained by the anti-convulsant effects of progesterone and the pro-convulsant effects of estrogens, respectively [42]. This finding indicates that female neutered epileptic dogs might have a prolonged lifetime when compared with non-neutered epileptic females [23,28,42]. The effect of sexual hormones on seizure threshold has been discussed in humans as well [43]. Nonetheless, neutered dogs tend to be less aggressive or hyperactive due to the declining rate of sexual hormones present in the organism, which may help controlling seizures, as hyperactivity and aggressiveness are considered as risk factors for triggering epileptic seizures [28].

Stress can also be a precipitating factor, thus breeds that are known for being more active and anxious may be more vulnerable [44]. A study analysed the relationship between seizures and possible precipitating factors in a group of dogs and stress was found to be strongly related with the occurrence of seizures within 24h after stress-related situations [39]. According to Forsgard *et al.,* stress-related situations possibly related with the occurrence of seizures in dogs with IE include sleep deprivation, emotional stress or changes in the average daily routine of the dog [39]. The same factors are known to increase the likelihood of seizures in humans that have epilepsy as well [45–47].

Weather conditions may be considered as a precipitating factor, however, there is little evidence in veterinary medicine. One study reported that nine of 50 of owners that replied to a questionnaire had identified weather conditions as a precipitating factor for seizures in their dogs [39]. Conversely, studies in humans have reported a relationship between heat and cold temperatures and the occurrence of seizures, where patients identified heat and cold temperatures as being precipitating factors [45,46].

1.3 PATHOPHYSIOLOGY OF EPILEPSY

Epileptogenesis is described as the process of conversion of a regular neuronal activity into a hyperexcitable, disorganised and synchronised neuronal activity which persists for a non-specific period [8,10,48]. Trauma, brain infections/tumours, brain malformations, metabolic disorders or oxygen/glucose deprivation may disorganise the normal neuronal activity leading to hyperexcitability that triggers a seizure [48,49]. The most frequent mechanism known for triggering seizures is the imbalance between excitatory and inhibitory neurotransmitters, with insufficient neuronal inhibition or excessive excitation [50]. Abnormal concentration of specific ions can also lead to epileptiform events [50]. A summary of alterations in the neuronal environment that may lead to abnormal neuronal discharge can be seen in Table 1.

Table 1: Summary of alterations in the neuronal environment that may lead to seizures (Adapted from Sanders 2015).

ALTERATIONS IN THE NEURONAL ENVIRONMENT	
ALTERATIONS IN..	Inhibitory/excitatory homeostasis
	Ions concentrations
	Neuronal homeostasis (e.g. brain infections/tumours)
	Function of neurotransmitters
	Neuronal transmission, leading to the spontaneous firing of large groups of neurons (e.g. trauma)
	Glucose or oxygen metabolism

The concept of threshold is essential to understand the epileptogenesis. According to DeLahunta *et al.,* a seizure threshold is considered as the minimum level of inhibition that when exceeded, may have the potential to originate an uncontrolled discharge of a particular group of neurons [51]. A seizure occurs whenever this threshold lowers after any disturbance [51]. Factors related with seizure threshold are illustrated in the Figure 1.

In most cases, the epileptic focus initiates in neurons localised in the forebrain but may be originated in other parts of the brain as well [51]. The cerebral cortex and hippocampus englobe groups of easily-firing neurons, which makes these regions the initial source for seizures in most of the cases [52].

There is an individual susceptibility for epilepsy and it varies among dogs [8]. In fact, under the same conditions, a dog with a lower threshold is more likely to experience an epileptic event [8] as a lower threshold will mean that the mechanism that balances the excitatory and inhibitory responses in the brain is less stable. Dogs with a lower threshold are more vulnerable to factors such as stress, exhaustion, oestrus and weather conditions [53]. The same dog may experience

both focal and generalised seizures [8]. Focal seizures arise from a specific area in the brain, whereas generalised seizures start in an epileptic focus, evolving to other regions of the brain [54]. The neuronal environment is constituted by various elements that are intimately connected between each other and guarantee the normal homeostasis in the brain. If one of these elements is disturbed, it may trigger a potential seizure by lowering the threshold [51].

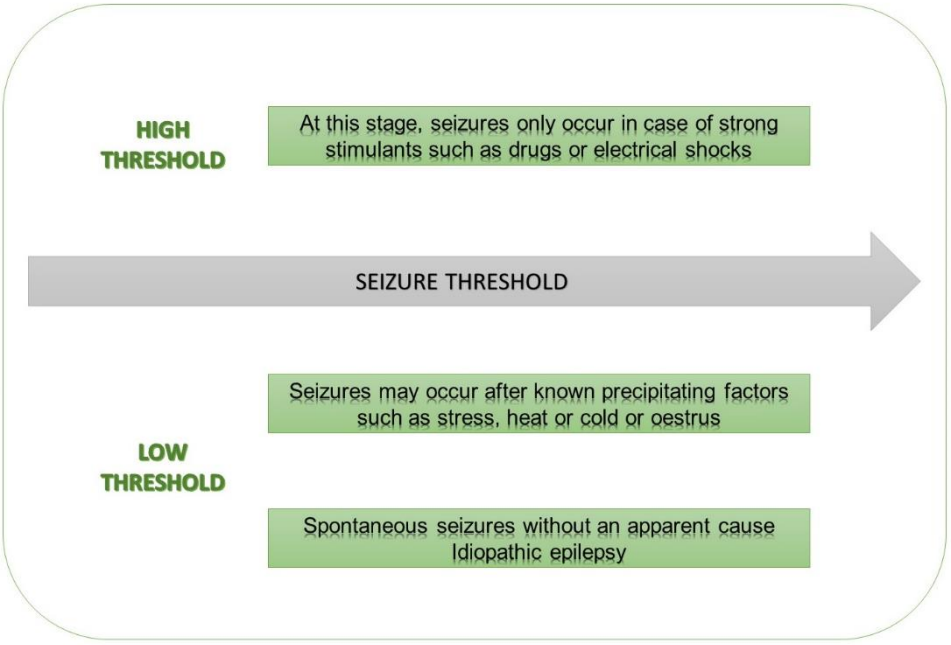

Figure 1: Factors that may affect the seizure threshold (Adapted from DeLahunta *et al.* 2015).

The sodium – potassium adenosine triphosphatase pump (Na^+K^+ ATPase) is the main enzyme that composes this neuronal environment, responsible for the flow of sodium (Na^+) and potassium (K^+) across the neuronal membrane in an energy-dependent process [51]. All these ions have their channels along the neuronal membrane, allowing them to freely move across it. Neurotransmitters may induce an excitatory or inhibitory response, being glutamate and GABA (Gamma-Amino-Butyric Acid) the most essential excitatory and inhibitory neurotransmitter, respectively. Nevertheless, astrocytes also play an essential role in this environment by metabolising neurotransmitters and allowing the flow of neurotransmitters and ions through capillaries [51]. Elements that constitute the neuronal environment are represented in the Figure 2.

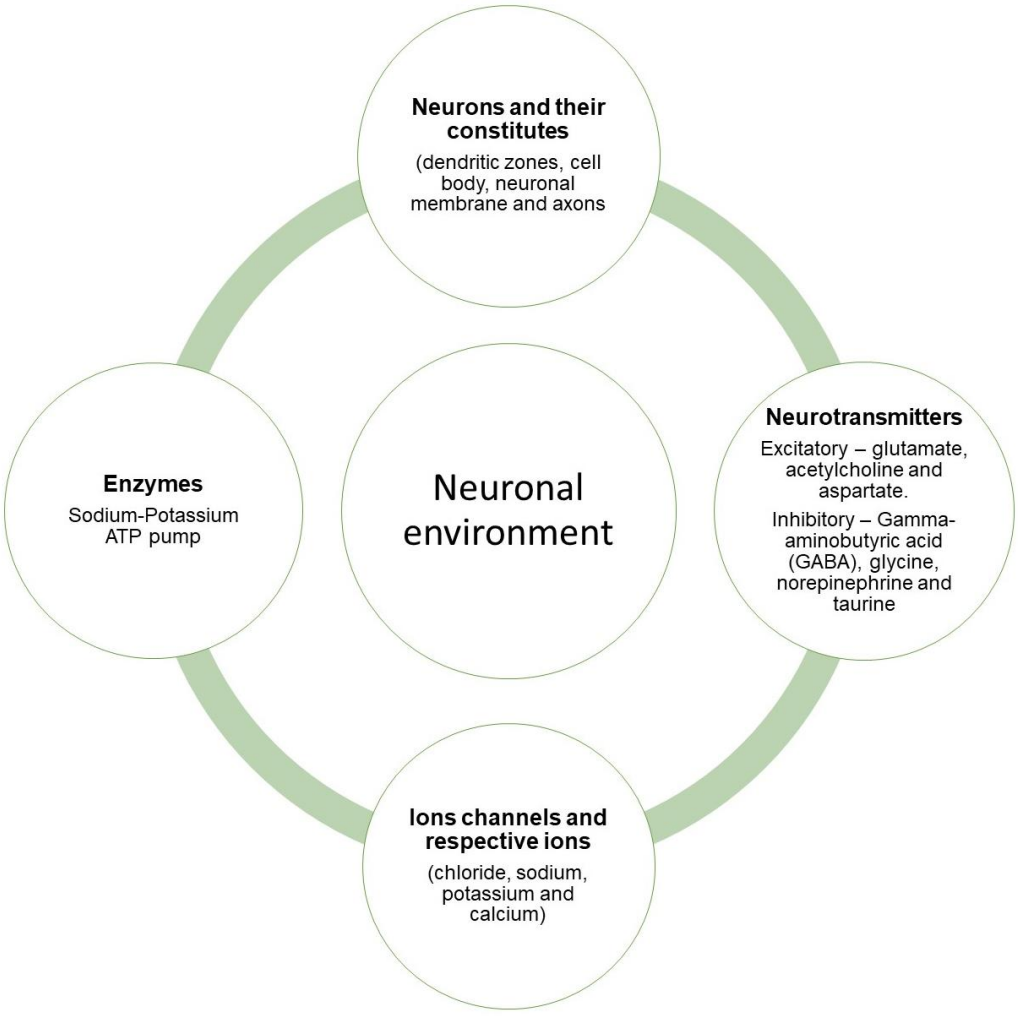

Figure 2: Neuronal environment constituted by different elements (Adapted from DeLahunta *et al*. 2015)

Understanding the physiology behind the neuronal transmission is essential to understand the mechanisms underlying the pathophysiology of the epileptic events. Neurons are capable of communicating between them, receiving and integrating signs received from other neurons and transmitting information in the form of electrical signs [55].

The neuronal membrane has a vital role in electrical transmission [55]. This neuronal membrane separates two spaces, the intra and extracellular space, each one mainly constituted by several ions differently charged [7,55]. During the resting membrane potential, the inner membrane is negatively charged, whereas the outer membrane is positively charged, mostly because of the presence of differently charged ions: Na^+, K^+ and chloride (Cl^-) [55]. In the absence of stimuli, the resting membrane potential is maintained along the neuronal membrane at a difference of -70 mV between inside and outside spaces [55]. Regarding the resting membrane potential, many factors contribute for its maintenance such as the presence of energy-dependent Na^+- K^+ ATPase pumps,

the presence of ions with different concentrations/voltages and the permeability of the neuronal membrane to these ions [7,55]. The K^+ flow throughout the neuronal membrane profoundly influences the resting membrane potential because of the higher permeability to this ion [55]. K^+ moves quickly through K^+ channels across the membrane by its concentration gradient and enables a dynamic balance [55]. Concerning the extracellular space, Na^+ and Cl^- are the primary ions existing, whereas K^+ ions are mostly concentrated in the intracellular space. Some anionic proteins within the neuronal cell can also contribute to a negative charge [55]. The regulation of these ions is mainly controlled by astrocytes, which are the dominant subtype of glial cells that help in maintaining the normal homeostasis within the brain [56]. These glial cells can work as buffers, regulating ions levels and metabolising neurotransmitters [7].

The Na^+- K^+ ATPase pumps allows the flow of Na^+ and K^+ ions throughout the neuronal membrane and against their concentration gradients [7,55,57]. This process is energy-dependent, requiring ATP as the primary source of energy that is provided by glucose metabolism and which represents the majority of the glucose consumption by the brain [55,58]. Changes in the average concentration of serum glucose could lead to seizures because neurons do not have appropriate mechanisms to maintain glucose for metabolism purposes [55].

As these pumps are a decisive factor to the normal function of neurons, many studies have been conducted to understand how alterations regarding Na^+- K^+ ATPase pumps can affect the neuronal activity [57,59]. One study reported that abnormalities in the functionality of these pumps can trigger seizures [59].

In the presence of a stimuli, neurons will depolarise leading to an action membrane potential that turns the intracellular space more positively charged than the extracellular space [55]. Consequently, this action membrane discharge will progress along the neuron until it reaches its presynaptic terminal [55,57]. Neurons are separated from each other by a synaptic cleft and in the presynaptic terminal, it is possible to find vesicles that are neuronal structures responsible for the storage and releasing of chemical substances denominated neurotransmitters [7]. These neurotransmitters are an essential communication between consecutive neurons and depending upon the electric impulse generated, different neurotransmitters may be released [7]. After the electric impulse reaches the presynaptic terminal, the ions channels will open and subsequently it allows the bonding between synaptic vesicles and the neuronal membrane in the presynaptic terminal [7]. After bonding, these vesicles are stimulated to release a particular neurotransmitter to the synaptic cleft [7]. Consequently, the released neurotransmitters will bond to specific receptors localised in the nearest post-synaptic neuron [7].

The pathophysiology of seizures is complex and several factors have been credited as inducing-seizures factors. Some studies reported the relationship between neurotransmitters and epilepsy where glutamate seems the excitatory neurotransmitter more involved in epileptogenesis [60–62].

In a healthy brain, excitatory and inhibitory mechanisms are balanced, which maintains a regular neuronal synchronisation and avoids the triggering of neurons without previous stimulating factors provided by other surrounding neurons [7]. When triggered alone, these neurons may initiate an uncontrolled excitatory response that could lead to a seizure. An imbalance between inhibitory and excitatory responses may increase the likelihood of epileptiform activity if excitatory responses are over-represented [63]. The simplest mode to inhibit an excitatory response is by releasing inhibitory neurotransmitters like GABA [52]. Typically, an inhibitory response precedes an excitatory response and it is when inhibition mechanisms fail in controlling excitatory messages that neurons become hyper-synchronised, thus originating an epileptic focus [52].

Glutamate is the most important excitatory neurotransmitter that is recycled by astrocytes and is ubiquitously distributed in the central nervous system (CNS) [8,50,56,64,65]. Glutamate is obtained from glutamine that is converted into glutamate by glutaminase, a mitochondrial enzyme [52]. Astrocytes have an important role in metabolising glutamate into glutamine, which is carried back to the presynaptic terminal to be reused [52].

It is possible that glutamate is involved in the mechanism underlying epilepsy because there is a remarkable increase of glutamate's concentration when a seizure occurs [8,56]. The overconcentration of glutamate might be toxic, contributing to neuronal death by inducing alterations in permeability to calcium (Ca^{2+}) ions [13,52,64,66]. Elevation of intracellular Ca^{2+} concentrations may lead to activation of certain enzymes such as proteases and phospholipases inducing proteolysis of proteins that constitute the neurons [62]. Moreover, during neuronal death, glutamate is released to the extracellular space as it is present in most of the neurons [67,68]. The resulting increase in extracellular glutamate overstimulates glutamate receptors especially the N-methyl-D-aspartate (NMDA) receptor, which allows the entrance of calcium ions into neuronal cells through this receptor [67,68]. The overconcentration of Ca^{2+} inside the neuronal cells will also contribute for oxidative stress and mitochondria's damage [67,68]. Neuronal death may expand the seizure focus increasing the likelihood of a seizure occurring and entering into a vicious cycle [52,62]. However, while it remains disputed as to whether neuronal death is a cause or a consequence of seizures, some people believe neuronal death could be both a cause and consequence.

Several types of receptors differ by their agonist/antagonist function or their permeability to specific ions [7]. According to Nakanishi, these receptors are involved in many cerebral functions such as synaptic transmission, brain plasticity, learning, memory, brain development and differentiation [69].

Concerning glutamate, inotropic receptors are one type of receptor that work as a Ca^{2+} channel that only opens when bonded to an affinity-neurotransmitter, causing an opening that allows ions to flow through the receptor [63,70,71]. These ionotropic receptors are related to a direct and faster response. NMDA is a subtype of ionotropic glutamate receptor and it has a high affinity to Ca^{2+}

ions [62]. The 5-methyl-4-isoxazole propionate (AMPA) receptor is another subtype of ionotropic glutamate receptor and it is related to fast excitatory responses [64].

On the other hand, metabotropic glutamate receptors (*mGluR*) are closely related to the membrane and coupled to a G-protein [70,71]. These receptors function as a second messenger system and when activated, they allow the flow of Ca^{2+} and Na^{+} ions which leads to depolarisation of neurons [63,70,71]. Because it depends on secondary messengers, the response is slower when compared with ionotropic receptors [70]. These receptors help to modulate the neuronal activity and they also regulate the releasing of neurotransmitters [64]. Contrary to what happens with ionotropic receptors, *mGluRs* are activated in the presence of prolonged and increased concentrations of glutamate, which is relatively frequent after brain injury induced by seizures or trauma [72].

GABA is synthesized from glutamate and it also has main receptors which are known as $GABA_A$ and $GABA_B$ [51,63,73,74]. The former is an ionotropic receptor and the latter a metabotropic receptor and, as previously discussed, they have different mechanisms of actuation [73]. The $GABA_A$ receptors allow the flow of Cl^{-} ions, leading to hyperpolarization of neurons whereas $GABA_B$ receptors operate via second messenger system, increasing the conductance of K^{+} ions while decreasing the conductance of Ca^{2+} ions [75]. Activation of both types of receptors leads to a hyperpolarization of neurons, therefore causing an inhibition of neurotransmission [63,75].

GABA is known to be neuroprotective against distressing situations such as brain hypoxia and status epilepticus (SE), having an essential role on ceasing seizures [76,77]. A study by Loscher *et al.,* concluded that the neurotransmitter GABA was hardly found in cerebrospinal fluid (CSF) of epileptic dogs when compared with healthy dogs [78]. Another study found that dogs with a low concentration of GABA in CSF appeared to develop antiepileptic drug (AED) resistance [79]. Some studies suggest that either inhibition of GABA receptors or excessive activation of glutamate receptors may underlie the process of epileptogenesis [50,64,67,73].

Epileptic seizures may aggravate over time, especially without proper treatment. Some mechanisms that may worsen epileptiform events have been widely studied. *Kindling* is one of those events, where repeated lower-intensity stimulus may induce spontaneous seizures by stimulating non-hyperexcitable neurons that are converted into a group of hyperexcitable neurons [50,64]. It is possible that dogs develop epilepsy after experiencing spontaneously seizures because of this mechanism [80]. The group of neurons that triggered the seizure will be considered as seizure focus [80]. A study conducted by Goddard *et al.,* concluded that discrete and lower-intensity electrical stimulation applied to some areas of mammalian brains overtime originated permanent alterations of brain function and lowered the seizure threshold, thereby increasing the likelihood of seizures with minimal stimulus [80]. Another mechanism is known as *mirroring* and as stated by Dewey and Thomas, it is the process where a group of neurons from the opposite brain hemisphere are drafted into the seizure focus via the corpus callosum [50]. Secondary focus originated through this previously process is called *mirror focus* [53]. This *mirror focus* might be a

consequence of the *kindling* process and this secondary focus has the potential to generate seizures just as the primary source [80].

Secondary brain abnormalities may result from seizures, mainly when occurring frequently and for an extended period. As referred previously, neurotoxicity resulting from overexcitation by high concentrations of glutamate may induce neuronal death [64,66,81]. This disturbance of normal neuronal function may induce brain oedema with subsequent increase of intracranial pressure (ICP), which may decrease the normal perfusion of the brain [81]. Therefore, the regular supply of energy and nutrients for neuronal cells is compromised, leading to anaerobic glycolysis, neuronal acidosis and neuronal dysfunction [81]. In a study by Hasegawa *et al.,* significant concentrations of glutamate were found in the CSF of epileptic dogs which suggests the relationship between this neurotransmitter and the pathophysiology of epilepsy [82]. Similarly, increased concentration of glutamate was found in CSF of epileptic dogs [83], emphasising the theory that glutamate could be a possible biomarker for the diagnosis of IE.

1.4 CLASSIFICATION OF EPILEPSY

Diagnosing epilepsy in dogs may be challenging for veterinarians considering the amount of different aetiologies and clinical manifestations involved. Therefore, an organised and detailed physical examination and signalment are necessary to ensure that noteworthy alterations are detected. A hemogram, routine serum chemistry and urinalysis are examples of simple and inexpensive exams that should be performed to exclude diseases that may mimic IE [11]. IE should only be considered when significant abnormalities are not detected throughout physical examination and with a normal interictal neurological examination [34,49]. In human medicine it was found that some types of epilepsy were possibly related to genetical abnormalities, resulting in changes of the existing classification regarding epilepsy by the International League Against Epilepsy (ILAE) [84]. Many studies in veterinary medicine suggest genetical abnormalities underlying IE [24,30,85–87]. These findings led to a new classification adopting the terms of StE, genetical epilepsy and unknown origin epilepsy in veterinary medicine.

1.4.1 CLASSIFICATION OF EPILEPSY ACCORDING TO ITS AETIOLOGY

Epilepsy can be classified according to its aetiology (Table 2). IE is classified as epilepsy of unknown cause when no other diseases are suspected [1] but recently IE has been divided into subgroups. According to these subgroups IE can be defined as purely genetic, which is usually related to channelopathies that are characterised by mutations in genes that encode K^+, Na^+, Ca^{2+} and Cl^- channels [1,12] but it can only be confirmed by specific genetic tests [1]. When in the absence of diagnostic tests that confirm genetic mutation, veterinarians should suspect of genetic-related epilepsy when the prevalence within a specific breed is higher than 2% [1]. A causative gene has been already confirmed as responsible for epileptic seizures [1].

Lastly, epilepsy can also be a consequence of any process that induces brain injury [1], being the latter defined as StE[1]. Typically, StE includes all the intracranial pathologies, regardless of aetiology. These pathologies can be either infectious, inflammatory, neoplastic, traumatic, vascular, degenerative or anomalous [1,7]. The seizures associated with StE can also be defined as secondary seizures, as they are secondary to other disorders that had inflicted direct or indirect damage to the brain [13].

Table 2: Epilepsy types defined by aetiology (Adapted from Berendt *et al.* 2015).

<table>
<tr><th colspan="4">CLASSIFICATION OF EPILEPSY ACCORDING TO ITS AETIOLOGY</th></tr>
<tr><td rowspan="3">IDIOPATHIC EPILEPSY</td><td colspan="3">Genetic Idiopathic Epilepsy</td></tr>
<tr><td colspan="3">Suspect Genetic Idiopathic Epilepsy</td></tr>
<tr><td colspan="3">Unknown Cause Idiopathic Epilepsy</td></tr>
<tr><td rowspan="3">STRUCTURAL EPILEPSY</td><td rowspan="3">Intracranial Pathologies</td><td>Vascular</td><td>Anomalous</td></tr>
<tr><td>Inflammatory/Infectious</td><td>Neoplastic</td></tr>
<tr><td>Traumatic</td><td>Degenerative</td></tr>
</table>

1.4.2 CLASSIFICATION OF SEIZURES ACCORDING TO LOCALISATION

The cause of seizures can be located inside or outside the CNS [11]. Causes of seizures according to its localisation are summarized in Table 3.

Intracranial causes directly affect the CNS and are intimately related to the brain [11]. Progressive brain disorders such as tumours, inherited diseases or inflammatory processes are defined as acquired epilepsy [11]. Tumours are one of the leading reasons for seizures and most of the dogs with brain neoplasia frequently experience seizures [88]. IE is defined by the occurrence of repeated seizures with an apparent unknown source and is also included in the intracranial causes [11]. Considering extracranial causes, toxins and metabolic disturbances are also responsible for epileptic episodes [11]. Usually, epileptic episodes induced by intoxication or metabolic disorders are classified as reactive seizures because they are a physiological response to a temporary disruption of inhibition and excitation neuronal processes that are provoked by systemic disorders [13,16,89].

Table 3: Causes of seizures in dogs (Adapted from Berendt et at. 2015 and Lorenz *et al.* 2011) *Induce reactive seizures

CAUSES OF SEIZURES IN DOGS		
INTRACRANIAL CAUSES	**Idiopathic epilepsy**	Genetic or unknown cause
	Acquired epilepsy	**Brain injury** (e.g. trauma, cerebral vascular accident)
		Progressive brain disorders (e.g. tumours, inherited or inflammatory diseases)
		Brain Development disorders (e.g hydrocephalus)
EXTRACRANIAL CAUSES*	**Toxins**	**External** (e.g. carbamates, lead poisoning, ethylene glycol, metaldehyde, permethrins, mycotoxins, chocolate, metronidazole, organophosphates)
		Internal, associated with a metabolic disease or organ failure (e.g. hepatic encephalopathy due to portosystemic shut, uraemia)
	Metabolic Disorders	Hypoglycemia, hyperglycemia, electrolyte disorders, hypoxia, thiamine deficiency, cobalamin deficiency

1.4.2.1 BRAIN DEVELOPMENT DISORDERS

Some brain abnormalities may happen during the normal development of dogs and even though it is not as common as other causes of seizures, they can be included in the possible differential diagnosis, especially when in the presence of some breeds like toy breed dogs [63,90]. For instance, chihuahua dogs are known for having a higher predisposition for congenital hydrocephalus and this disease is the most commonly diagnosed brain development disease that may induce seizures [63,90]. Most of the brain development diseases are severe, so generally it is possible to find abnormalities on the neurological examination such as proprioceptive deficits and visual impairment [63]. Ataxia is also a common sign observed in dogs with brain development diseases [90]. These brain development disorders are normally seen in young dogs and imaging diagnostic exams like MRI and computed tomography scan (CT-scan) are of great value to detect these structural dysfunctions in the brain [91].

1.4.2.2 BRAIN INJURY

In humans, acquired epilepsy is normally a result of brain injury [92]. Brain injury is life-threatening and usually is a consequence of direct head trauma caused by car accidents, gunshot, falls or blows inflicted to the head [92]. In other circumstances, brain injury can be the result of a cerebral vascular accident (CVA) that in most cases is a consequence of thrombosis, haemorrhage or embolism [93]. Nevertheless, CVA is not as common in dogs as in humans [93]. Brain injury may be reversible or not, depending upon its severity. The likelihood of developing seizures after brain injury will be higher the more severe the injury [92]. In situations where skull fractures are presented, the chances of developing epilepsy as a result of brain injury are higher [92]. Brain injury alters the brain homeostasis, which deregulates the physiological mechanisms of inhibition and excitation and consequently originating seizures [93,94]. Currently, it is difficult to predict whether a dog that suffered a brain injury will develop seizures in the future, but owners must be aware that chances are higher in such cases. Seizures might occur within an extended period after brain injury episode and pharmacological therapy should be prescribed as soon as seizures appear [94].

1.4.2.3 PROGRESSIVE BRAIN DISORDERS

Brain tumours are known to be the most common causes of progressive brain disorders [92]. Brain tumours are a common condition that may affect dogs of all ages but usually is more common amongst elderly dogs [63]. Brain tumours can be primary or secondary (e.g. metastasis from another tumour) and they can start seizures by inducing compression on the surrounding brain

tissues, which may disorganise the usual structures and also prevent the normal blood flow in the vessels [63]. Veterinarians should suspect brain tumours whenever elderly dogs are presented with seizures, especially when it is the first onset [95]. In some cases, seizures are the first symptom showing up in dogs with brain tumours [95]. When a brain tumour is suspected, imaging diagnostic exams should be performed to detect these abnormalities in the brain. MRI is usually the best diagnostic tool available for diagnosing tumours because of its high sensitivity and specificity for soft tissues [63]. Veterinarians should be aware that dogs with brain tumours or other intracranial lesions may have an unchanged neurological examination [96]. According to Ghormley *et al.,* the neurological examination had 74% sensitivity and 62% specificity on previewing StE [96].

Inflammatory brain diseases are also a common cause of seizures, especially in non-vaccinated young dogs. All inflammatory processes have the potential to start seizures whenever neuroinflammation is presented. Many inflammatory diseases may induce brain inflammation, whether they are infectious or not. Inflammation-related with bacteria (e.g. ehrlichiosis), virus (e.g. distemper and rabies), parasites, protozoa (e.g. toxoplasmosis and neosporosis) or fungi (e.g. cryptococcosis) are relatively common in young animals, but these diseases may affect dogs of all ages [63]. Generally, seizures are not the first sign of inflammatory processes induced by infectious agents, but they are common in dogs in chronic stages [63]. Nonetheless, seizures might be the first and only clinical sign in diseases like toxoplasmosis or neosporosis [63]. Veterinarians should suspect inflammatory diseases whenever young dogs are presented with seizures, usually without a proper vaccination protocol, with other systemic signs and with remarkable alterations on the neurological examination [97]. Systemic signs may vary depending upon the aetiology, but gastrointestinal disturbances such as vomiting and diarrhoea, cough and anorexia are the most commonly observed [97]. Generally, neurological deficits are non-specific, so further thorough examination is necessary. As inflammatory diseases are progressive, it is expected that clinical signs worsen along time without treatment. For a dog who has been having seizures for an extended period, without any abnormalities detected in the interictal period, the cause of the seizures is unlikely to be inflammatory [97].

Non-infectious inflammatory diseases inducing seizures are less common than infectious inflammatory diseases [63]. The most common non-infectious inflammatory disorders in dogs are granulomatous meningoencephalitis (GME) and the necrotising meningoencephalitis (NME), affecting dogs of all ages. However, there is a higher prevalence of NME in younger dogs [63,97]. Ideally, analysis of CSF is advised when inflammatory processes are suspected and serology or polymerase chain reaction (PCR) should be performed whenever an infection is suspected [63,97]. Imaging diagnostic exams such as CT-scan or MRI should be performed as well, whenever appropriate. A definite diagnosis of non-infectious inflammatory diseases can be only made by histopathology, so a diagnosis in life is not possible [97].

1.4.2.4 TOXINS

One study reported that intoxications and hypoglycaemia were the most frequent causes leading to reactive seizures [98]. Intoxications may lead to seizures by changing the normal excitatory/inhibitory mechanisms or interfering with the metabolism of neurons [99]. Toxins may increase excitatory processes within the neuronal environment by acting on excitatory neurotransmitters [100]. Conversely, toxins also act in inhibitory receptors, leading to an inhibition of these same receptors [100].

1.4.2.4.1 EXTERNAL TOXINS

Intoxication may increase the risk of developing a SE and the risk is higher when compared with dogs with concomitant idiopathic epilepsy [14]. Usually, intoxications have an acute onset and systems like cardiovascular, gastrointestinal or urinary are affected, triggering systemic abnormalities together with neurological deficits [101]. Depending upon the toxin, clinical manifestations may vary but neurological and systemic abnormalities are typically presented at the same time [102]. When left untreated, clinical signs may worsen within hours, leading ultimately to death. Unless in the presence of an aggressive SE, dogs with IE will not experience their health deteriorating so rapidly compared to an intoxication [102]. According to Zimmermann *et al.,* intoxications with organophosphates and insecticides appeared to be the most common amongst dogs [101]. These toxins inhibit the action of acetylcholinesterase in the cholinergic synapses, which destabilise the normal homeostasis of the neuronal environment [101]. Rodenticides are another common cause of intoxications in dogs. Crimidine is a common rodenticide widely available and it is considered as a pro-convulsant because it acts as a vitamin B antagonist, which is essential for the synthesis of GABA and also inhibits the action of acetylcholinesterase [101,103]. Intoxication with permethrins is relatively common in cats because these insecticides are highly toxic for this species for reasons yet unknown [104]. Permethrin toxicity mainly happens when topical spot-on products formulated for dogs are applied in cats [105]. Clinical signs related to toxicosis are mainly neurological due to the effects of permethrin in the presynaptic nerve ending [105,106]. Intoxication with permethrins leads typically to an aggressive SE and most of the cats die when not treated.

1.4.2.4.2 INTERNAL TOXINS

Many metabolic disorders may be responsible for the occurrence of seizures in dogs, but most of the cases are reversible with the appropriate treatment. Metabolic-related disorders are a

common cause of seizures in dogs and are provoked by the accumulation of endogenous toxins in the organism [92,99].

Alterations in average blood glucose are one of the most common causes of reactive seizures in dogs and hypoglycaemia is more frequently seen in these same dogs [98]. Seizures induced by hypoglycaemia should be suspected whenever blood glucose levels are repeatedly low, especially after seizures and when seizures are reversed by glucose supplementation [98]. Hypoglycaemia may lead to a seizure due to the lack of glucose, which is the primary energy source for energy-dependent metabolisms occurring in the brain [99]. Generally, neoplasia is the most common cause for persistent hypoglycaemia and imaging exams such as abdominal ultrasound or CT-scan should be performed to rule-out this diagnosis. Insulinoma is the most common pancreatic neoplasia that affects beta cells which are responsible for insulin production, causing an overproduction of this hormone, therefore inducing hypoglycaemia [107]. Insulinoma should be suspected whenever an elderly dog is presented with seizures [107]. Hypoglycaemia may also be a consequence of inadequate production of glucose due to hepatic failure, so it should also be considered during the diagnosis [99].

Hepatic encephalopathy (HE) is a metabolic disorder normally provoked by the existence of a portosystemic shunt that is typically associated with either congenital or acquired hepatic abnormalities [108,109]. HE develops when abnormal levels of ammonia are presented in the blood. Ammonia is highly liposoluble and can easily cross the blood-brain barrier (BBB), therefore leading to alterations of normal brain homeostasis [108]. When in the presence of a portosystemic shunt, ammonia is not converted into urea, that is water-soluble and less toxic, going directly to the systemic circulation without being metabolised by the liver [108]. HE is relatively common in dogs and one of the most common metabolic causes of seizures in Yorkshire Terriers dogs [108]

Uremic encephalopathy (UE) is another common metabolic disorder that is closely related to renal failure [99]. Usually elderly dogs are the most affected however it can be seen in young dogs especially in cases of acute renal failure. Seizures may be a sign of renal failure when in the presence of high levels of urea in the blood, due to a compromised excretion of this metabolite in the urine [99]. An imbalance of acid-base homeostasis and electrolytes concentration as a consequence of renal failure are also a cause for seizures [99].

1.4.3 CLASSIFICATION OF SEIZURES ACCORDING TO THEIR PRESENTATION

Seizures can be classified according to their presentation, which allows a better understanding of affected areas of the brain as well as their severity [63]. Depending upon the affected areas of the brain, the clinical manifestation will vary and even with the same affected area, different dogs may

express different signs [63]. Even though impairment of consciousness can be detected in some situations, this evaluation is not as accurate as in humans because it relies on owners reports, which sometimes may not be precise [1]. There is no reliable scientific evidence supporting a relationship between clinical manifestations and aetiology of the source [8]. Classification of seizures according to its presentation may not influence the treatment but it may help not only identifying which region of the brain is possibly affected but also it allows the perception of whether there is improvement associated to treatment and progression of the disease [3].

1.4.3.1 FOCAL EPILEPTIC SEIZURES

Focal epileptic seizures (FES) are originated by abnormal electrical activity in a localised, specific group of neurons within one cerebral hemisphere and their clinical presentation may help to identify the affected areas of the brain [1]. Focal seizure rises from an epileptic focus, but the abnormal electrical activity is not propagated to the other cerebral hemisphere [63]. Depending upon the localisation of the epileptic focus, any region of the body may be affected [52]. FES are more likely to occur in the presence of StE as a consequence of other diseases, which emphasises the importance of searching for these diseases when in the presence of FES, especially if consciousness stays unchanged [4,35,37,63]. This presentation usually manifests into a motor and focal muscle activity that begins laterally [1]. FES can also be present as autonomic or behavioural and these presentations may coexist with motor presentation [1]. FES may initiate after a sensory experience like lights, smells, sounds or touch [102]. These seizures may pass unnoticed by owners or can be misinterpreted as behavioural abnormalities [110].

1.4.3.2 MOTOR FOCAL EPILEPTIC SEIZURES

This type of presentation is mainly characterised by involuntary, unilateral focal motor episodes that result in atypical movements of specific muscles, such as facial twitches, head movements (e.g. flexion/extension), contraction of masticatory muscles or facial jerking [1,9]. Some of these signs may be defined as hyperkinetic when there is an involuntary contraction of a group of muscles of one side of body, which may lead to an abnormal posture and dislocation of the midline [3].

On the other hand, hypokinetic signs are described as sudden atonia in a group of muscles, which may lead to falls [3]. It is believed that this type of seizures might start from an epileptic focus that is near to a primary motor area in the frontal cortex [9].

1.4.3.3 AUTONOMIC FOCAL EPILEPTIC SEIZURES

In this type of presentation, it is possible to observe autonomic signs such as mydriasis, hypersalivation, urination, defecation or vomiting [1,110,111]. Other less visible signs like salivary gland enlargement, dysphagia or oesophageal spams may be associated with autonomic focal seizures [112,113]. These autonomic signs may be associated to injured areas in the limbic system [3].

1.4.3.4 BEHAVIOUR FOCAL EPILEPTIC SEIZURES

In humans, this type of presentation is characterised by psychic and sensory episodes, where people experience sensations such as agitation, fear, anger or anxiety [114]. According to the ILAE, focal onset can be subclassified as non-impaired or impaired awareness, depending on whether consciousness is impaired or not [115]. In dogs, the evaluation of behaviour changes and consciousness is difficult to assess since it is based only on the owners reports or video-documentation [110].

For this reason, the International Veterinary Epilepsy Task Force (IVETF) decided that FES should not follow the same classification used in humans since it is difficult to do an accurate evaluation of these alterations [1]. Nonetheless, some studies have reported behaviour changes in dogs associated with focal seizures such as anxiety, seeking/attention or avoidance behaviour, confusion, fear, fatigue, bitting or licking imaginary objects and compulsive tail-chasing [110,111,116].

1.4.3.5 GENERALISED EPILEPTIC SEIZURES

Generalised epileptic seizures (GES) are described as the most common presentation in dogs and indicates that both cerebral hemispheres are affected [1,11,117]. In contrast, other studies suggest focal seizures as the most common [26,28]. This opposition may be related to many factors such as discrete clinical manifestation, non-agreement between veterinarians regarding seizure's classification, lack of experience or population bias.

In GES, epileptic activity may propagate from an epileptic focus to other parts of the brain and other groups of neurons may be affected [1]. This presentation usually reveals itself as a tonic-clonic muscle activity that begins bilaterally, often symmetrical and usually consciousness is impaired [9,63]. However, GES can also be present as only clonic, tonic, atonic and myoclonic but these presentations are less frequent [1,63]. There may be cases where consciousness stays unmodified during GES [1]. However, evaluation of consciousness in dogs is complicated because they cannot express their experiences as humans do [49]. Because of little use of

electroencephalogram (EEG) recordings in veterinary medicine, the correct classification should rely on the differentiation between a focal and generalised seizure and more complex classifications are not useful and may be confusing [49]. Normally, GES will follow a particular pattern for a specific dog but may vary between different dogs [3].

Frequently, autonomic signs are noticed during GES and defecation, urination and hypersalivation the most commonly observed [1]. According to the IVETF, GES is identified in the presence of both clinical and EEG changes demonstrating possible affection of both sides of cerebral hemispheres [1]. A generalised seizure is precepted by owners as a potentially life-threating condition for their pets when compared with a focal seizure, which can remain unnoticed most of the time [32]. Most of the owners believe that generalised seizures decrease the quality of life (QoL) of their pets, which could lead to premature euthanasia if owners are not available to cooperate in the diagnosis and treatment [3]. Furthermore, they can easily detect a generalised seizure rather than a focal seizure [32].

1.4.3.6 GENERALISED TONIC-CLONIC EPILEPTIC SEIZURES

This type of presentation was formerly called *grand mal* seizures because of its generalised, aggressive muscle movements [111,117]. There is a high variability on the duration and clinical manifestations of these episodes [9]. Generalised tonic-clonic seizures may cease in less than five minutes but the majority of them do not persist for more than one minute [3].

The tonic phase associated with this presentation is characterised by a generalised muscle contraction and increase of muscle hardness, which leads to opisthotonos that generally does not last more than one minute [1,9,63]. Before this stage starts, there is a loss of consciousness [9]. According to Lorenz *et al.,* consciousness may be measured through responsiveness, attentiveness and disorientation [63].

The clonic phase follows the tonic phase and is characterised by rhythmic, repetitive contractions of the muscles, that leads to jerking and chewing movements of limbs and jaw, respectively [9,11]. Some dogs may experience specific movements such as running or may vocalise [9]. During the seizure, tonic and clonic phase may alternate after one another [9]. Autonomic signs are relatively common during a generalised tonic-clonic seizure and hypersalivation, defecation or urination are normally observed [9].

In the post-ictal phase, dogs may experience abnormal behaviour such as confusion, aggressiveness, fear or anxiousness [9]. Also, it has been reported that some dogs might have proprioceptive deficits, temporary blindness and ataxia [9]. If neuronal deficits persist for more than 24 hours after the epileptic episode, one should suspect of StE [63].

1.4.3.7 GENERALISED TONIC EPILEPTIC SEIZURES

In this type of presentation, dogs experience an increase in muscle hardness but without being followed by a clonic phase [9]. Just as generalised tonic-clonic seizures, loss of consciousness and autonomic signs may also occur [9]. Pre and post-ictal signs usually are similar to other presentations and generally it only lasts for seconds [3,9].

1.4.3.8 GENERALISED CLONIC EPILEPTIC SEIZURES

Clonic seizures are characterised by involuntary and repetitive contractions of the same group of muscles, which are often aggressive and intense [1,9]. Consciousness is also impaired and autonomic signs are similar from the ones observed in other presentations [3].

1.4.3.9 MIOCLONIC EPILEPTIC SEIZURES

Clinical manifestations associated with this presentation include repetitive myoclonic jerking of some muscles of the head, neck and thoracic limbs and these movements are generally abrupt [118]. It can happen as a response to a visual, sound or light stimulus [119]. Normally, these muscles spasms are bilateral, symmetrical and rhythmic [3]. According to Sanders, consciousness is not impaired in most of the times, which may lead to a misclassification as focal seizure [3]. Some studies reported the presence of this type of presentation in miniature wirehaired dachshunds, basset hounds and beagle dogs, associated with Lafora disease [120–122].

1.4.3.10 ATONIC EPILEPTIC SEIZURES

Previously known as *petit mal*, atonic seizures are the least frequent of all presentations [63]. This type of presentation is characterised by a sudden loss of muscle tone, together with a loss of consciousness [3,63]. This atonia may persist for several minutes until the dog is able to recover from it [3]. Given that clinical manifestation is very tenuous, this presentation might pass unnoticed by owners [3].

1.4.3.11 FOCAL SEIZURES WITH SECONDARY GENERALISATION

Sometimes, a focal seizure can evolve into a generalised seizure when the abnormal electric activity spreads from the epileptic focus to the bilateral neuronal environment [1]. Generalised seizure can evolve from a focal seizure within a short space of time, so focal seizures may stay unnoticed, leading to misclassification as a generalised seizure [9]. This type of presentation will start with a focal seizure that may present motor, behavioural or autonomic signs and will suddenly progress to a convulsive phase with bilateral tonic-clonic, clonic or tonic movements, with loss of consciousness [1]. Classification of seizures according to their presentation can be observed on Table 4.

Table 4: Epilepsy classification by seizure semiology (Adapted from Berendt *et al.* 2015).

CLASSIFICATION OF SEIZURES ACCORDING TO THEIR SEMIOLOGY			
FOCAL EPILEPTIC SEIZURES	Unilateral and regional signs such as motor, autonomic or behaviour signs alone or in combination	**Motor**	Focal motor event (e.g. muscles twitch repeated jerking movements, rhythmic blinking, twitching of specific group of muscles
		Autonomic	Parasympathetic influence (e.g. dilated pupils, urination, defecation, hypersalivation
		Behavioural	Changes in normal or expected behaviour depending on breed/species/personality (e.g. anxiousness, fear, aggression, disorientation)
GENERALISED EPILEPTIC SEIZURES	Bilateral signs can be either tonic, clonic, tonic-clonic, myoclonic or atonic (rare)	**Tonic**	Muscle hardness and contractions lasting seconds to minutes
		Clonic	Regularly repetitive contractions from the same muscles groups and can be rhythmic
		Tonic-Clonic	A sequence starting in a tonic followed by a clonic phase
		Myoclonic	Involuntary contractions that can be either single or multiple (a specific muscle or a group of muscles)
		Atonic	Abrupt loss or decrease of muscle tone and can involve different groups of muscle. It can be misunderstood as a syncope

According to Sanders, 85% of the focal seizures in dogs later progress to generalisation [3]. The duration of a focal seizure is generally concise and lasts seconds to minutes, followed by a secondary generalised seizure [1].

Owners are less likely to witness the focal seizures, which emphasise the importance of an accurate report by owners to help the veterinarian on the right approach [9]. As pointed out by Berendt and Gram, most of the focal seizures evolve to secondary generalised seizures [117].

1.4.4 CLASSIFICATION OF SEIZURES ACCORDING TO THEIR CHRONOLOGICAL DISTRIBUTION

Prodrome is the phase that precedes the seizure and it is the first manifestation that can be seen by owners. Generally, during prodrome, dogs experience behaviour changes like anxiousness, increased vocalisation, seeking/attention behaviour, fatigue or chewing unusual objects [1,3,50]. Prodrome phase signs are variable and it may last from hours to days previous to the epileptic event itself [1]. There may be behaviour changes that are easily detected by the owner for being uncommon in their pets, which may help to detect the onset of an epileptic episode. On the other hand, these changes can remain unnoticed by owners [3]. According to Berendt and Gram, prodromes typically reflect an increase of excitability in an epileptic focus or in the entire brain, just before the ictus phase [117]. In humans, the prodrome is described as a mood change that happens minutes to days before the epileptic episode and no significant changes are normally detected on EEG [3]. The prodrome phase can be misclassified as a behaviour focal seizure, but the latter tends to be shorter in time compared with the former [1]. Generally, this phase can pass unnoticed because most of the owners will not think these behaviour abnormalities as something pathological, especially if associated to the first episode of seizures [50].

There is a small phase between the prodrome and ictal phase called aura [63,117]. This phase may be hard to identify and distinguish from prodrome because both involve behaviour changes, but generally the aura may also involve autonomic and motor changes, lasting for seconds to minutes [63,117]. Therefore, the aura phase is an early manifestation of the seizure [9]. According to the new classification by the ILAE in humans, the aura is classified as a focal seizure mostly because some noteworthy changes are detectable on EEG [84].

Ictus is the phase when the seizure occurs, which may consist in the different types described previously [1,117]. The ictus phase may last from seconds to minutes and there is a high variability of manifestation amongst dogs [3]. The damage provoked by seizures is in part related with the length of the episode, so the longer the seizure, the higher probability of secondary lesions. These secondary lesions are mostly a consequence of prolonged muscles contractions, apnoea, metabolic disorders, hypertension and hyperthermia [3].

The post-ictal phase comes just after ictus and dogs may appear confused, disorientated, ataxic or may urinate and defecate [1,9]. Also, some neurological deficits can be detected during the post-ictal phase such as decreased or absent menace response and temporary blindness [9]. The post-ictal phase may vary amongst dogs in duration and clinical manifestation [9]. Some dogs may rapidly recover from it within seconds to minutes while others may have clinical signs associated with the seizure lasting for hours to days [3]. It is during this phase that the normal brain function returns progressively [9,11]. In one study, visual impairment and excessive alertness were found to be the most common detected abnormalities during the post-ictal phase [123]. In other study with 125 epileptic dogs, the most observable signs during the post-ictal phase were fatigue, abnormal hunger or thirsty, mydriasis and disorientation, which lasted from minutes to hours [37]. It seems that there is no connection between aura/post-ictal phases and severity/aetiology of the seizures [3,117].

After the post-ictal phase, the interictal phase begins. According to Sanders, it is described as the space of time between the end of the post-ictal phase and the beginning of the next ictus [3]. This phase is important in dogs that experience seizures frequently [3]. During this phase, dogs may demonstrate some proprioceptive deficits and behaviour abnormalities [3]. When a dog experiences a prolonged and aggressive ictus, it can consequently result in brain damage which may be reflected in prolonged or permanent post-ictal or interictal abnormalities [3]. If these neurological deficits persist for more than 5 to 7 days after an epileptic seizure, a structural brain injury should be suspected [3].

1.4.4.1 STATUS EPILEPTICUS

SE is defined as a severe and life-threating condition that usually involves generalised tonic-clonic seizures that are more intense and long-lasting [13]. SE is the term used to describe an epileptic episode that lasts longer than five minutes or when two or more seizures occur successively, without any recovery between them [13,63]. Usually, physiological mechanisms within the brain are capable of stopping seizures, but when these mechanisms cease, SE ensues [53]. In one study, the prevalence of SE within a group of dogs that were hospitalised was 0.7% [14]. The greater the frequency and the longer the duration, the more severe is the injury, increasing the chances of brain injury and systemic abnormalities [13,124]. Severe forms of epilepsy characterised by recurrent SE and cluster seizures were associated with some breeds like Border Collies and Australian Shepherds [30,125]. Most of the episodes of SE in dogs are related to StE provoked by different causes such as tumours, encephalitis or trauma [126]. In one study, 45.1% of dogs that suffered SE as their first manifestation of seizures were diagnosed with StE [14].

SE is considered as an emergency that should be seen immediately by a veterinarian, to avoid possible neurological damage as consequence of some dysregulations like hypoglycaemia,

hyperthermia, acidosis, renal failure or cardiopulmonary failure following the event [14]. SE may be associated with IE, however it can also be involved in other disorders such as infections, trauma, toxins, neoplasia, metabolic or vascular diseases [127]. Thereby it is essential to realise a thorough examination to exclude these differential diagnoses. Intoxications may lead to SE in most of the cases, regardless of the type of toxins and it is a common diagnosis in dogs that are experiencing their first SE [14].

In a study by Zimmermann *et al.,* dogs with IE were less likely to develop SE when compared with dogs that had StE [14]. It appears that dogs that suffer from epileptic seizures arising from a secondary disorder are more likely to develop SE than dogs that are affected by a primary disorder such as IE [13,35]. Also, according to Platt and Haag, almost half of the dogs that experienced SE had not experienced any seizure beforehand [13]. This finding supports the hypothesis that SE may be more related to StE rather than with IE. Nonetheless, it appears that SE may be associated with AEDs failure to control seizures in dogs with IE [4].

There is no risk factor such as sex or age associated with a higher prevalence of SE [128,129]. As stated by Arrol *et al.,* dogs with IE that experienced SE have a worse prognosis compared with those who have IE but did not experience SE [130]. Most of the deaths related to episodes of SE were a result of euthanasia [131]. Euthanasia is frequently performed in dogs with uncontrolled SE or cluster seizures, mainly due to lack of financial conditions or cooperation by owners [132]. Although less common, euthanasia may also arise due to lack of experience of veterinarians, who may feel frustrated in chronic and non-responsive to treatment cases [126].

When assessing a patient in SE at the hospital, the main goal of treatment is to stop seizures because prolonged SE may induce functional changes that may be damaging for the brain. It is crucial to ensure that airways, breathing and cardiovascular function are safeguarded [53]. Also, blood pressure, temperature and blood glucose should be measured as soon as possible to allow early detection of abnormalities [53]. After ensuring the airways-breathing-circulation (ABC), catheterisation should be performed so fluids and intravenous (IV) drugs can be administered rapidly, therefore allowing higher serum concentrations [126].

Initially, benzodiazepines (BZPs) are the first drugs of choice for the management of SE because of their fast-action [129]. When intravenous access is not available, BZPs may be given intramuscular (e.g. midazolam) or intrarectally (e.g. diazepam (DZP)) in an attempt to stop seizures [129]. DZP is the first-choice drug used in the treatment of SE and it can be given either IV or intrarectal [133]. DZP is mainly used by owners at home, in emergency cases. Administration of intranasal midazolam is becoming a promising alternative option for home-treatment. In a study by Charalambous *et al.*, midazolam given intranasally at a dosage of 0,2 mg/kg seemed to be more effective than DZP given via-rectal [134]. DZP is given intravenously in a dosage of 0,5 to 2 mg/kg, which may be repeated twice or three times [53,63]. It is advised to double the dosage of DZP in case of dogs that are undergoing a chronic treatment with phenobarbital (PB) because the

standard dosage of DZP may not be enough to control seizures [133]. When a IV route is not available, DZP should be given intrarectal at a dosage of 1-2 mg/kg [126]. If seizures keep occurring, additional boluses of BZPs may be necessary [53]. A constant rate infusion (CRI) may also be a good option, especially because it enables a continuous serum concentration and the recommended dosage of DZP as a CRI is 0,25-0,5 mg/kg/h [63].

Generally, BZPs are not effective in controlling chronic cases of SE because of pharmacological resistance [129]. The duration of action of BZPs is short, so in most of the cases, maintenance with stronger AEDs such as PB is required [53]. For dogs that are not undergoing treatment with PB, a loading dosage may be needed [53]. The loading dosage recommended of PB in dogs is 15 mg/kg and should be given IV slowly and diluted with sterile saline solution during five to ten minutes [126]. PB can be concurrently administered with DZP and the recommended dosage is 2-4 mg/kg given IV every 20 to 30 minutes until it reaches a total of 18-20mg/kg [53]. At that point and if seizures stopped, a maintenance dosage of 3-5 mg/kg of PB should be given IV every 12 hours for 24 to 48 hours [53]. If the dog recovers its consciousness and capability to properly swallow, oral AEDs may be given [53]. If seizures persist, administration of stronger AEDs such as levetiracetam in a dosage of 20-60 mg/kg may be required [126]

When any of these AEDs do not control seizures, boluses of propofol in a dosage of 4-6 mg/kg IV is recommended and additional precautions should be taken to avoid apnoea [63,129]. According to Sanders, a CRI of propofol should be administered following the first boluses in a rate of 6-10 mg/kg/h [126]. According to Lorenz *et al.,* CRI administrations of DZP or propofol should continue for six to eight hours [63]. If CRI succeeds in controlling seizures, the rate of infusion should be lowered to the lowest rate that allows effective control of seizures [63]. Ketamine is also an option and may be given IV in a dosage of 5 mg/kg [126]. In one study, a bolus of ketamine at a dosage of 5mg/kg IV followed by a CRI of this same drug at a rate of 5mg/kg/h was successful in controlling seizures in a dog that was unresponsive to both DZP and propofol [135].

As a last resort, when any of the previously mentioned drug fails in controlling seizures, coma should be induced with pentobarbital at a dosage of 5-15 mg/kg given IV until it reaches the desirable effect [126]. In this situation, artificial ventilation should be implemented as well as maintenance of blood pressure to avoid hypotension [126].

Monitorisation and intensive supportive care of the epileptic patient are important to ensure that complications do not occur. Some standard blood tests such as hemogram or serum chemistry should be performed, especially blood electrolytes with the aim of detecting electrolyte imbalance [129]. When intoxication is suspected, toxicity screen tests should be done [129]. Hypoxia and hypotension are likely to happen after seizures, so it is crucial to ensure a proper monitorisation of these two parameters with pulse oximetry and oscillometer, respectively [129]. If the animal is hypoxic, oxygen may need to be supplemented by face masks or nasal cannula [63]. Frequently, hyperthermia occurs secondary to seizures, so it is essential to cool down the patient through

fluids and active cooling [126,129]. Analysis of arterial blood gases is another vital exam that should be performed to detected metabolic acidosis as a consequence of the seizures [129]. Hypoglycaemia may be a cause of seizures, so supplementation with 5% dextrose fluid should be done intravenously [129]. Hyperglycaemia induced by glucose supplementation may be damaging to the brain if hypoxia is present, so thiamine (vitamin B1) should be given intramuscularly before administering fluids supplemented with glucose [129]. During brain hypoxia, there is a persistent anaerobic metabolism over aerobic metabolism due to oxygen deprivation [136]. An overload of blood glucose will contribute for a hypermetabolism that will prefer anaerobic over aerobic metabolism [136]. Thiamine is an important coenzyme in oxidative metabolism, thus will contribute for a higher utilisation of this energy pathway [137] .

Dogs that experience SE or cluster seizures are likely to develop cerebral oedema as a consequence of severe seizures so that mannitol may be an option for treatment [53]. Dogs with severe neurological deficits such as coma or stupor may need intubation and artificial ventilation [63].

1.4.4.2 CLUSTER SEIZURES

According to Sanders, the term cluster seizures refers to an abnormal sequence of seizures in the same individual [3]. Cluster seizures are characterised for being a sequence of several successive seizures where the dog recovers to its normal status between each episode [3]. Therefore, cluster seizures differ from SE because, in the latter, dogs do not recover between episodes [3]. These cluster seizures may be a group of multiple seizures that occur within different spaces of times going from days to months [3]. German shepherds and Boxers seem to be more likely to suffer from cluster seizures than other breeds [131]. In a study, dogs with cluster seizures were more likely to be diagnosed with structural brain disease [33]. Similarly, according to Pákozdy *et al.,* a diagnosis of StE was likely when in the presence of cluster seizures [35].

1.5 DIAGNOSIS

There is no standard gold test that can be used in the diagnosis of epilepsy [18]. For well-understanding of the seizure type, clinicians are enforced to get an appropriate clinical history as also a general detailed examination of the animal. A diagnosis of IE is achieved whenever other possible causes of seizures are excluded [12]. The underlying cause of seizures can be assessed by a thorough clinical history, physical and neurological examination and diagnostic exams that should be performed based on the main differential diagnosis [6]. Preferably, extracranial causes should be ruled out first and then more specific exams such as MRI or CT-scan should be performed to exclude intracranial causes [63].

Ideally, some elementary exams such as hemogram, serum chemistry and urinalysis should be performed in cases where epilepsy is suspected because, with these exams, some extracranial causes of seizures can be easily excluded [6,63]. One study said that dogs are more likely to be diagnosed with IE when the space of time between the first and second seizure is longer than four weeks [16]. StE can be diagnosed by a variety of diagnostic imaging tools (e.g. MRI, CT-scan, x-rays), CSF examination and post-mortem exam [1]. In a new study, there was a higher agreement between veterinarians about seizure's semiology when identifying a primarily generalised seizure than a focal seizure [5]. On the other hand, there was a low percentage of agreement regarding the presence or not of consciousness [5]. The level of diagnosis accuracy depends on the veterinarian experience and specialists are less likely to misinterpret the signs of epileptic seizures [5].

1.5.1 DISEASES THAT MAY MIMIC EPILEPTIC SEIZURES

In the beginning, an epileptic seizure may be easily confused with other pathologies that may mimic epileptic seizures (Table 5). Some examples of these conditions are behaviour disturbances, cataplexy/narcolepsy, neuromuscular weakness, compulsive disorder, paroxysmal dyskinesia, idiopathic head tremor, syncope/heart disease and vestibular disease [7,138,139]. To clarify whether the dog experienced an epileptic seizure, the veterinarian should undertake an exhaustive clinical examination including a neurological examination and collect a thorough anamnesis. It is important that the veterinarian can understand whether the events described by owners are indeed an epileptic seizure because it will therefore affect the diagnostic approach. It is advised that veterinarians have a standard questionnaire prepared for owners to get as much information as possible from owners [139].

A syncope happens as a result of an unexpected decrease of brain blood perfusion which manifests in a sudden loss of consciousness, commonly described as collapse [140]. The most common cause of syncope in dogs is heart disease [102]. When differentiating an epileptic seizure from a syncope, many details may be essential to exclude one of those events. Generally, a syncope is shorter in duration when compared with an epileptic event and dogs can rapidly recover after a syncope whereas in an epileptic event, they normally exhibit post-ictal signs such as confusion and disorientation [9,102]. When a dog experiences a syncope, owners commonly describe an exercising event such as running, as well as coughing beforehand [138]. The age of the dog may also give important information regarding the aetiology of the event. Generally, heart disease is diagnosed in older dogs whereas IE is more frequently diagnosed in young dogs [140]. Some breeds such as Cavalier King Charles Spaniel, Boxer and Doberman Pinscher dogs may be predisposed to heart disease [141] and therefore the likelihood of experiencing a syncope is increased in these breeds.

Paroxysmal dyskinesias (PDs) are movement disorders characterised by sudden and involuntary episodes of muscle movements that leads to abnormalities in the normal gait and posture but without consciousness impairment [140,142]. Normally, it is hard to distinguish from an epileptic seizure because the clinical manifestations are similar between each other [40,139]. However, it is known that some differences exist, which may help establishing the diagnosis. Typically, during a PD episode, consciousness is not affected, autonomic signs are absent and there are no abnormalities regarding post-ictal behaviour [40,139]. Dogs with PDs may be able to return to their previous activity since consciousness is not impaired [139]. Generally, these changes are not frequent in the case of epileptic seizures [40]. It is possible to perform some genetical exams in some breeds like Cavalier King Charles Spaniel with the purpose of PD's diagnosis since PD is frequently seen in this breed [143].

Idiopathic head tremor is included in the PDs disorders [142] and it has been mainly identified in Doberman Pinscher [144] and English Bulldogs [145] and it is characterised by fast and rhythmic myoclonus of the head, that may move vertically or horizontally [144]. The aetiology of this disease is still unknown and this condition may be easily confused with a focal seizure. However, in cases of idiopathic head tremor, the consciousness is not impaired [144,145]. A study reported that owners were able to stop the episode by simply calling them or asking them to get up [146].

Narcolepsy is a sleep disorder often underdiagnosed in dogs characterised by excessive somnolence and it is normally accompanied by cataplexy, which is defined as a sudden flaccid paralysis [50,63]. Cataplexy may happen alone and it is normally triggered by emotions (e.g. anxiety, anger, excitement) [81]. Diagnosing narcolepsy in dogs may be challenging because not only its symptoms are difficult to identify but also because owners may see an excessive sleepiness as something normal.

Myasthenia gravis is a neuromuscular junction disorder that may be acquired or congenital [147]. The acquired form is more commonly observed in dogs and is characterised by an autoimmune response against acetylcholine receptors at the neuromuscular plate [147,148]. The clinical presentation is often confused with a focal seizure since it also leads to muscle weakness and abnormalities in the gait and posture [50]. Episodes are frequently observed after stimulating-situations such as exercise and it may worsen while it intensifies [50,147].

Vestibular syndrome is another disorder that may be confused with epileptic disorder if a thorough neurological examination is not performed [149]. Even though it is more frequently seen in cats than in dogs, vestibular syndrome is mainly characterised by ataxia, which may be further classified in cerebellar or peripheral, depending upon its aetiology [149]. Generally, some signs of vestibular syndrome are distinctive of this disorder such as head tilt, circling and nystagmus [50]. Also, consciousness is never impaired in case of vestibular syndrome, so these signs may be sometimes enough to exclude epileptic disorder [149].

Compulsive and behaviour disorders are frequently associated with epileptic disorders but sometimes these behaviour disorders may happen singly. For this reason, it is important to understand whether these behaviour changes are induced by mechanisms underlying epilepsy or if they are provoked by other diseases or environmental factors [140]. Factors such as stress, genetics, pain or conditioned behaviour may be responsible for behaviour disturbances such as tail chasing, foot or hand chewing, excessive licking, aggression, excessive vocalisation, among others [140,150]. Some of these disturbances may also be seen during the prodrome or in the post-ictal phase, so it is important to understand whether the other phases of the epileptic episodes are present. Also, behaviour changes associated with epilepsy cannot be stopped by the owner and dogs will manifest these disturbances regardless the presence of the owner [140].

Even though some disorders may have identical clinical presentations that could possibly be misunderstood as an epileptic episode, there are still some characteristics that may strengthen the identification of an epileptic seizure. For instance, generalised seizures may have a distinctive clinical presentation. Generally, they are defined as short in time, may occur during resting time and usually the post-ictal phase is associated with autonomic signs such as hypersalivation, urination or defecation [139]. Autonomic signs are generally associated with epileptic seizures [15]. Also, some behaviour abnormalities are generally evident in the post-ictal phase like aggressiveness, disorientation, anxiousness or seeking behaviour [139]. Ictus phase is described as intense and non-reversible and, while owners may revert other disorders like PD or idiopathic head tremor during the event, ictus phase in IE will not cease regardless owners' efforts [139]. Another difference regarding epilepsy is the fact that, on the post-ictal phase, animals will not be able to return to the activity they were performing previously. This happens because dogs usually lose their consciousness [139].

Focal seizures may be more harder to distinguish between other disorders because sometimes consciousness is not impaired or autonomic signs are absent, which may be challenging for the veterinarian [139]. In this case, the age at the onset as well as the presence of post-ictal signs should help to establish a definitive diagnosis [139,142].

Table 5: Different aetiologies that may present similar clinical manifestation to IE (Adapted from De Risio *et al.* 2015).

PARAMETERS	SYNCOPE	NEUROMUSCULAR WEAKNESS	NARCOLEPSY/ CATAPLEXY	COMPULSIVE DISORDER	VESTIBULAR DISORDER	PAROXYSMAL DYSKINESIA	IDIOPATHIC HEAD TREMOR	EPILEPTIC SEIZURES
Clinical signs between events	Normal or arrhythmia, pulse deficits, heart murmur, cyanosis, abnormal lung auscultation	Normal or generalised weakness, muscle atrophy, pain, decreased reflexes	Altered sleep/wake cycle, normal clinical examination	Normal	Normal	Normal	Normal	Normal or forebrain signs
Precipitating event	Exercise, excitement	Activity, exercise	Excitement, eating	Behaviour triggers	None	None or activity, exercise, excitement, stress	None or stress, fatigue, overstimulation	None or flashing lights, anxiety, stress
Pre-event changes	None	None	None	None	None	None	None	Pre-ictal signs like: anxiety, vocalisation, aggression, contact-seeking, hiding
Event description	Brief, sudden collapse and rapid recovery	Stiff, stilted gait before collapse	Sudden collapse	Pacing, barking, licking, chasing imaginary objects or tail, chewing	Head tilt and collapse towards the affected side nystagmus, vestibular ataxia, head	Dystonia, ballismus, tremors, impaired posture, ataxia	Vertical or horizontal rhythmic head movement	Focal or generalised, tonic-clonic movements are most common
Level of consciousness	Reduced to absent	Normal	Normal if only cataplexy. Absent in narcolepsy	Normal	Normal or disorientated	Normal	Normal	Often impaired
Autonomic signs	Possible abnormalities of heart rate and rhythm	None	None	None	None	None	None	Hypersalivation, defaecation, urination
Muscle tone	Flaccid	Often flaccid or spastic	Flaccid	Normal	Unilateral decrease in extensor muscle tone	Hypertonicity	Normal	Tonic or alternating tonic-clonic movements
Lateralising signs	No	No	No	No	Yes	Possible	No	Possible
Duration	Seconds	Minutes to hours	Seconds to minutes	Minutes to hours	Second to hours	Seconds to hours	Seconds to hours	Seconds to minutes or over 5 minutes in case of SE
Post-episodic changes	None	None	None	None	None	None or tiredness	None, tiredness or restlessness	Disorientation, aggression, lethargy, deep sleep, ataxia, hunger/thirst
Other signs that may accompanied	Cough, increased respiratory noise	Dysphagia, dysphonia, regurgitation, dyspnoea	Young purebred dogs	History of anxiety disorder	Signs of vestibular disease	Interaction with owner may alleviate the episode	Episodes can be interrupted by the owner	Facial muscles often involved during the ictus

1.5.2 SIGNALMENT AND CLINICAL EXAMINATION

When receiving a dog at the hospital that might have suffered a seizure, the main question to answer is whether that dog experienced indeed an epileptic seizure. Good and clear communication between owners and veterinarian plays a key role in helping formulate a list of possible differential diagnoses [6]. Most of the cases, the veterinarian does not witness the seizures, so the clinical classification of seizures must rely on owner's observation and description of the event as well as in video-recordings.

The veterinarian should ask the owner to describe the episode as accurately as possible, including physical manifestation, duration of the episode, frequency, abnormal behaviours preceding and succeeding the episode and its severity [17,63]. During the appointment, routine questions about the patient should be asked as well as more specific questions such as existence of clinical history of seizures in other animals genetically related with the patient, possible trauma, noteworthy behaviour changes, type of diet, changes in the normal daily routine, if the patient has been diagnosed previously with other diseases, treatments that the patient has done or is currently doing, possible access to toxins, among other questions [17,63,151].

Details about the patient such as species, gender, breed and age at the first episode are of great value to determine whether possible IE is presented. As previously discussed, some breeds have a genetical predisposition for IE, which is mostly diagnosed in young dogs, especially in those aged between six months to six years. In some cases, dogs may start exhibiting seizures at an earlier stage of their life and under these circumstances, StE related to congenital or genetic abnormalities is likely [22]. Therefore it is vital to ensure a proper distinction between StE and IE is made, especially in small breed dogs that seemed to be more predisposed to structural abnormalities [22].

Also, some breeds are predisposed to certain diseases that may induce seizures as well. For example, Boxers have a high incidence of intracranial tumours whereas Maltese dogs are predisposed to inflammatory diseases that may affect the brain [63]. Hydrocephalus and hypoglycaemia are relatively common in toy breeds, so both should be considered as a differential diagnosis [151]. It is also known that the older the dog, the higher the likelihood of StE. Even though that is unlikely to detect abnormalities at the MRI in young dogs, this exam should still be performed if possible, to strengthen the diagnosis of IE. According to De Risio, in dogs that exhibit neurological abnormalities, it is important to ask the owner about the onset of the episode to distinguish between acute and chronic situations and also about its course to distinguish between static, progressive, relapsing or regressive [151].

A detailed general examination may be helpful to detect underlying abnormalities in vital organs, that may be the origin of seizures or may induce similar symptoms that mimic an epileptic seizure. This general examination should be performed during the interictal period [51]. As stated by

DeLahunta *et al.,* focal asymmetrical manifestations may suggest neoplasia, vascular compromise, trauma, focal infection or non-infectious meningoencephalitis [51].

1.5.3 NEUROLOGICAL EXAMINATION

A thorough neurological examination may indicate which part of the nervous system is affected and therefore it helps to identify the localisation and distribution of the lesion [151]. The presence of interictal neurological deficits indicates that other causes other than IE are likely, especially if these deficits persist for a long period [35,151]. However, the absence of abnormalities on the neurological examination does not completely exclude StE [6]. The presence of irregularities on the neurological examination may happen in dogs with IE, although these do not extend for a long period of time [150].

Through neurological examination, it is possible to evaluate the integrity and function of the regions that constitute the nervous system. Neurological examination includes evaluation of mentation, gait, posture, muscles mass/tonus, spinal nerve reflexes, nociception, proprioception, behaviour, cranial nerve function and postural reactions [6,151]. Normally, a seizure indicates that the forebrain is somehow affected but after recovering from the episode, dogs do not often exhibit neurological abnormalities unless noteworthy brain injury has occurred [6,151]. Thus, it is expected that a dog with IE does not show neurological deficits during preictal stages. According to Dewey and Costa, unilateral menace response deficits, hemiparesis, circling, head tilt and unilateral decrease of proprioceptive placing may be indicative of StE [50]. Patient signalment and physical as well as neurological examination would influence the diagnostic approach that will be followed later [6]. The majority of metabolic diseases that may lead to seizures do not induce neurological abnormalities during the interictal period [51]. In one study, dogs that presented sporadic single seizures were more likely to be diagnosed with IE than dogs that were presented with cluster seizures [33].

1.5.4 ELECTROENCEPHALOGRAM

EEG can give an absolute, definitive diagnosis of IE together with clinical manifestations of epilepsy and diagnostic exams [139]. EGG is especially useful for the diagnosis of IE because it allows differentiating epilepsy from other paroxysmal diseases since it evaluates the functionality of the brain [53,152]. However, EEG is not widely available for veterinarian use mainly because it requires extensive knowledge to be used. Conversely is widely used in humans in the diagnosis of epilepsy, most of the times together with video-recording [153]. In most of the dogs, general anaesthesia is required to perform an EEG since any movement may compromise the results,

which is another inconvenient for the use of the EEG in veterinary practice [154]. Factors like muscle contractions, restraint methods, type of electrodes and sophisticated EEG instrumentation may difficult the interpretation of results [139]. In human medicine, video-EEG is a reliable and useful tool for the diagnosis and characterisation of epileptic seizures [5]. However, in veterinary medicine video-EEG is not currently used because its reliability is not as good as in humans [5].

1.5.5 HEMOGRAM, SERUM CHEMISTRY, URINALYSIS AND BILE ACIDS LEVELS

As previously said, the minimal database including hemogram, serum chemistry, urinalysis and bile acids levels are a key role in excluding frequent extracranial causes of seizures, most related with metabolic disorders [63]. The required minimal database may vary among patients, especially taking into consideration their age [17]. For example, in dogs younger than one year old that were not dewormed, the faecal exam could be performed when severe parasitism is suspected [17].

If the seizures are being provoked by a hypoglycaemia or by electrolytes imbalance such as hypocalcaemia or hyponatremia, these can all be easily identified on a serum chemistry [63]. Hepatic failure is likely in the presence of hyperammonaemia together with increased levels of hepatic enzymes [51] and non-regenerative anaemia [63]. Increased levels of serum urea, non-regenerative anaemia and abnormalities on urinalysis such as increased protein-creatinine ratio and isosthenuria may also be suggestive of renal disease [51]. Abnormalities on normal values of pre and post-prandial bile acids and triglycerides may also suggest the existence of a portosystemic shunt or hepatic failure, especially when together with another anomaly such as increased hepatic enzymes and hyperammonaemia [63]. The presence of increased levels of white blood cells (WBC), especially neutrophils, are indicative of inflammatory/infectious processes in the majority of the cases [51].

1.5.6 VIDEO-DOCUMENTATION

Regarding the clinical history of the patient, a video-documentation should be asked to owners to analyse certain characteristics such as frequency, duration and event patterns that can strongly suggest a diagnosis of epilepsy [18]. Video-records have been considered as an essential, inexpensive and useful tool for the clinician as it can help to identify the seizure type [5]. Video recordings are especially helpful when it comes to distinguish between a primary generalised seizure from a secondarily generalised seizure that was preceded by a primarily focal seizure [5]. However, video recordings should not be the unique source to characterise the seizure type. Sometimes owners do not record videos from the beginning of the episode and focal seizure can

be missed because its signs are not as visible as in a generalised seizure. Therefore this can lead to a misconception of the seizure type [5,110].

1.5.7 IMAGING AND CSF ANALYSIS

Advanced imaging such as MRI or CT-scan are often used to help the veterinarian identify intracranial causes of seizures. The introduction of MRI and CT-scan for diagnostic purposes has allowed the detection of structural and morphologic brain anomalies that may be the cause for epileptic episodes [155]. According to the IVETF, these exams are important for ruling-out StE and in cases of resistance to AEDs [139].

In one study, 26% of dogs that experienced an epileptic episode before the age of one year were posteriorly diagnosed with a structural brain disease that could only be identified by the MRI [130]. These same dogs had normal neurological exams, which highlights the importance of these advanced imaging exams on the diagnosis.

Preferably, any dog with recurring seizures should undergo an MRI and a CFS analysis to exclude possible StE [63]. CSF analysis is way more important in the absence of remarkable brain anomalies and when inflammatory/infectious diseases such as meningoencephalitis are strongly suspected [63]. However, CFS analysis is not indicated when increased ICP is suspected [51]. Tumours and encephalitis were described as the most common detected causes of StE [35]. Even though MRI is performed with the aim of detecting causes of StE, it is possible to detect abnormalities secondary to epileptic events. In a study, abnormalities caused by seizures were observed as hyperintense zones on T2-weighted images in brain regions that are normally affected by over-concentration of excitatory events [156]. The fact that these lesions were presented after seizures events and without any inflammatory or infectious process highlighted this hypothesis [156]. As said by Smith *et al.,* a low percentage of abnormalities (2.2%) was found during the MRI in dogs younger than six years with recurrent seizures but with an unremarkable neurological examination [34].

Although MRI and CFS are crucial to exclude many causes of StE, these exams are costly and may not be performed if owners cannot bear the financial cost [6]. The recommendations by the IVETF is to perform an MRI in the presence of the following circumstances: first episode occurring before six months of age or after six years old; interictal abnormalities detected during the neurologic examination; presence of SE or cluster seizures and when resistance to AEDs is suspected [139].

Similarly, abdominal ultrasonography and radiographs may be helpful in excluding causes of StE, with the advantage of being reasonably inexpensive exams. Abdominal radiographs may show possible abnormalities like metastasis, primary tumoral masses or evidence microhepatica/hepatomegaly [17]. On the other hand, three-view thoracic radiographs (right and left

lateral and ventrodorsally) may also show metastatic processes, primary tumoral masses and microcardia/cardiomegaly [17]. Abdominal ultrasonography is particularly useful to diagnose portosystemic shunts or supporting the diagnosis of renal failure, that are both common anomalies that may induce seizures. Rectal scintigraphy may also be suitable for the diagnosis of the portosystemic shunt [51]. A summary of possible diagnostical approach for IE can be seen on Figure 3.

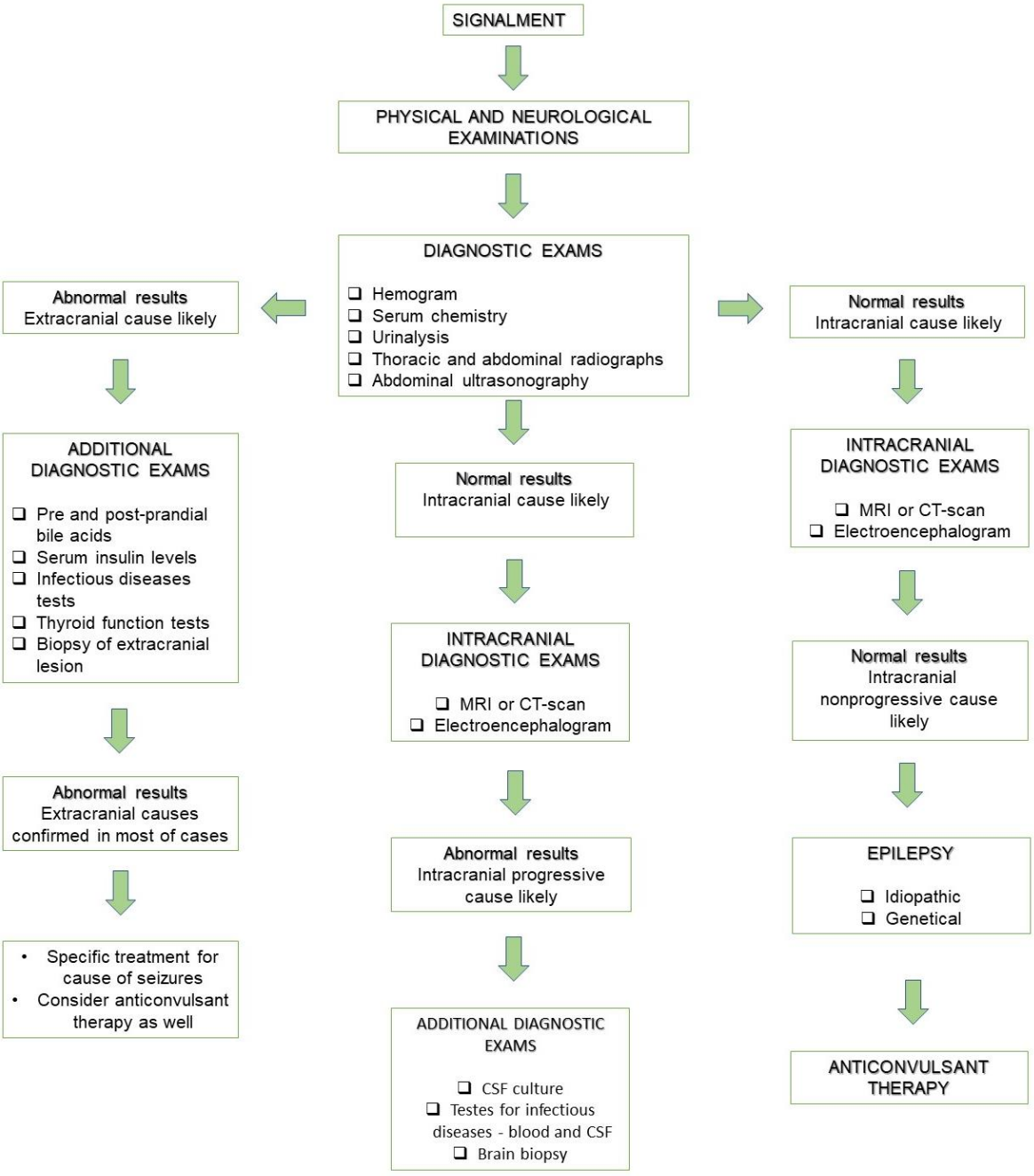

Figure 3: Diagnostic approach to idiopathic epilepsy (Adapted from Vernau 2015).

1.5.8 TIER-SYSTEM

The IVETF defined a three-tier system of confidence criteria regarding the diagnosis of IE (Table 6). This system may assist the veterinarian in identifying the appropriate diagnostic exams as well as improving the accuracy of diagnosis.

The tier I confidence level is the minimal requirements that should be assured to properly diagnose IE. In what concerns to the diagnostic exams, to be classified as a tier I, minimal database is required. According to De Risio *et al.,* the minimal database requirements include a hemogram, complete serum chemistry (e.g. electrolytes analysis, hepatic enzymes, urea, creatinine, total protein, albumin, glucose, cholesterol, triglycerides, ammonia and fasting bile acids) and urinalysis [139]. If the patient has a family history of IE, this finding may support the diagnosis [139].

The tier II confidence level adds all the diagnostic exams that should be performed for a tier I confidence level in addition to a brain MRI and CSF analysis.

The tier III confidence level is considered when tier I and II requirements are achieved, without no remarkable abnormalities and in the addition of an EEG [139]. When abnormalities are detected on these exams, a diagnosis of StE is likely. In order to accurately diagnose IE, a tier II confidence level should be achieved, however some owners may not be able to afford some diagnostic exams such as an MRI, so in these cases a tier I confidence level should be attained. CT-scan may be considered over an MRI since it is normally cheaper. The EEG is widely used for scientific rather than medical purposes, so in most cases, a tier III confidence cannot be achieved in a normal daily-basis clinical routine.

Table 6: Tier system of confidence for diagnosis of IE (Adapted from De Risio *et al.* 2015).

TIER SYSTEM OF CONFIDENCE CRITERIA FOR A DIAGNOSIS OF IE	DIAGNOSTIC COMPLEMENTARY EXAMS
TIER I	History of two or more unprovoked epileptic seizures separated by at least 24 hours Dog aged between six months and six years Unremarkable interictal physical and neurologic examination Normal hemogram, serum chemistry and urinalysis
TIER II	Tier I factors Unremarkable fasting and post-prandial bile acids Brain MRI CSF analysis
TIER III	Tier II factors Abnormalities detected on EEG

1.6 TREATMENT

In human medicine, the main objective of the treatment is to achieve total remission of seizures in the patients and provide a higher QoL with minimisation of side effects related with AEDs [49]. However, in veterinary medicine, achieving a total remission of seizures is unlikely due to AEDs resistance, that is evident when compared with human medicine [49]. According to Potschka *et al.,* less than 50% of dogs that had been treated for IE achieved remission [157]. Veterinary medicine continues to work towards trying to decrease seizure frequency, severity and duration as well as decreasing the adverse effects associated with AEDs [4,139]. There is no cure for epilepsy and spontaneous remission is rare. Thus a lifelong treatment based in AEDs that provide suppression of epileptic seizures needs to be implemented in most cases [158]. Therefore, it is crucial to ensure that safety regarding AEDs is accomplished.

There are more AEDs available in human medicine than in veterinary medicine, mostly because some AEDs are rapidly metabolised and excreted in dogs, hence the importance of using drugs with a long half-life time in this species [159].

In contrast to what happens in human medicine, epileptic seizures are not classified into different presentations in veterinary medicine, so AEDs are used regardless of seizure's type [15]. Typically, the use of a single drug on treatment is considered enough for proper management of seizures [49]. Nevertheless, some dogs may need combined therapy when a satisfactory response to the first-line drug is not achieved [49].

Some studies claim that around 30% of the dogs diagnosed with IE are refractory to the treatment, showing resistance to the most commonly used AEDs such as PB and potassium bromide (KBr), thereby making it harder for veterinarians to find a suitable, efficient treatment for some dogs [157,160,161]. AEDs only suppress seizures and do not act against epileptogenesis, so they do not prevent the development of the disease, which highlights the importance of developing more efficient and tolerable drugs [159,162]. Also, tolerance and resistance to the AEDs are particularly common and even dogs that have achieved remission can again experience seizures, despite a good therapy and management from both veterinarian and owner [163]. According to Hülsmeyer *et al.,* 71% of dogs were found to have resistance to AEDs and recurrent cessation of medication was related to therapeutic failure [30].

Dogs that had experienced a more significant number of seizures before treatment are more susceptible to develop tolerance against AEDs [164]. These problems are generally resolved by simply increasing the dosage of the chosen drug, especially when seizures suddenly occur after reaching to a steady-state [163].

The veterinarian should consider factors related with the AEDs such as their availability, effectiveness, tolerability and side effects, ensuring owners are aware of possible complications with the treatment. Also, the factors related with the patient such as its general health and factors related with the owner such as its financial status and cooperation should also be considered before starting the treatment [132,165,166]. According to the IVETF, a treatment should be administered when in the presence of the following conditions: interictal period shorter than six months (e.g. when two or more epileptic seizures occur within a six-month period); when in presence of SE or cluster seizures; when the post-ictal signs are severe or last for more than 24 hours or when the epileptic seizure frequency and/or duration increases [165,166]. Before initiating the treatment, it is essential to certify the absence of underlying diseases that may originate seizures as well. Most of the times, owners overreact to the diagnosis of epilepsy because epilepsy is typically seen as a severe disorder. Veterinarians play a key-role to ensure that unnecessary euthanasia is not performed when dealing with such cases [132]. Also, veterinarians should explain to owners that epilepsy might be a life-threatening condition when in the absence of treatment but that seizures can be controlled with a suitable and appropriate treatment.

Furthermore, owners should be aware that treatment may not be entirely successful, despite the efforts from both parties [132]. The sooner the treatment starts, the more effective it is likely to be [132,165]. Male dogs are likely to have an AEDs resistance compared with female dogs, leading to a poorer prognosis [167]. A study reported that AEDs might affect canine cognition and, therefore, it could weaken their performance on routine actions such as learning tricks or complete tasks [168]. Without appropriate treatment, frequency of seizures may increase as time passes, thereby worsening the symptoms and leading to a poorer prognosis. The *honeymoon effect* is used to mention the tolerance against some AEDs during a chronic treatment, which may vary among

dogs, with some of those developing resistance to specific AEDs while others do not [165]. This effect is commonly seen in dogs with refractory epilepsy, when a chronic treatment with AEDs leads to a functional tolerance against these drugs, thus to a failure in controlling seizures [169].

1.6.1 PHENOBARBITAL

PB is a barbiturate derivate and is commonly used in the treatment of IE in dogs, with a reported effectiveness of 60% to 80% in controlling seizures [49,170]. Monotherapy with PB is commonly used in both human and veterinary medicine, despite its side effects [49,171]. Many formulations are available for use. However, oral formulations are undoubtedly the most used. When PB is orally given, the peak serum concentration is achieved 4 to 8 hours after administration [172,173]. IE is a chronic disease, requiring continuous treatment to ensure seizures cessation, thus oral administration is the simplest way to owners provide medication to their pets. As PB is mainly metabolized in the liver, it intensifies the activity of cytochrome P450, a potent enzyme that leads to the production of reactive oxygen species that are potentially damaging to the liver, thus the importance of regular monitorisation of PB serum concentration and hepatic enzymes [170,174]. The median half-life known for PB is 88.7 to 99.6 hours [173]. As PB may be potentially hepatotoxic, its administration should be avoided in dogs with pre-existing or suspected hepatic disease. PB should not be used in combination with other drugs that are metabolised by cytochrome P450 because it may decrease those drugs concentrations, therefore decreasing their efficacy [175]. Similarly, PB should be avoided in combination with drugs that inhibits cytochrome P450's activity as it may lead to toxicity [176].

When choosing the best treatment, PB is the first option for individual therapy and KBr remains an add-on drug because PB provides lower seizure recurrence when compared with KBr [176]. Nonetheless, a study suggests that KBr may be a reasonable choice as a first-choice AEDs, even though seizure freedom is not as effective when compared with the use of PB [176]. Like all drugs, some side effects may be seen like polyuria, lethargy and ataxia [170,176,177]. These side effects usually are dose-dependent and the higher the dosage, the higher the likelihood of developing side effects [178]. PB is associated with an increase of hepatic enzymes such as serum alkaline phosphatase (ALP), serum alanine transaminase (ALT) and Ɣ-glutamyl transferase (GGT) [179]. The reason for such is related to enzyme induction by PB's metabolization in the liver [179]. Even though PB provokes some side effects during long-term treatment, this drug is relatively safe and moderately reduces the frequency of the seizures [172,173]. A scheme regarding the management of PB treatment is observed in Figure 4.

According to Bhatti *et al.,* the recommended starting oral dosage should be 2.5 to 3 mg/kg twice a day, however, it may need to be adapted according to seizure's control, side effects and serum concentrations [165]. After initiating the treatment, PB serum concentration should be monitored

every 2 weeks until an acceptable serum value is achieved, which is known to be between 25-30 mg/L in dogs [165]. This range of values has been identified as the optimal concentrations regarding seizure control and minimal side effects [165]. Hemogram and serum chemistry exams are recommended every six months after initiating treatment to detect abnormalities that may be induced by chronic use or high dosage of AEDs [165,170]. Before starting combined drug therapy, PB dosage can be increased if seizure frequency does not reduce after initiating treatment, however, PB serum concentrations should not overcome 35 mg/L due to a higher likelihood of hepatic damage [165,176]. Monotherapy with PB should be stopped if the dog starts developing adverse effects or an add-drug should be implemented if PB serum concentrations are constantly above 30mg/L [165,170,176]. Revaluations and optimal timing for blood collecting for PB serum measurement, hemogram and serum chemistry may vary among studies.

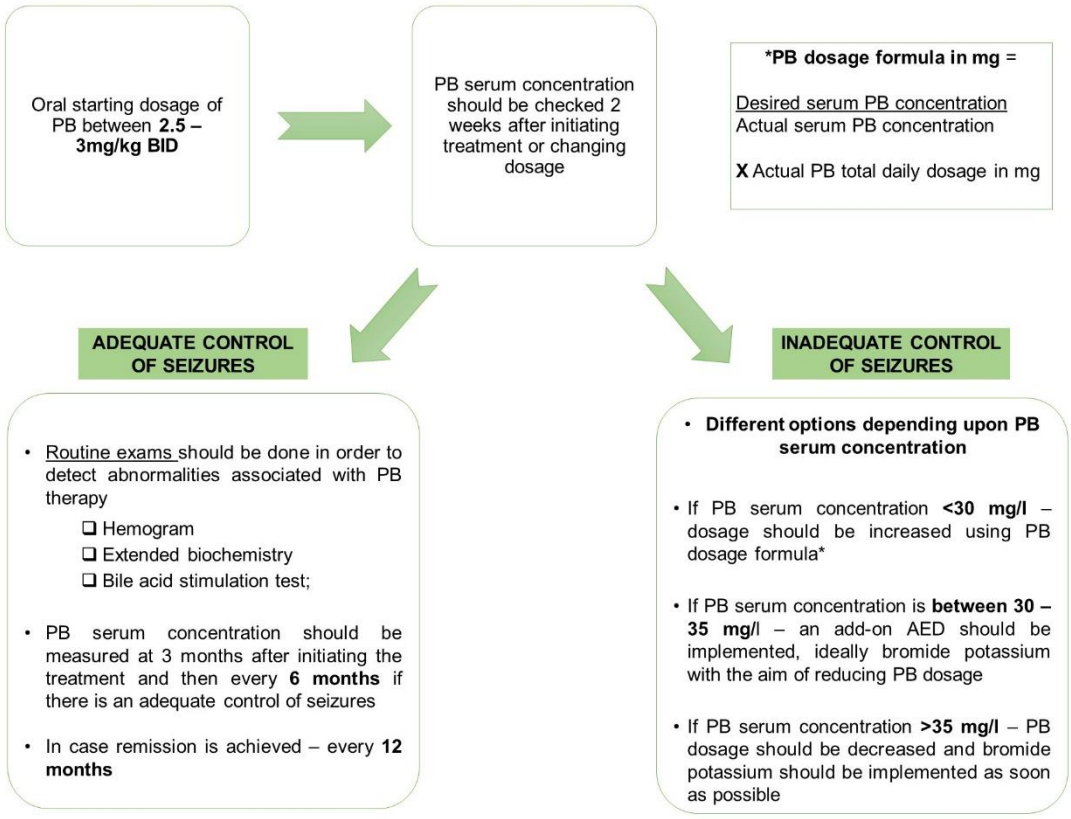

Figure 4: Treatment approach using phenobarbital as monotherapy (Adapted from Bhatti *et al.* 2015)

One study reported an acute PB intoxication where the affected dog developed severe haematological abnormalities such as leukopenia, thrombocytopenia and anaemia [180]. As the main objective of treatment is to achieve a partial or total remission of seizures, the dosage of PB

should not be changed if seizures are controlled even if PB serum concentrations are below the optimal amount [165]. PB serum concentrations should remain at the lowest possible concentration that is effective in controlling seizures and their frequency, whilst minimising the possible side effects [181]. Every time that remission is achieved, routine exams may be performed every 12 months [165]. Treatment with PB is often associated with functional resistance and tolerance, hence leading to a failure in controlling seizures [182].

1.6.2 POTASSIUM BROMIDE

Nowadays, there are more AEDs available for the management of IE. KBr is still one of the best options since it is inexpensive, relatively safe, well-tolerated when chronically used and efficient [177,183]. A combination of KBr with PB is the most commonly combined therapy used when PB per se does not provide seizure freedom and the synergetic effect between both drugs allows a better control of seizures, leading to at least 50% reduction of seizures [44,49,184]. When compared with PB, KBr is not as effective as PB in controlling seizures [170,176]. A monotherapy with KBr was already reported in a study [176] although its single-use in treatment of IE in dogs has not been approved in the majority of the European Union (EU) countries [165]. Nevertheless, it is approved as an add-on drug in combination with either PB or imepitoin [165,176,177].

KBr is available in oral formulations that allow an easier way of administration. When compared with PB, KBr has a slower metabolization after oral administration, with a median half-life of 46 days [185], but other studies suggest different values between 24 to 46 days [170,183,185]. KBr is completely excreted in the urine, thus it is not hepatotoxic and it may be administrated in animals with hepatic abnormalities [165,166,170]. However, precautions should be taken when giving KBr to dogs with renal disease as the excretion is compromised and this may lead to toxicity [186]. Moreover, some side effects have been associated with KBr such as polyphagia, polydipsia/polyuria (PD/PU) and sedation [170,177]. The measurement of KBr serum concentration should be performed three months after introducing this drug, taking months until steady-state values are achieved [165,170]. An interval range of 1000 – 2000 mg/L is desirable when undergoing a combined therapy with PB [165,170,185]. KBr serum concentration should be measured three and six months after initiating the treatment but it can also be performed every 12 months if remission is attained [165,170].

During a combined treatment with PB, KBr should be given orally, twice a day, in a dosage of 15 mg/kg and should be given alongside food, avoiding gastrointestinal disturbances like vomiting or diarrhoea [165,187]. It is recommended that the administration of a loading dose is given to allow for a steady-state concentration to be achieved at an earlier stage, resulting in better control of seizures [170]. According to the IVEFT, a loading dose of 125 mg/kg/day divided in three to four times a day, during five consecutive days is advised and owners must be aware of possible side

effects that may occur such as gastrointestinal signs or sedation [165,170]. As previously stated for PB, routine exams should be performed such as hemogram and serum chemistry before initiating treatment with KBr and every six months afterwards [165]. In specific situations when seizures are frequent even when undergoing treatment with PB or when side effects may be life-threatening, a loading dosage of KBr may be administered to control seizures or to discontinue the treatment with PB, respectively [165]. This loading dosage will allow for a faster increase of KBr serum concentration, resulting in a quicker response [170]. During a combined treatment with PB and KBr, it may be necessary to readjust KBr dosages, depending upon seizures frequency and side effects presented.

1.6.3 IMEPITOIN

A new drug that has been approved by EU for the treatment of IE in dogs is imepitoin, a low-affinity partial agonist of GABA receptors drug that was initially developed for humans [165,166]. Imepitoin seems to be well-tolerated and efficient in both dogs and humans, however, its use was discontinued in humans due to variability in pharmacokinetic properties [188]. Its effectiveness and fewer side effects make it a safe and reliable option for treatment [166,177,188]. Imepitoin is not normally chosen as a first-drug line in the treatment of IE but it is frequently used as add-on drug when other standard AEDs fail in controlling seizures or when side effects provoked by those AEDs are life-threatening, so dosage of the main AED has to be reduced [177]. However, a study suggested the use of imepitoin on monotherapy that was proven to be more efficient than in combined therapy with other AEDs [171].

Moreover, a study found that imepitoin is more stable and shows better results in controlling seizures when compared with KBr as an add-on drug [159]. Infrequently, imepitoin may be used as a first-line drug and a study demonstrated that either PB or KBr could be used as add-on therapy in cases where dogs are refractory to the treatment with a maximum dosage of imepitoin [177]. According to the IVETF, imepitoin is mainly used in cases of recurrent generalised seizures [165]. Some side effects associated with imepitoin may include sedation, PD/PU and polyphagia [158,188,189]. However, these side effects are uncommon which makes it an advantage when compared with other AEDs [158,188,189]. In one study, hyperactivity appeared to be the most common side-effect associated with imepitoin when compared with other AEDs [188].

In contrast to what frequently happens with other AEDs, dogs that had been treated with imepitoin did not present behaviour changes during treatment and therefore owners may prefer this course of treatment [159]. As imepitoin has an anxiolytic effect, it is possible that behaviour abnormalities such as anxiety or aggression, which are commonly described in epileptic dogs, are well-controlled [158,159]. Some studies have not discovered significant alterations in both hemogram, serum chemistry and urinalysis, which suggests that imepitoin does not influence metabolic

pathways regarding vital organs such as liver or kidneys [158,159,188]. Imepitoin is mainly metabolised by the liver and its excretion mostly happens through the faeces but no noteworthy alterations have been detected in the liver that could be induced by the metabolization of this drug [158,165]. There is no satisfactory evidence on safety regarding the use of imepitoin in dogs with concomitants diseases such as heart or gastrointestinal disease, despite undetectable abnormalities in vital organs [165]. Therefore, the use of imepitoin in such cases should be avoided.

According to the European Medicines Agency (EMA), the initial recommended dosage of imepitoin is 10 mg/kg, twice a day, that could be increased up to 30 mg/kg if necessary [190]. In a study by Rundfeldt *et al.*, a dosage of 30 mg/kg, given orally twice a day, resulted in an active stabilisation of seizures in dogs that were suffering from generalised tonic-clonic seizures [188]. Another study claimed that imepitoin at a dosage range of 5 to 30 mg/kg was sufficiently efficient in decreasing the frequency of seizures and was more tolerated than other AEDs [159]. This therapeutic range was found to be safe in other studies and the therapeutic range where side effects are non-existent or tolerable was found to be between 1 mg/kg to 30 mg/kg [189,191]. However, higher doses have shown to be more efficient in controlling seizures when compared with lower dosages but may be sensible to initiate therapy with lower dosages as higher dosages may increase the risk of developing undesirable side effects [188]. The same study suggested that imepitoin may be used in chronic therapy because it is unlikely that antiepileptic properties are lost throughout the treatment [188].

It is considered unlikely that tolerance against imepitoin develops during a prolonged treatment, which enables its use on chronic treatments for IE [159,191]. The daily dosage of imepitoin can be moderately increased up to 30 mg/kg in cases where there is an unsatisfactory control of seizures [165]. In contrary to what happens with PB and KBr, serum concentrations of imepitoin are not routinely controlled because an ideal range of serum drug concentration have not yet been defined [165]. Equally to what happens with other AEDs, routine blood analysis should be performed every six months after initiating treatment with the selected drug, with the objective of early detection of side effects [165]. According to Rundfeldt *et al.,* the median half-life time was found to be two hours [159]. Even though there is no evidence of pharmacological interactions between imepitoin and other drugs, especially other AEDs, precaution should be taken if DZP is added to a dog that is submitting a treatment with imepitoin because both drugs are GABA receptors agonists, therefore undesirable interactions may happen [158].

1.6.4 LEVETIRACETAM

Levetiracetam is one of the licensed UE drugs for the treatment of IE and its use as an add-on drug has been recommended in some studies [166,171]. According to Volk *et al.,* 57% of epileptic dogs that were resistant to first-line AEDs positively responded when levetiracetam was added to

the treatment [169]. Some studies claimed that levetiracetam represents a safe, well-tolerated drug and side effects are practically non-existent but sedation and ataxia had been described as the most common adverse effects [164,192]. The kidneys quickly metabolise levetiracetam and its half-life time varies between four to eight hours [165,193]. Because its metabolization mainly occurs via the kidneys, it should be avoided in dogs with renal disease as the clearance of this drug may be affected and could possibly lead to toxicity [194].

On the other hand, levetiracetam may be a good choice for dogs with hepatic disturbances as hepatic metabolization is minimal [194]. Levetiracetam does not stimulate hepatic enzymes' activity, therefore tolerance is less likely to occur in contrast to what happens with AEDs such as PB [163]. According to Patterson *et al.,* the recommended dose of levetiracetam given orally is 20 mg/kg twice or four times a day due to its short half-life time and it can be given parenterally to dogs without complications [193]. However, the dosage range is 5 to 30 mg/kg, twice or three times a day [195], so dosage may be adjusted to every situation. A study reported that levetiracetam was associated with tolerance in the long-term, frequently called the *honeymoon effect*, which may be a disadvantage in a chronic treatment [169]. Similarly to what happens with imepitoin, the ideal range of serum concentration is not yet known for levetiracetam [192]. According to Moore *et al.,* it may be necessary to increase the dosage of levetiracetam when in a combined therapy with PB since PB seemed to slightly change the normal pharmacokinetics of levetiracetam [196]. This finding may suggest that the actual recommended dosage possibly is not sufficient to maintain a therapeutic serum concentration of levetiracetam, when in combination with PB [192].

1.6.5 DIAZEPAM

BZDs are a class of drugs that may be used in the treatment of IE, although its efficiency is poorer when compared with other drugs and BZDs are associated with a high risk of developing resistance and tolerability, thus reducing their efficacy [159]. Nevertheless, these drugs, especially DZP, are primarily chosen for treatment of SE and not for prolonged treatments [163,172]. Since DZP is easily absorbed through the BBB, its therapeutic concentration within CNS is rapidly achieved, which makes it a useful choice for the treatment of SE [172].

1.6.6 NON-LICENSED EU DRUGS SUGGESTED FOR USE IN THE TREATMENT OF IE IN DOGS

Other drugs have been suggested for the treatment of IE in dogs, however, there still is limited clinical and scientific evidence that could support the utilisation of these same drugs. These drugs are used in human medicine and some examples are zonisamide, felbamate, topiramate,

gabapentin and pregabalin [165]. Even though many studies have been conducted with the aim to clarify whether these drugs could be used in the treatment of IE in dogs, none of these studies could totally support their efficacy and safety [197–203]. Among these drugs, zonisamide is the only one that has been approved for the treatment of IE in dogs but it is only licensed in Japan [165]. According to the IVETF, zonisamide was approved as an add-on drug in cases where therapy with PB alone or together with KBr induces undesirable side effects [165,201,203]. This drug has the potential to decrease the PB dosage, therefore reducing some of its side effects [201]. There are fewer side effects associated with zonisamide. However, an increase of hepatic enzymes, apathy or ataxia may occur [201]. Zonisamide is known to have a half-life of 15 hours [50]. One disadvantage associated with the use of this drug is its high cost when compared with other drugs, which in chronic treatments may be a deciding factor given the owner's financial circumstances [201]. Zonisamide can be given orally every 12 hours, maintaining a good serum concentration between administrations [203].

1.7 ALTERNATIVE TREATMENTS

In humans, the ketogenic diet (KD) is one of the alternative treatments available to help control seizures. This diet is characterised for having an elevated percentage of fats (75-90%), decreased percentage in carbohydrates (5%) and moderate in proteins (10-20%) [204,205]. This diet exhibits anti-inflammatory properties that may help control seizures, mainly when inflammation is associated [204]. Thus, these anti-inflammatory properties decrease the interleukin (IL) 1B expression, which decreases inflammation response and therefore contributes to seizure control [204]. A low carbohydrates diet can be useful in the management of seizures and a study describe this diet as an alternative treatment to the medication that can have some unwanted side effects [206]. In one study, dogs fed with KD experienced a reduction of their seizure frequency when compared with dogs that were fed with regular commercial diets [207,208]. Also, the addition of KD as an add-on to PB treatment, in cases of recurrence, helped reduce seizures by 85% [209].

1.8 PROGNOSIS

The personal opinion of owners concerning their pet's QoL may influence the decision of euthanasia or not [25,210]. Owners are likely to choose euthanasia if there is a decrease in their pet's QoL [25,210]. QoL is one of the factors that may be crucial to the management of the disease, so cooperation from owners is crucial. Many owners may find it difficult to cope with the fact that their pets have an incurable disease that requires life-long treatment [25,210]. Some owners may not have financial conditions to ensure that adequate treatment is provided to their pets [210]. Thus, the

prognosis relies not only on the treatment success but also on owner's cooperation, economic status and veterinary expertise [25].

Some factors that influence the prognosis may include age at first seizure, frequency, treatment failure and the presence of SE or cluster seizures [30,128]. The prognosis is closely related to the number and frequency of seizures. The higher the number and frequency of seizures, the worse the prognosis [11]. According to Heske *et al.,* age is also a risk factor that influenced the survival-time and patients that had been belatedly diagnosed with IE had a shorter survival time following the diagnosis when compared with those whose epilepsy was diagnosed earlier [18]. As stated by Lengweiler *et al.,* most of the dogs responded well to the treatment if treated in the early stages [211]. The owner's economic status is another factor that may affect the prognosis of IE [20]. The management of IE from the diagnosis to the treatment can be very expensive and some owners may not be able to afford such expenses, which may influence the outcome if the protocol for diagnosis and treatment of IE is not followed.

In one study, dogs that had experienced cluster seizures were more likely to be resistant to AEDs, which worsens the prognosis in such cases [167]. According to Kwan *et al.,* a strong and positive response to the first AEDs administered, with proper management of seizures, may be an indicator of satisfactory prognosis [212]. Most of the euthanasia performed in epileptic dogs are related not only with treatment failure but also with the decrease of dog's health provoked by chronic treatment with AEDs [25]. SE is also a prognosis factor depending upon its manifestation, duration and aetiology [126]. According to Sanders, the prognosis is poorer in the following situations: dogs that experience a SE that last longer than six hours [129], dogs that have another episode of SE less than six hours after the first and dogs that have StE, especially tumours and inflammatory processes due to trauma or infection [126].

Dogs diagnosed with epilepsy may have a shorter life span when compared with healthy dogs. One study reported seven years as the median age for dogs with epilepsy [25]. Alternatively, Heske *et al.,* reported 1.5 years of median survival time following the diagnosis of epilepsy [18]. This study said that dogs kept as pets presented a higher median survival time when compared with dogs that were kept for other purposes such as hunting [18]. This finding was probably because owners might feel more emotionally connected with their companion pets than with pets that are kept for working/hobbies purposes.

1.9 HEMOGRAM

The hemogram is a diagnostic exam that evaluates, quantitatively and qualitatively, the main cells that constitute the blood: red blood cells (RBC), WBC and platelets (PLT). The evaluation of these parameters should not be interpreted alone but together with other diagnostic tests and physical examination. Through hemogram it is possible to evaluate the RBC, the WBC count, the haemoglobin (HGB), the haematocrit (HCT), red cells indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) mean corpuscular haemoglobin concentration (MCHC) and red cell distribution width (RDW), PLT and PLT indices such as mean platelet volume (MPV) platelet distribution width (PDW) and plateletcrit (PCT).

Each of these parameters has different reference values that may vary between blood analysers and species [213] . Other factors like age, diet and environment may influence these values between patients [213], thus the importance of having reference values for blood parameters. All these cells originate from the haemopoietic cells, that are produced in the bone marrow [214]. Within the bone marrow there are two different types of cell lines: myeloid line and lymphoid line [215]. The myeloid line consists of granulocytes cells (neutrophils, basophils and eosinophils), monocytes, macrophages, erythrocytes, megakaryocytes and mastocytes [215]. The lymphoid line consists of lymphocytes B, T and natural killer cells (NK cells) [215].

The hemogram gives information about primary diseases that directly affect the bone marrow but also expresses haematological abnormalities resulting from other processes such as infection, inflammation, toxicity or neoplasia [216]. As hemogram parameters are affected by these factors, it is expected that some of these parameters may work as possible biomarkers for some diseases.

1.10 HEMOGRAM PARAMETERS, INFLAMMATION AND SEIZURES

Red cell distribution width (RDW) refers to the size disparity of erythrocytes. A human study suggests there might be a correlation between the RDW and inflammation since this parameter was increased in many cases of inflammatory diseases such as systemic lupus erythematosus or Crohn's disease [217]. As said by Gurler and Aktas, the correlation between the RDW and inflammation might be due to the fact that, during inflammation, inflammatory cytokines are released within the blood circulation, therefore causing changes in erythropoiesis in bone marrow [217]. Consequently, it results in RBC of different dimensions [217].

Also, during inflammation, PLT become activated and there is a tendency for these activated PLT to be larger than non-activated PLT, hence an increased MPV [217].

During inflammation, WBC are activated and recruited from the bone marrow and circulation to the damaged foci [218]. The Neutrophils-Lymphocytes Ratio (NLR) may also be an indicator of inflammation because it is associated with oxidative stress and cytokines production [218]. In one study in humans, more than one third of patients with generalised seizures had a significant increase of WBC following seizures and a relationship between duration and elevation of WBC was detected, so the longer the seizure, the higher the WBC [219]. In addition, the elevation of WBC was temporary and strictly related to the seizure [219]. Another study in humans also found a temporary increase of lymphocytes and neutrophils in epileptic patients following a seizure, which persisted for 24 hours [220]. According to Sarkis *et al.,* a higher WBC and monocyte count was found in patients with generalised seizures compared to those with focal seizures [221].

Finally, another study in humans that evaluated the relationship between EEG and hemogram found an increase of WBC and neutrophils in patients that shown abnormalities in the EEG [222]. This finding emphasises that seizures may alternate the hemogram parameters by inducing an inflammatory cascade following the epileptic event.

1.11 NEUROINFLAMMATION

The neuronal environment is known for being a unique and privileged for having its own immune mechanisms, distinct from the rest of the body [223]. According to Platt, the neuronal environment can be considered as immune-privileged due to the existence of a BBB, absence of a conventional lymphatic systemic and limited transferring of peripheral immune cells from the blood to the brain [223]. Neuroinflammation may be defined as an inflammatory process within the CNS [224]. Many different cells are involved in the neuronal immune response such as microglia, astrocytes, neurons, endothelial cells presented in the BBB and peripheral immune cells that cross the BBB [223,225,226]. All these cells have the capacity of producing and releasing inflammatory mediators as well as upregulating their respective receptors [223,225,226]. Any damage inflicted directly or indirectly to the brain induces acute inflammation, which may evolve to chronic inflammation when these mechanisms become exacerbated and prolonged [2]. During chronic neuroinflammation there is an exacerbated release of cytokines and other inflammatory mediators that may cause neuronal injury and consequently neuronal death [2].

The fact that seizures may trigger inflammation and recurrent seizures may lead to an underlying state of chronic inflammation evidence a firm relationship between these processes. In fact, one study suggested that inflammation can lead to epilepsy and vice versa, so both may be intimately related [223]. As inflammation is a physiological mechanism to ensure protection against threatening situations, it is not surprising that inflammation is triggered after a seizure episode. For many years the relationship between epilepsy and neuroinflammation has been widely discussed by

many neuroscientists around the world. The fact that inflammation may trigger seizures and vice versa [2], prompted neuroscientists to carry out several studies with the aim to understand the pathophysiology behind it [74,100,227–229]. A study in humans found a relationship between neuroinflammation and some neurological diseases such as Alzheimer disease and multiple sclerosis [230].

In the brain, the inflammatory response is initiated by resident cells of CNS known as glial cells [229]. These glial cells are involved in neuronal modulation of injured neurons, production of myelin, buffering of electrolytes/neurotransmitters and play an essential role in modulating the immune response in the brain [229,231]. Within the glial cells, astrocytes and microglia are key role to neuroinflammation [229,232,233]. Microglia cells are mainly responsible for innate immunity in the brain, but astrocytes and neurons are involved as well [233]. According to DiSabato *et al.,* microglial cells are presented in both grey and white matter of the CNS*,* representing at least 10% of the total CNS cell population [229]. Microglial cells are typically activated when a noxious stimulus like infection, inflammation and trauma occurs [234], releasing inflammatory mediators such as cytokines or chemokines [229,232]. Cytokines such as ILs, tumour necrosis factors (TNFs) and transforming growth factor (TGF) are some inflammation mediators released by glial cells, following epileptic episodes [2,235]. IL-1β, IL-6 and TNF-α are known for being the most important inflammatory mediators [236,237]. As stated by Platt, there are many mechanisms of regulation for cytokines, that include gene transcription, cleavage of their precursors by proteolytic enzymes, cellular release and through receptors [223]. Microglial cells have phagocytic capacity and are able to phagocyte apoptotic neuronal cells to ensure the normal proliferation of neurons and remove toxins deleterious to the brain [236,238]. However, the perpetuation of microglial activity may lead to dysregulation of their normal function. When overstimulated, they have the potential to release reactive oxygen species, nitric oxide and inflammatory mediators that, when overconcentrated, may be neurotoxic [234,239,240].

Astrocytes are CNS cells that allow a connection between neuronal and glial cells and regulate the synaptic transmission by releasing chemical transmitters [241] that were involved in the activation, differentiation and morphology of microglial cells [239]. Accordingly, astrocytes produce neurotrophic factors that regulate the activity of microglial cells, providing nutrition and protection [242]. When activated by a noxious stimulus, astrocytes become activated and move to the damaged foci. Consequently, astrocytes induce the release of cytokines and chemokines that are responsible for the activation and recruitment of microglial cells [243]. Astrocytes also have the capacity of producing nitric oxide and reactive oxygen species that are harmful for the normal neuronal regeneration after injury, therefore contributing to the neuroinflammation [244].

Neuroinflammation may also lead to the recruitment of peripheral inflammatory cells [245]. Chemokines work as chemo-attractants for peripheral leucocytes [232,246], that move throughout a damaged BBB. Chemokines have an important role in regulating microglial cells as well as

promoting different mechanisms such as angiogenesis and neurogenesis [247,248]. The BBB is a structure composed mainly by endothelial cells and it is first-line protection of the brain against injury and pathogens [231]. These components regulate whether some blood elements can move from blood circulation into vessels and capillaries that are directly related to the brain [231]. BBB can breakdown during inflammation, changing its permeability and allowing pathogens to cross the BBB and reach the brain [229]. This increase in permeability is mainly due to the action of cytokines and consequently it allows proteins like albumin and other cells like leucocytes to circulate from the peripheral blood to the neuronal blood circulation [229,249]. Generally, inflammation starts in endothelial cells in the brain as a consequence of seizures and mostly cytokines are upregulated by perivascular glia [223].

Many studies found a correlation between seizures and inflammation. During SE, the exaggerated electric stimulation activated glial cells, causing an upregulation of proinflammatory mediators such as IL-1B, IL-6 and TNF-α [2,235,249]. Generally, inflammatory mediators persist in low concentrations in a healthy neuronal environment, but these concentrations can easily increase when inflammation is triggered [236]. In other study, some hallmarks of chronic inflammation like reactive gliosis and overconcentration of cytokines and chemokines have been found in the resected brain from epileptic patients, which emphasises the role of neuroinflammation in epilepsy [232]. A study conducted by Sakurai *et al.,* reported evidence of neuronal death induced by seizures in cerebral tissue from dogs with epilepsy [250]. This neuronal death was probably related to abnormal concentrations of inflammatory mediators such as TNF-α and IL-6 that were identified in these cerebral tissues [250].

This finding suggests that seizures are responsible for the initiation of neuroinflammation that can persist and evolve to chronic inflammation. The latter, in advanced stages, may be responsible for neuronal death and neurodegeneration [240,250]. In a study by Bartels *et al.,* levels of inflammatory cytokines in the CSF were significantly higher in dogs with IE than in healthy dogs, which emphasises the role of inflammation in the pathogenesis of IE [251]. Similarly, high concentrations of TNF-α and IL-6 were found in the CSF of dogs with IE [252].

2. STUDY – EXPLORING THE IMPACT OF CANINE IDIOPATHIC EPILEPSY ON HEMOGRAM PARAMETERS

2.1. INTRODUCTION

IE is known to be the most common neurological disease in dogs and humans [1,2,155]. IE decreases the QoL of epileptic dogs when not treated, so adequate treatment with AEDs should be applied and formulated for each patient [210]. Several AEDs are available for use in the treatment of IE but none of them provide cessation of epileptogenesis but instead only prevent seizures from occurring. Nonetheless, the relationship between epilepsy and inflammation has been discussed by many neuroscientists around the world and it is known that inflammation can trigger seizures and vice-versa [74,227,230]. It is also well-known that hemogram can give valuable information regarding inflammatory processes in the organism, which makes it a useful tool in the diagnosis of several diseases.

2.2. OBJECTIVE

The focal objective of this study is to understand if there are relevant changes in the hemogram of epileptic dogs and whether these possible changes are related with inflammation. For this purpose, hemogram parameters of dogs who had experienced at least one seizure 24 hours before blood collection were evaluated and compared with non-peri-ictal values and with healthy animals, with the aim of detecting possible abnormalities in the hemogram related to inflammation.

2.3. MATERIALS AND METHODS

At the beginning of the study, three groups were hypothetically created. The control group or C1 would be constituted by healthy dogs. The epileptic dogs included in this study will further be include on the epileptic group, divided into two different subgroups according to their chronological distribution. The epilepsy group after seizure would be listed as group E2 and the free-seizure epilepsy group would be listed as group E3. In order to be included in group E3, the selected epileptic dogs could not have had any seizure in the last three days prior to the blood collection. The interval of three days was selected based on half-life of neutrophils, which are closely related with inflammation.

A total of 22 dogs were enrolled in this study between November 5th 2018 to April 5th 2019. From these 22 dogs, 16 were purebred (16/22) and six were cross-breed (6/22). Within purebred dogs, there were six Labrador Retrievers, two French Bulldogs, three Estrela Mountain dogs and one of the following breeds: Yorkshire Terrier, Chihuahua, Maltese, Pinscher and Boxer. From those 22 dogs, 13 were male (59%) and nine were female (41%). The median age was 2.45 years (range 0.5-7.5 years). A total of 14 dogs (14/22) were included in the control group. From these 14 dogs, six were male (6/14) and eight were female (8/14). Selected dogs to be included in the control group were dogs that had been admitted to the hospital to realise elective surgeries such as neutering. All these dogs were healthy with a normal physical examination and there were no abnormalities in both hemogram and serum chemistry.

Eight of these 22 dogs (8/22) were included in the epilepsy group and seven of those dogs were male (7/8) and one was female (1/8). The eight dogs englobed in the epilepsy group had approximately a tier II level of confidence for the diagnosis of epilepsy. According to De Risio *et al.,* a level of tier II confidence includes a history of two or more seizures, dogs aged between six months and six years, unremarkable interictal physical and neurologic examinations and undetectable abnormalities on hemogram, serum chemistry, urinalysis, fasting and post-prandial bile acids as well in the MRI [139]. However, no measurement of fasting and post-prandial bile acids was performed since there were no detectable abnormalities on hemogram, serum chemistry and physical examination that could justify the necessity for such exams. The urinalysis was not also performed for the same reason. Another reason that justifies the absence of these exams was the financial constraints of owners, which could have had a negative impact on both diagnosis and treatment. All the eight dogs enrolled in the epileptic group have performed an abdominal ultrasonography. Out of the eight dogs, only two dogs had undergone an MRI and six had undergone a CT-scan because the latter is a cheaper imaging diagnostic exam. In the absence of abnormalities found in either a CT-scan or an MRI, analysis of CSF would not be performed.

Some dogs have been excluded from this study since they were not suitable for the epileptic group due to a variety of reasons such as insufficient level of tier confidence, lack of collaboration from owners or the presumptive diagnosis was other than IE (e.g. distemper, intoxication and brain tumour) or concomitant diseases (e.g. chronic hepatitis).

All epileptic dogs included in this study were medicated with appropriate AEDs and all them started treatment with PB. In two of these eight dogs, it was necessary to add an additional AED, with one undergoing a combined therapy with KBr and another one with levetiracetam. All blood samples related to the subgroup E3 were collected during the treatment with AEDs.

2.3.1. BLOOD COLLECTION AND HEMOGRAM PARAMETERS

All blood samples for hemogram were collected by jugular venepuncture with a sterile, disposable 23-gauge needle and 2mL syringe. Prior to blood collection, the puncture zone was shaved and disinfected with a pad impregnated with water-diluted 1% chlorhexidine. Alcohol was generally avoided since some animals are sensitive to it and alcohol may have led to an allergic reaction. Therefore, the blood sample collected was put onto a purple EDTA-tube containing 2% of K2 Ethylenediaminetetraacetic Acid (EDTA). K2EDTA is an anticoagulant widely used and it is a good choice for blood analyses since it does not significantly change the blood parameters [253]. Hemogram was performed straightway after blood collection to avoid alterations in the results, especially due to haemolysis. Hemogram was obtained from a BC-2800Vet auto haematology analyser, at the hospital laboratory (Table 7). This analyser is adapted for veterinary use and allows the evaluation of blood parameters of different species and it only requires 0,5 mL of the blood sample.

Table 7: Reference blood parameters for dogs in a bc-2800 Vet Analyser.

PARAMETERS	VALUES
White Blood Count (WBC)	6.0 - 17.0
Lymphocytes	0.8 - 5.1
Monocytes	0.0 - 1.8
Granulocytes	4.0 - 12.6
Lymphocytes %	12.0 - 30.0
Monocytes %	2.0 - 9.0
Granulocytes %	60.0 - 83.0
Red Blood Count (RBC)	5.50 - 8.50
Hemoglobin (HGB)	110 - 190
Hematocrit (HCT)	39.0 - 56.0
Mean Corpuscular Volume (MCV)	62.0 - 72.0
Mean Corpuscular Hemoglobin (MCH)	20.0 - 25.0
Mean Corpuscular Hemoglobin Concentration (MCHC)	300 - 380
RDW (Red Distribution)	11.0 - 15.5
PLT (Platelets)	117 - 460
MPV (Mean Platelets Volume)	7.0 - 12.9

2.3.2. QUESTIONNAIRE

The questionnaire was based on the available literature about precipitating/risk factors associated with IE in dogs, as well as characterisation of seizures [8,25,39,47]. The questionnaire comprised of 17 questions of different categories: yes/no, multiple choice and short answers. The owners voluntarily answered the questionnaire and a face-to-face dialogue between the veterinarian, student and owner was established to obtain all the necessary information regarding each selected epileptic dog. Most of the owners have replied to the questionnaire while undergoing a check-up appointment, while others have gone to the hospital by request of their veterinarian, who offered a free appointment in exchange for their participation. Only seven from the eight owners of dogs with IE have completed the questionnaire. One owner left some answers regarding seizure's characterisation blank because he did not witness any of the episodes.

2.3.3. STATISTICAL ANALYSIS

The dependent variables evaluated in this study were WBC, lymphocytes, monocytes, granulocytes, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, PDW and PCT while the independent variables were the occurrence of a seizure or not. The SPSS software was used for the statistical analysis. Non-parametric tests have been chosen due to the small size of our samples. For independent samples, the Mann-Whitney test was used whereas the Wilcoxon Ranked test was used for dependent samples. Our level of confidence was settled to 0.05 and any value equal or lower to 0.05 would reject the null hypothesis.

2.4. RESULTS

2.4.1. AGE, BREED, SEX AND WEIGHT

In the epilepsy group, six of the eight dogs were under 20 kg (Figure 5). These results agree with their breed because most of the epileptic dogs were considered as small to medium breed whereas within the control group, 10 of the 14 dogs were considered as a large breed.

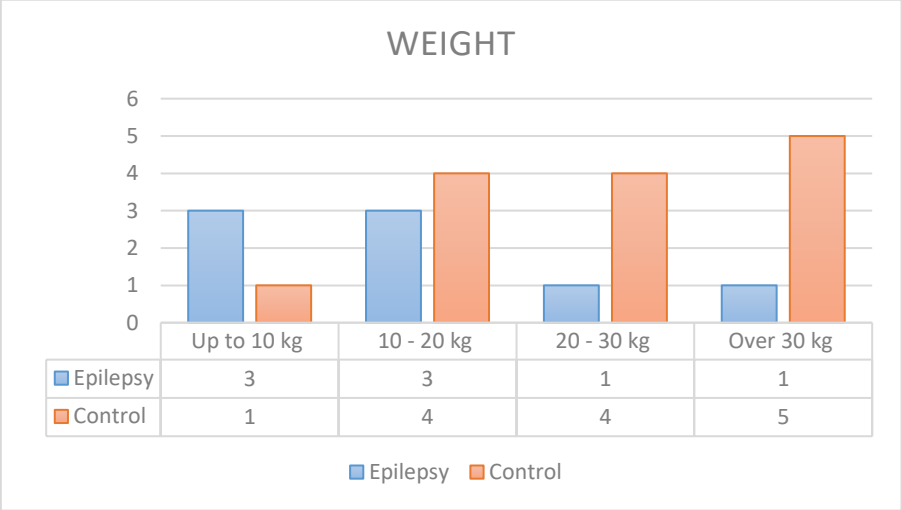

Figure 5: Characterization of the variable weight in both epileptic and control groups.

From the 22 dogs englobed in this study, 13 dogs (13/22) were male while the other nine were female (9/22) (Figure 6). Within the epilepsy group, the incidence of male dogs was higher (7/8) than the female (1/8).

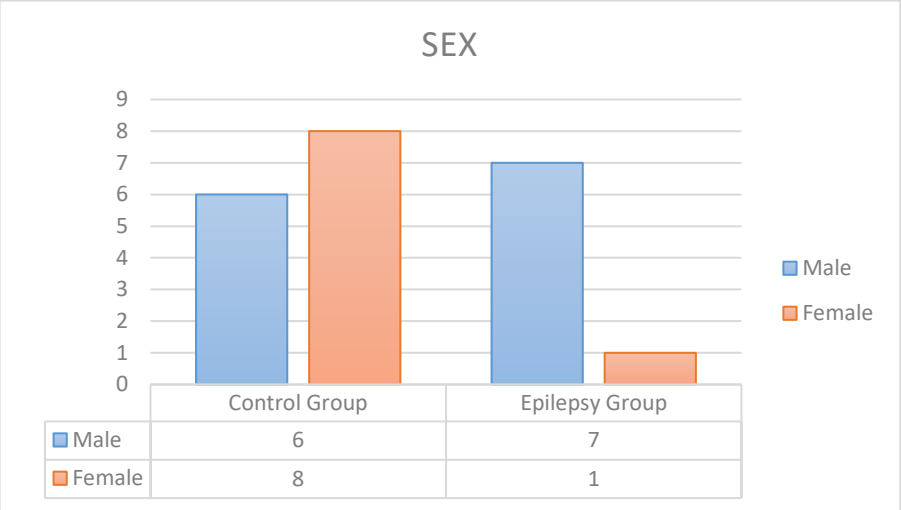

Figure 6: Characterization of the variable sex in both epileptic and control groups.

From the 22 dogs enrolled in this study, 16 were pure breed (16/22). From these 16 pure-breed dogs, seven of them belonged to the epilepsy group (7/16). There is heterogeneity in breeds regarding epilepsy group, probably due to the small size of this population (Figure 7). The most common breed was Labrador Retriever (6/16) followed by the Estrela Mountain dog (3/16).

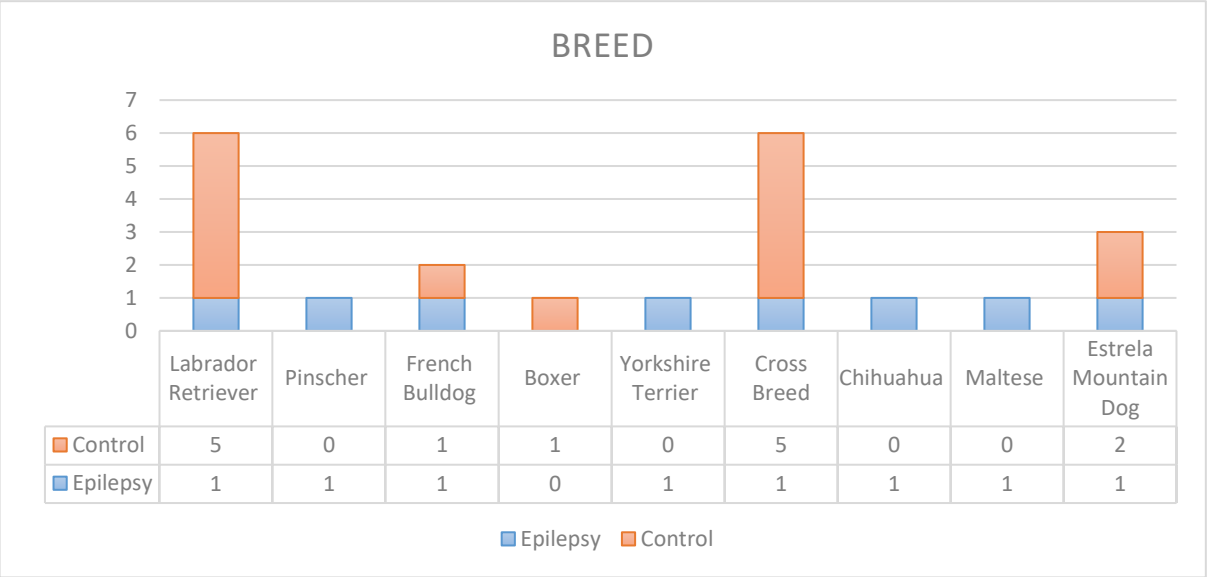

Figure 7: Characterization of the variable breed in both epileptic and control groups.

In what concerns to the age at first seizure, most of the epileptic dogs experienced their first seizure between one year and five years old (Figure 8). One of these dogs experienced its first seizure earlier whereas two dogs had experienced their first seizure after their five years of age.

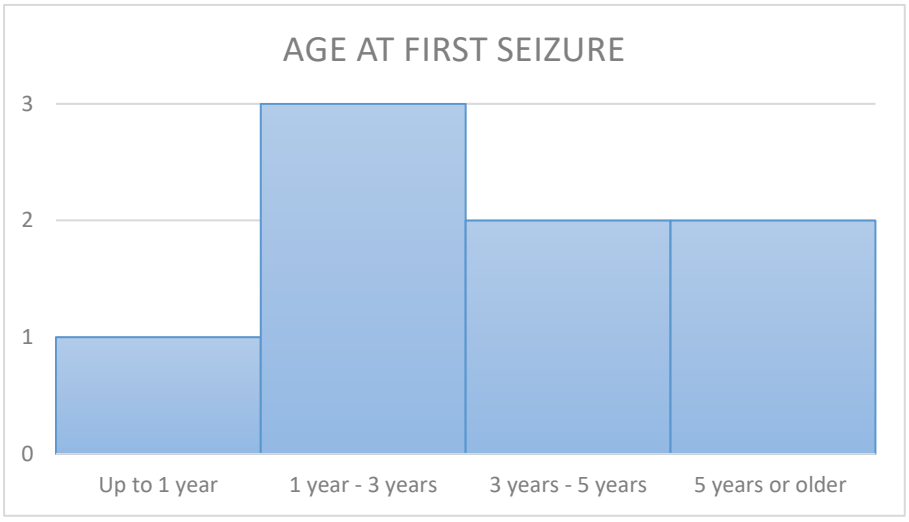

Figure 8: Age at the first seizure in the epileptic group.

2.4.2. QUESTIONAIRE

Regarding the signalment of the patient, none of the owners reported a family history of epilepsy. Similarly, none of the owners reported any trauma nor infectious/inflammatory/metabolic disease before the onset of seizures. One of the owners reported a reaction to the vaccination as possible trigger of seizures in his dog. Finally, none of the owners reported access to possible toxins or poisons but two of the eight epileptic dogs had access to the garden.

Considering the duration of seizures, all owners reported that most of the epileptic episodes were shorter than five minutes (Figure 9).

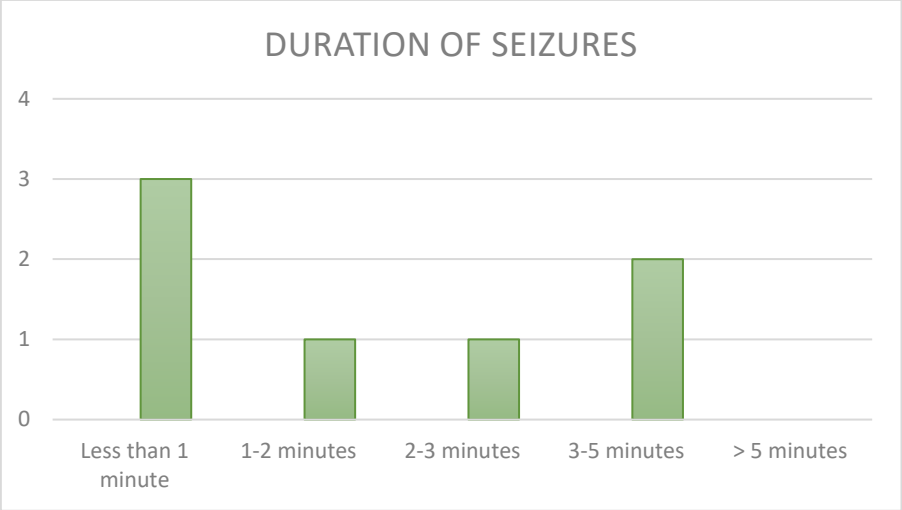

Figure 9: Duration of seizures in the epileptic group.

Regarding the frequency of seizures, three of the owners reported a frequency of once per month while the other three reported seizuring events every six months. Only one owner reported a seizure per week (Figure 10). Two of the owners reported an increase in seizure's frequency after the first onset of seizures.

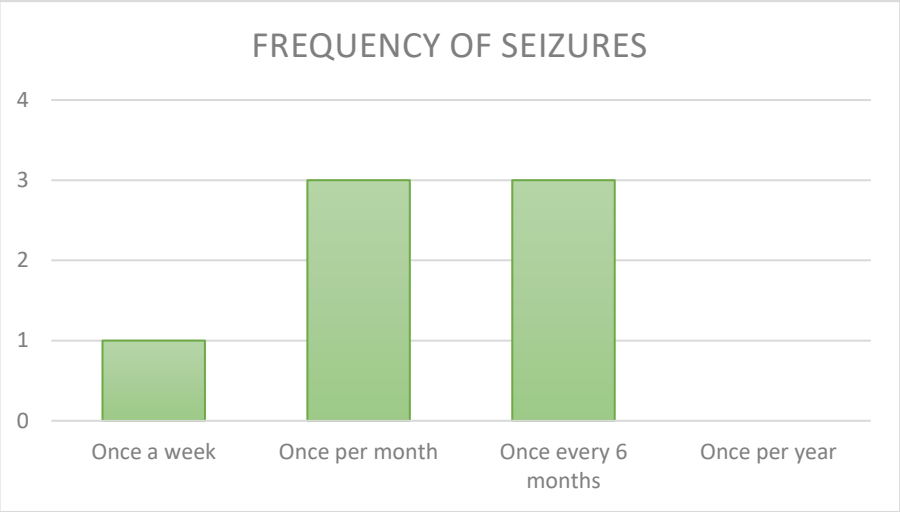

Figure 10: Frequency of seizures in the epileptic group.

Four owners interviewed described a higher incidence of seizures while their dogs were sleeping/resting or straight after they awoke.

Only two owners reported possible precipitating factors that possibly triggered seizures in their dogs. One of the owners stated that the onset of seizures was preceded by a change in the usual diet while another owner said that seizures occurred after moving to a new house and after using the vacuum cleaner.

Loss of consciousness during the epileptic episode was reported by all owners in this study. The fact that all owners reported loss of consciousness together with a hard muscle tone, emphasises the hypothesis that all dogs enrolled in this study had suffered generalised seizures. Only two owners noted behaviour abnormalities following the epileptic episode. Both owners reported confusion and not recognising the owner/surrounding space as the main identifiable changes in the normal behaviour of their pets.

When asked to write a brief description of the epileptic event, three of the owners described that their dogs were shaking during the episode, while the other four owners reported that their dogs were stiff. All owners have identified their dog's muscle tone as rigid/hard. One of the owners observed pedalling/jerking movements during the episode and another one noticed that his dog had his tongue out of his mouth. One of the owners reported an improvement in the clinical manifestation of the seizures after starting the treatment. Initially, the seizures were classified as aggressive and autonomic signs were remarkedly visible whereas after initiating the treatment, the seizures stopped or were practically unnoticed.

Most of the owners noted some autonomic signs associated with the epileptic episode (Figure 11). The most-reported autonomic sign was hypersalivation (6/7), followed by urination (2/7). Dilation of pupils, vomit and defecation was reported only once (1/7). Some owners reported more than one autonomic sign in their dog and one of the owners did not report any autonomic sign.

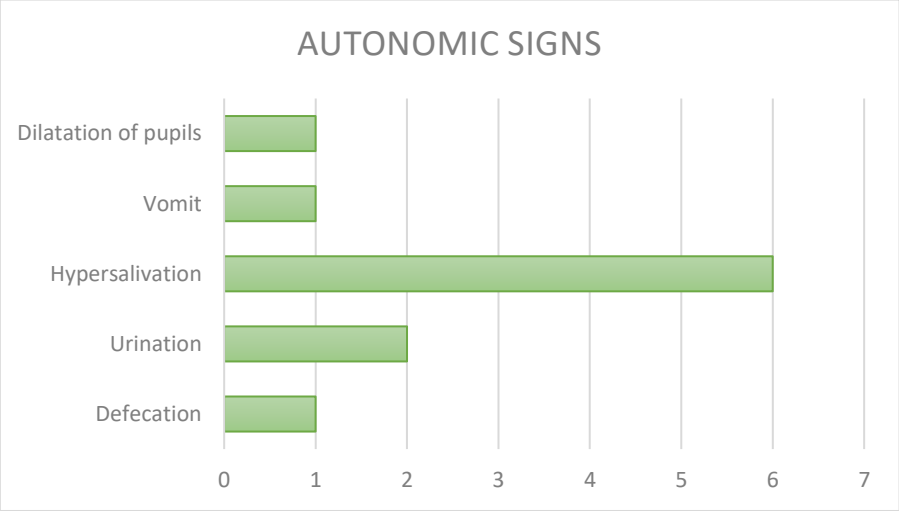

Figure 11: Autonomic signs observed in the epileptic group.

2.4.3. HEMOGRAM PARAMETERS

MCHC was significantly higher in the group C1 compared with the group E2 (p-value <0.05). On the other hand, RDW was significantly higher in the group E2 compared with the group C1 (p-value < 0.005). The median of RDW was higher in the group E2 but an outliner was observed (Figure 12). The medians of WBC and granulocytes were higher in the group E2 whereas the median of lymphocytes was higher in the group C1 (Table 8).

Table 8: Comparison of hemogram parameters between the group control and epilepsy group after seizure.

Mann-Whitney test	**C1 (n=14)**	**E2 (n=8)**	**p-value**
	Median ± *SD*		
WBC	11.2 (8-15.9)	12.9 (5.6 – 16.5)	0.707
LYMP	2.05 (1-3.6)	1.95 (0.8 – 2.6)	0.338
MON	0.5 (0.3 – 0.6)	0.4 (0.2-0.6)	0.550
GRAN	8.6 (4.5 – 12.9)	10 (4.6 – 13.8)	0.561
RBC	7.6 (5.63-10)	7.805 (6.96-8.79)	0.393
HGB	180 (145-198)	182 (144-209)	0.758
HCT	56.85 (44.9 – 61.6)	59.3 (48.1-66.1)	0.161
MCV	73.75 (71.3-80.2)	75.3 (69.2-78.4)	0.584
MCH	23.85 (21.5-25.8)	23.35 (20.6-24.5)	0.193
MCHC	317.5 (296-342)	308 (294-316)	0.018
RDW	14.05 (9.7-15.3)	15.35 (10.5-15.9)	0.015
PLT	403.5 (125-581)	361.5 (269-536)	0.473
MPV	8.55 (7.8-9.8)	8.85 (7.3-10.7)	0.706
PDW	16.3 (16-17)	16.4 (16.1-17.3)	0.679
PCT	0.3475 (0.102-0.48)	0.32 (0.24-0.41)	0.682

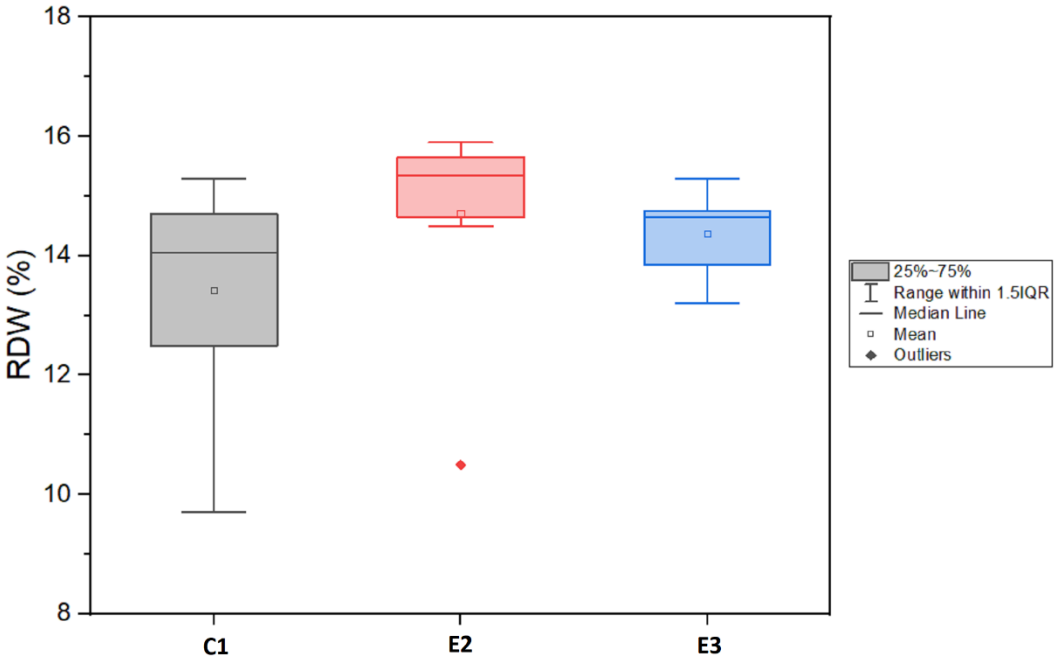

Figure 12: RDW absolute values distribution in the three groups.

There were no significant statistical differences between parameters from the group C1 and the group E3. The median of lymphocytes was slightly higher in the group E3 compared with the group C1. The HBG and HCT medians were also higher in the group E3 whereas the medians of red cells indices like MCV, MCH and MCHC were higher in the group C1 (Table 9).

Table 9: Comparison of hemogram parameters between the control group and free-seizure epilepsy group.

Mann-Whitney test	C1 (n=14)	E3 (n=8)	p-value
	Median ± *SD*		
WBC	11.2 (8-15.9)	11.4 (8.2-15.6)	0.972
LYMP	2.05 (1-3.6)	3 (1.3 – 4)	0.150
MON	0.5 (0.3 – 0.6)	0.5 (0.3-0.6)	0.887
GRAN	8.6 (4.5 – 12.9)	7.3 (6.5 – 12.2)	0.339
RBC	7.6 (5.63-10)	8.075 (6.33 – 8.87)	0.133
HGB	180 (145-198)	194 (141-208)	0.132
HCT	56.85 (44.9 – 61.6)	58.15 (45.3-66.1)	0.494
MCV	73.75 (71.3-80.2)	72.4 (69.4-79.7)	0.231
MCH	23.85 (21.5-25.8)	23.7 (22.2-24.8)	0.452
MCHC	317.5 (296-342)	314.5 (311-342)	0.891
RDW	14.05 (9.7-15.3)	14.65 (13.2-15.3)	0.192
PLT	403.5 (125-581)	423.5 (292-548)	0.538
MPV	8.55 (7.8-9.8)	9 (8.6-9.9)	0.055
PDW	16.3 (16-17)	16.55 (16.1-17)	0.192
PCT	0.3475 (0.102-0.48)	0.4 (0.26-0.53)	0.356

The lymphocytes were significantly higher in the E3 group compared with the E2 group (p-value < 0.05). The median of lymphocytes was smaller in the group E2 and the dispersion of values is higher in the E3 group (Figure 13). The medians of WBC and granulocytes from the E2 group after seizure were higher when compared with the E3 group. The median of RBC and HGB were significantly higher in the E3 group but the median of HCT was higher in the E2 group (Table 10).

Table 10: Comparison of hemogram parameters between epilepsy group after seizure and free-seizure epilepsy group.

Wilcoxon Ranked Test	**E2 (n=8)**	**E3 (n=8)**	**p-value**
	Median ± *SD*		
WBC	12.9 (5.6 – 16.5)	11.4 (8.2 – 15.6)	0.484
LYMP	1.95 (0.8 – 2.6)	3 (1.3 – 4)	0.021
MON	0.4 (0.2 – 0.6)	0.5 (0.3 – 0.6)	0.340
GRAN	10 (4.6 – 13.8)	7.3 (6.5 – 12.2)	0.483
RBC	7.805 (6.96 – 8.79)	8.075 (6.33 – 8.87)	0.889
HGB	182 (144 – 209)	194 (141 – 208)	0.207
HCT	59.3 (48.1 – 66.1)	58.15 (45.3 – 66.1)	0.484
MCV	75.3 (69.2 – 78.4)	72.4 (69.4–79.7)	0.674
MCH	23.35 (20.6 – 24.5)	23.7 (22.2 – 24.8)	0.123
MCHC	308 (294 – 316)	314.5 (311 – 342)	0.630
RDW	15.35 (10.5 – 15.9)	14.65 (13.2 – 15.3)	0.262
PLT	361.5 (269 – 536)	423.5 (292 – 548)	0.237
MPV	8.85 (7.3 – 10.7)	9 (8.6 – 9.9)	0.233
PDW	16.4 (16.1 – 17.3)	16.55 (16.1 – 17)	0.725
PCT	0.32 (0.24 – 0.41)	0.4 (0.26 – 0.53)	0.068

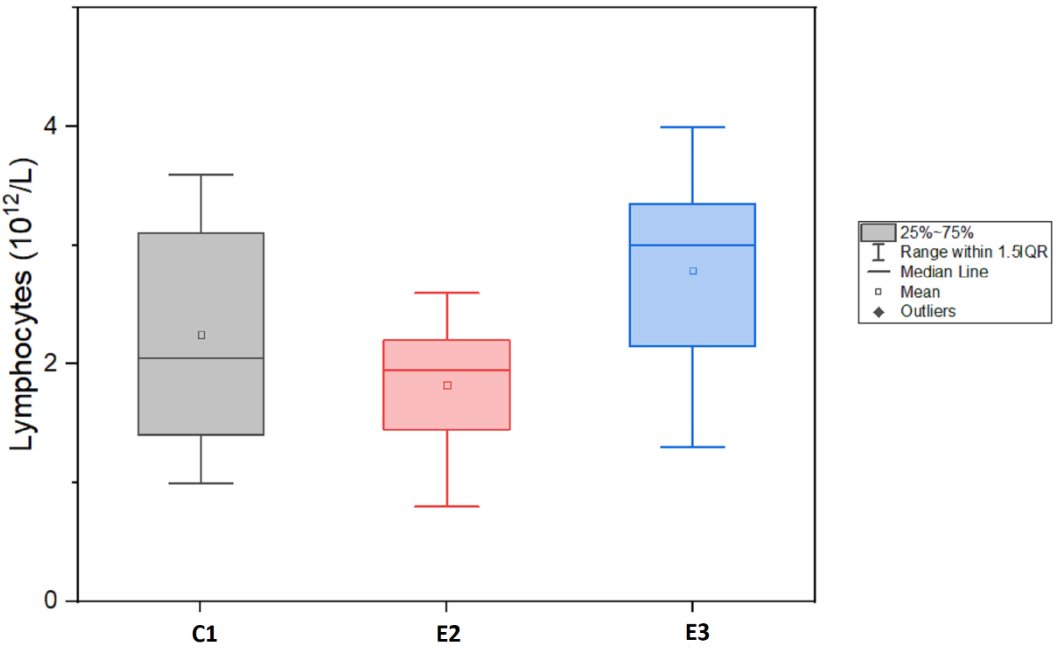

Figure 13: Lymphocytes absolute values distribution in the three groups.

2.5. DISCUSSION

The results obtained in this study demonstrate few statistically significant differences between groups, yet these results are interesting. In addition, the findings that are not deemed to be statistically significant are still worth considering.

In the study, most of the dogs included in the epilepsy group were small breed dogs. This differs from what has been discussed in a study by Podell *et al*. that found a higher prevalence of IE in medium to large breeds, mostly over 15 kg [16]. However, these results may be related to the popularity of small to medium breeds in Portugal, especially French Bulldogs, Yorkshire Terriers and Chihuahuas.

Different breeds were included in the epilepsy group but despite this heterogeneity, most of the breeds observed in the epilepsy group have been previously described as predisposed to IE. Some of these breeds include the Labrador Retriever [18,23,26], and the Chihuahua [15]. Other breeds like Maltese dog, Yorkshire Terrier and French Bulldog are not described as predisposed to IE, but are associated with other diseases that may mimic seizures like hydrocephalus [254]. Additionally, pinscher dogs have been previously associated with cataplexy [254]. The Estrela Mountain dog has only been associated to a higher predisposition for hip dysplasia [255] and there is a lack of information regarding other diseases in this breed. Given it is a common breed in Portugal, this increases the likelihood of finding this breed in a clinical setting. A study conducted by Kearsley-Fleet *et al.,* investigated the possible role of coat-colour type and coat length as risk factors for IE [20]. A statistically significant association was found between long-coat/multicolour-coat and IE. Whether these results suffer from a bias effect is not clear. No other studies have analysed the relationship between coat's characteristics and IE.

Regarding gender, almost all dogs included in the epilepsy group were male. Three of these eight dogs were neutered and all the neutered dogs were male. These results are in accordance with other studies, where the proportion of male dogs with IE was higher [15,23,24]. Notwithstanding, the relationship between neutering and IE remains unclear. One study reported that neutered female dogs were less likely to have seizures than non-neutered female dogs [23,42], while another study reported that neutered male dogs with IE had a shorter survival time compared with non-neutered male dogs [256]. Nonetheless, the relationship between gender and IE is still unknown and many believe that sex hormones like testosterone may be involved. The higher incidence of females in the control group may be explained by the fact that most of owners wanted to neuter their bitches, thereby avoiding undesirable pregnancies.

Most of the dogs included in the epilepsy group experienced their first seizure within their first to fifth year of age. This finding is in agreement with other studies, which reported the likelihood of diagnosing IE in dogs aged between six months to six years old [33,34]. The fact that most dogs

diagnosed with IE are young makes the diagnosis of StE less likely. Also, all dogs included in the epilepsy group underwent an MRI or a CT-scan and none structural anomalies in the brain were detected. According to Smith *et al.,* the prevalence of clinically relevant abnormalities detected from an MRI is relatively low in animals younger than six years old [34].

None of the owners reported a family history of epilepsy. However, this does not exclude the possibility of genetic factors associated with the epileptic dogs since owners may not have access or be aware of that information. One of the owners reported a reaction to the vaccination who may have triggered seizures in his dog, however there are no studies in veterinary literature reporting such a situation. Nonetheless, seizures induced by vaccination have been reported in humans, which might be explained by physiological stress induced by the immunological response [257]. Therefore, in this case, vaccination might be seen as a precipitating factor.

All owners reported that most of epileptic events were not longer than five minutes and this finding is comparable to what has already been reported by Berendt and Gram that most of the seizures last up to 2 minutes [117]. In other study, Heynold *et al.* reported a mean duration of three minutes and a half [44].

Three owners reported seizure frequency of once per month while the other three owners reported a seizure every six months. This is similar to what was been found in a study by Heynold *et al.,* where the reported average frequency was one seizure every 65 days [44] and in another study by Jaggy and Bernardini, the frequency of seizures ranged between two seizures every day to one every six months. However, most of the dogs had less than one seizure per month [37]. Only one owner reported a seizure per week and as previously said, this was related with the fact that his dog was recently diagnosed.

Two owners reported an increase of frequency after the first episode. An increase in frequency after the first seizure has been reported in some studies, but mostly related to either treatment failure [258] or absence of treatment. A reasonable explanation may be the fact that sometimes the time between diagnosis and initiation of treatment is longer, possibly due to economic restraints that prevent the realisation of more specific exams such as a CT-scan or an MRI.

Four owners described a higher incidence of seizures during sleeping/resting or straight after awaking. This agrees with what has already been described by Pákozdy *et al.,* who said that a diagnosis of IE was likely when dogs experienced seizures during their period of rest [35]. Also, Podell *et al.,* said that, in a non-referral-based population, most of the seizures occurred between midnight and 8 am, which includes some moments of resting and sleep [16].

Only two owners identified possible precipitating factors that could have possibly triggered seizures in their dogs. These precipitating factors are not completely understood but have already been reported in some studies and are mainly associated with stress [39]. Stress changes the normal homeostasis, leading to a response of the organism to this imbalance [259]. Any situation

that leads to physiological stress may have the potential to trigger seizures. Precipitating factors may vary between dogs, depending upon their temperament, age, sex, among others. In a study by Forsgard *et al.,* owners reported stress as being the most common precipitating factors that were observable [39]. In the same study, changes in the normal daily routine were another common precipitating factor identified by owners, which agrees with what was reported by one of the owners in this study.

All owners reported loss of consciousness during the epileptic episode. In a study by Heynold *et al.,* 41 of the 49 dogs enrolled lost their consciousness during the epileptic episode [44]. In another study by Jaggy and Bernardini, 75% of the dogs with generalised seizures lost their consciousness [37]. Loss of consciousness is generally associated with generalised seizures and, in some focal seizures, consciousness may be unimpaired [1]. Whether consciousness is impaired or not, is difficult to assess since dogs do not express their feelings about their experiences. Also, owners may misunderstand those signs and think that their dogs are conscious when they are not and vice-versa. For this reason, the IVETF downplay the importance of evaluating consciousness in veterinary medicine. In the questionnaire, all the owners described their dog's muscle tone as rigid/hard during the epileptic episode. During generalised seizures, which are known to be the most common presentation in dogs, the tonic phase is characterised by a generalised increase in muscular tone as a consequence of multiple electrical discharges during the episode [1].

The most common autonomic sign identified was hypersalivation, followed by urination. This agrees with what was already been found in other studies, which identified these autonomic signs as common [1,9,139].

From the eight dogs included in the epilepsy group, six of them were on monotherapy with PB and two of them on a combined therapy with PB, one with KBr and another one with levetiracetam. Blood samples from group E3 were collected after initiating treatment so blood parameters could be affected by AEDs. Some studies have associated PB to megaloblastic anaemia, which is mainly observed in cases of deficiency in folic acid or cobalamin vitamins [260,261]. Folic acid and cobalamin are vital to erythropoiesis because of their role in the proliferation and differentiation of erythroblasts [262]. PB induces the cytochrome P450 enzyme which has the potential to increase the metabolism of folic acid, leading to a decrease of its serum levels [263]. Without acid folic and cobalamin, the erythropoiesis is compromised leading to a megaloblastic anaemia. According to Moore, megaloblastic anaemia is defined by a pancytopenia in association with macrocytic erythrocytes that are not fully differentiated [263].

None of the dogs included in this study presented values of blood parameters that could suggest an underlying anaemia. However, RDW was significantly higher in the group E2 when compared with the control group. RDW refers to the red cell width distribution and represents the size heterogeneity of RBC. RDW may increase in situations that require a high demand of RBC

because reticulocytes, that are bigger than mature erythrocytes, are released into the bloodstream. On the other hand, RDW may be decreased in situations where production and maturation of RBC are impaired. RDW may also be affected by inflammation because an increase in the inflammatory cytokines contributes to a non-regenerative anaemia by inhibiting the production of erythropoietin and its receptors as well as affecting the normal iron metabolism [264,265]. It would be expected to see a decreased RDW in the group E2, induced by a hypothetical chronic inflammation but these results are not in agreement with that hypothesis.

RDW has also been associated with hypoxia and this blood parameter has been suggested as a possible biomarker for hypoxia-inducing diseases [266–268]. During seizures the uncontrolled contraction of multiple groups of muscles such as the respiratory muscles may lead to airway obstruction and breathing issues, consequently initiating a status of hypoxemia [269]. The underlying hypoxemia during seizures may increase the risk of cerebral hypoxia, aggravating the symptoms if not treated. A study conducted by James *et al.,* described a decrease of 14.5% of arterial oxygen saturation in humans during seizures, which easily returned to normal values after the epileptic event. A status of hypoxemia increases the RDW by leading to a widespread release of erythrocytes into the bloodstream with the aim to increase the inflow of the circulating oxygen. In this study, none of the dogs demonstrated decreased levels of the number of erythrocytes, HCT or HGB, which rules out the possibility of anaemia that could increase the RDW by inducing regenerative response. Therefore, the RDW elevation in the E2 group may be due to the brief status of hypoxia caused by seizures or a result of inflammation-inducing seizures.

MCHC refers to the mean corpuscular HGB concentration and it was significantly lower in the E2 group when compared with the control group (p-value <0.05). MCHC is related with HCT and HGB and this relationship is expressed in the formula $MCHC = \frac{HGB}{HCT}$. HCT and HGB medians in the E2 group were slightly higher compared with the control group, which does not explain why MCHC is lower in the E2 group. Nonetheless, RDW was significantly higher in the E2 group, which may explain a lower MCHC if in the presence of a significant increase of erythrocytes' size. If reticulocytes are mainly released into the bloodstream during the seizures, it is expected to see a diminution of MCHC as consequence of an increase of erythrocytes' size, which makes the MCHC appear lower. The MCV refers to the mean corpuscular volume and the median of this blood parameter was slightly increased in the E2 group when compared with the control group, which empathises the hypothesis of a lower MCHC as consequence of larger, younger erythrocytes in circulation.

Other haematologic abnormalities that have been associated to PB administration included leukopenia and thrombocytopenia [180,270,271], which have not been observed in any of the dogs undergoing treatment in this work. The mechanism responsible for leukopenia and thrombocytopenia induced by PB remains unclear. A study reported that leukopenia could be

induced by destruction of mature granulocytes [272] while other study suggested that such anomalies could be explained by bone marrow necrosis provoked by PB [273].

On the other hand, monotherapy with KBr has not been associated with haematological abnormalities. Levetiracetam is a common choice as an add-on drug but some studies have reported haematological abnormalities associated with this drug when used in human medicine. In one study, monotherapy with levetiracetam was associated with thrombocytopenia [274], while in a case-report study it has been associated with pancytopenia [275]. Nevertheless, there is minimal evidence regarding the haematological abnormalities induced by levetiracetam in dogs.

Lymphocytes were significantly higher in the E3 group compared with the E2 group (p-value < 0.05). Lymphocytes are cells of major importance for the acquired immune response. In acute inflammation, the innate immune system is the first pathway activated in response to a noxious stimulus against the organism. Nonetheless, acquired immunity is mainly associated with a late and specialised response, which may be mainly involved in chronic processes. It is hypothesized that, in dogs suffering recurrent seizures, the acquired immune response is activated, leading to an increase of free-circulating lymphocytes in the bloodstream and chronic inflammation. Consequently, discrete lymphocytosis can be observed on the hemogram. The fact that lymphocytes were significantly higher in group E3 compared with group E2 supports the hypothesis that these dogs may experience underlying chronic inflammation, related with recurrent seizures. Decreased lymphocytes in group E2 when compared with group E3 is also compatible with stress leukogram changes due to recent seizures.

Even though there was no statistically significant relationship observed in WBC between groups, the median of WBC was slightly higher in group E2 compared with the group C1 and group E3. It is not clear which mechanism leads to an increase of WBC following seizures, however, one study suggested that WBC increases as a result of intense muscular activity and subsequently the release of epinephrine leads to demargination of leukocytes [219]. Stress and inflammation could also explain this increased in leukocyte number, regardless of the mechanism.

The neutrophils/lymphocytes ratio (NLR) could not be measured since the blood analyser used in this study did not provide the absolute value of circulating neutrophils, only granulocytes and eosinophils. NLR has shown to be a biomarker for some inflammatory diseases in humans [276,277] and could have been useful when assessing possible underlying inflammation in the epileptic dogs. The median of granulocytes was higher in group E2 compared with the control group and group E3. Since neutrophils are the most abundant granulocytes [278], it is hypothesised that the median value of granulocytes may be mainly changed by the absolute number of circulating neutrophils, leading to speculation of a higher NLR in the group E2 compared with the other groups, possibly reflecting inflammation. However, blood smears from these dogs were not analysed so it is not possible to conclude whether a higher absolute value of granulocytes was due to a major concentration of neutrophils, eosinophils or basophils. Nevertheless, it is not

common to observe basophilia on hemogram and percentage of eosinophils was not elevated in any of these dogs. As stated by Rebar, basophilia is rarely seen on the hemogram and when present, it is observed together with eosinophilia [278].

Stress leukogram is normally seen after stress-related situations that induce inflammation and is characterised by lymphopenia, mild mature neutrophilia, eosinopenia and mild monocytosis [214]. In dogs, it is common to see a marked neutrophilia and mild polycythaemia in the stress leukogram [214]. The granulocytes were higher in group E2 compared with group E3, which supports the theory of stress leukogram in dogs that suffered seizures. The median of HCT was elevated in group E2 which may suggest mild polycythaemia that may be associated with stress leukogram.

Because epilepsy has been linked to inflammation in several studies in humans [74,100,227–229], it is thought that non-steroidal anti-inflammatory drugs (NSAIDs) may have an important role in stopping epileptogenesis, especially in cases where the conventional AEDs do not work [249]. However, it is not yet known how NSAIDs should actuate on inflammatory pathways to avoid triggering seizures [279].

This study was performed during a 5-months externship in a clinical setting at a single veterinary hospital in Portugal, thereby limiting the number of animals included in this study. Therefore, the information and data from these dogs was collected during normal daily-basis clinical routine. All blood samples were collected during medical appointments and all owners were aware of the procedures.

The fact that this study was performed during this externship limited the sample of our population, as well as the diagnostic exams performed due to financial restraints of the owners. During the normal daily clinical routine, some diagnostic exams are preferred over others because some of these exams may be avoidable if no other abnormalities are found. For instance, if no abnormalities are found on serum chemistry regarding the hepatic functionality, fasting and postprandial bile acids may not be performed since the likelihood of finding abnormalities on this exam will be lower. The same may be applied for urinalysis if no alterations are found regarding creatinine and urea serum concentrations. The CSF analysis is another diagnostic exam included in the tier II confidence level that is normally not performed if no abnormalities are found on either a CT-scan or an MRI. It is often the case in Portugal, that most owners have financial restraints so most of the times, our diagnosis must be directed to the most important exams that should be performed with consideration given to the potential cost to owners. Some owners may have the willingness to have a definitive diagnosis and treat their pets but some of them may give up on some decisions due to financial constraints. Therefore, it is of great importance to initially select the most important diagnostic exams that are likely to reveal the most amount of information regarding the diagnosis. The fact that not all the dogs underwent the same diagnosis protocol may have affected the results of this study. The budget was also limited since this study was not financed by any institution but the hemograms performed were offered by the veterinary hospital.

Moreover, not all the dogs underwent the same treatment of epilepsy, which could have also affected the results.

At the beginning of this study, it was thought to include C-reactive protein (CRP) as a possible biomarker for inflammation. In humans, CRP has been identified as a high-sensitivity biomarker for inflammation. CRP is one of the main acute phase proteins (APP) recognised and it is widely used as biochemical tests for supporting diagnosis of several diseases such as leishmaniosis, leptospirosis or babesiosis in dogs [280,281]. Also, diagnosis of other non-infectious inflammatory diseases may be supported by serum concentrations of CRP including arthritis and canine inflammatory bowel disease. [280]

However, measurement of serum CRP is an expensive and low-specificity biochemical test [281] since it may be increased in the presence of several inflammatory conditions. Given these enumerated reasons, from which the cost was the foremost, it was decided to not include CRP measurement in this study, despite its potential benefits in identifying inflammation.

Assessing an empirical albumin/globulin ratio could have been another interesting analysis that could have been included in this study. A decreased albumin/globulin ratio could reflect inflammation due to a markedly concentration of globulins over albumin [282], which may also be decreased in inflammatory processes. However, this could not be assessed because not all the veterinarians that were following these epileptic dogs have analyzed both serum total protein and albumin while undergoing their diagnosis.

2.6. CONCLUSION

The existence of a relationship between epilepsy and inflammation is becoming more widely recognised. The fact that inflammation may trigger epilepsy and vice-versa makes the inflammatory mediators possible biomarkers for this disease. Also, a better understanding of inflammatory pathways in epilepsy could be of a great value to produce novel AEDs. Because epilepsy has been linked to inflammation in several studies in humans [74,100,227–229], it is thought that NSAIDs may have an important role in stopping epileptogenesis, especially in cases where the conventional AEDs do not work [249]. However, it is not yet known how NSAIDs should actuate on inflammatory pathways to avoid triggering seizures [279].

Currently there is a lack of effective drugs with little side effects and higher effectiveness praise the necessity to seek novel drugs that could be used in the treatment of IE. The new consensus regarding IE in veterinary medicine brought a better understanding between veterinarians regarding the classification and management of this disease, from the diagnosis to the prognosis. As epilepsy is a syndrome that is often misunderstood, especially regarding its pathophysiology, makes it harder to manage and treat such a disease. In addition, the fact that the costs of

diagnosing and treating IE are expensive due to the lack of government funding makes it even more difficult to ensure that this correctly managed.

Nowadays, most clinics and hospitals have blood analysers available for immediate use alongside large amount of data on hemogram parameters. However, it is not possible to directly relate hemogram parameters with IE unless in ruling-out other diseases. For this reason, a hemogram cannot be used alone to diagnose IE and since this is a complex disease, it is likely that the hemogram will never be considered as a gold standard test. Nevertheless, the fact that inflammation is suggested through hemogram parameters on these epileptic dogs highlights the relationship between epilepsy and inflammation. This relationship is interesting and may be helpful for the development of new novel drugs that act directly on specific inflammatory pathways.

Idiopathic epilepsy may not be defined as an inflammatory disease but its relationship with inflammation is becoming widely accepted, so assessing some inflammation biomarkers could be important in understanding a possible relationship between epilepsy and inflammation. Currently, some of the APPs known include CRP, serum amyloid A, alpha 1-acid glycoprotein and haptoglobin, which have a satisfactory diagnostic sensitivity for inflammation in dogs [280]. Thus, these potential biomarkers could be studied to understand whether they are markedly expressed following epileptic events. Inflammatory mediators such as ILs, chemokines and TNF-α could be of great value to understand their potential effects on the neuronal environment and its constituents. Measuring these inflammatory mediators could clarify whether they contribute for reactive gliosis and neuronal death, potentially aggravating the seizures. Glutamate could be also a potential target to assess its effects when overconcentrated in the brain tissue of epileptic dogs.

Finally, it is widely accepted that idiopathic epilepsy is strictly related with genetic mutations and some genes have been identified in humans and dogs. In Lagotto Romagnolo dogs, a mutation on the *LGI2* gene, encoding a protein strictly related with synaptic transmission, has been identified as a genetic cause for epilepsy [31,283]. Similarly, a type of epilepsy in humans was found to be induced by a mutation on the LGI1 gene [283]. Even though the gene involved is different from the one found in dogs, the proteins encoded belong to the same family, which highlights the relationship between these proteins and epilepsy. Mutations on these genes that lead to dysfunctions of these specific proteins are of a great interest as well.

This study suggested a relationship between epilepsy and changes in hemogram parameters, possibly triggered by underlying inflammation following seizures. However, it is not possible to guarantee the existence of a relationship between epilepsy and inflammation in this study since our sample was small and data limited to haematology. The fact that this is a preliminary and exploratory study encourages more studies on this subject, especially because some statistically significant results were obtained despite a small sample.

More studies on this topic should be encouraged in the future, enlarging the sample and assessing inflammation biomarkers, so that management of epilepsy can be easier for both veterinarians and owners and more importantly to allow for an improvement in the QoL of epileptic dogs.

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APPENDICES

Patient's Number	*Sex*	*Breed*	*Age at the first onset*	*Weight*	*Group*
1	Male	Labrador Retriever	4 years	36.5 kg	**Control**
2	Female	Cross-Breed	1-year e 5 months	36 kg	**Control**
3	Male	Labrador Retriever	6 years	38.6 kg	**Control**
4	Female	French Bulldog	2 years	12.45 kg	**Control**
5	Male	Estrela Mountain Dog	7 months	42.4 kg	**Control**
6	Male	Cross-Breed	2 years	6.7 kg	**Control**
7	Male	Cross-Breed	1-year and half	20.9 kg	**Control**
8	Female	Cross-Breed	1-year	19.5 kg	**Control**
9	Female	Estrela Mountain Dog	1 year and half	34.6 kg	**Control**
10	Female	Labrador Retriever	5 months	13.2 kg	**Control**
11	Female	Boxer	2 years	25.3 kg	**Control**
12	Female	Labrador Retriever	7 months	21,3 kg	**Control**
13	Male	Labrador Retriever	2 years	22 kg	**Control**
14	Female	Cross-Breed	1-year and 6 months	16 kg	**Control**
15	Male neutered	Pinscher	4 years and 5 months	3.80 kg	**Epilepsy**
16	Male neutered	Maltese Dog	5 years and 5 months	12.50 kg	**Epilepsy**
17	Female non-neutered	Yorkshire Terrier	1-year and 4 months	1.95 kg	**Epilepsy**
18	Male non-neutered	Estrela Mountain Dog	10 months	26.5 kg	**Epilepsy**
19	Male neutered	Labrador Retriever	5 years and 9 months	34.40 kg	**Epilepsy**
20	Male non-neutered	French Bulldog	3 years	17.30 kg	**Epilepsy**
21	Male non-neutered	Cross-Breed	3 years and 5 months	11.00 kg	**Epilepsy**
22	Male non-neutered	Chihuahua	2 years	2.65 kg	**Epilepsy**
23	Male neutered	German Shepherd	6 years	36,7	**Excluded** – Chronic hepatitis
24	Male non-neutered	Chihuahua	3 years and 1 month	3.25 kg	**Excluded** – Insufficient level of tier confidence

25	Female neutered	Labrador Retriever	4 years	32 kg	**Excluded** – Owner did not want to collaborate
26	Male non-neutered	Estrela Mountain Dog	9 months	29.9 kg	**Excluded** – Signs compatible with distemper but without definitive diagnosis
27	Female non-neutered	German Shepard	1-year and 7 months	25 kg	**Excluded** – Intoxication of unknown origin
28	Male non-neutered	Boxer	9 years	23 kg	**Excluded** – Possible brain tumour

1 QUESTIONÁRIO – HISTORIAL MÉDICO E SINTOMATOLOGIA

Este questionário tem como principal objetivo tentar recolher informações necessárias para a avaliação clínica de cães diagnosticados com epilepsia idiopática ou em casos em que haja forte suspeita deste diagnóstico. Este questionário está inserido num estudo que integrará a dissertação de mestrado integrado em medicina veterinária da aluna Daniela Pinto. Estes dados serão apenas utlizados para fins analíticos e estatísticos. Ao responder ao questionário concorda que estes dados sejam posteriormente divulgados na dissertação.

Nome do paciente: Número clínico do paciente (clínica/hospital):

Raça:

Idade:

Sexo: Castrado: Inteiro:

Peso:

1. **Suspeita ou diagnóstico definitivo de epilepsia em animais hereditariamente ligados ao paciente:**

Progenitora (mãe): ☐ Progenitor (pai): ☐

Irmãos: ☐ Outro:

2. **Relato de trauma físico prévio ao primeiro episódio convulsivo:**

Sim ☐ Não ☐

Se sim, tipo de trauma (zona afetada, forma de trauma, etc):

3. **Relato de doenças anteriores ao primeiro episódio convulsivo (por exemplo: doenças infeciosas, entre outros):**

Sim ☐ Não ☐

Se sim, responda de que doença/problema se tratou:

R:

Para efeitos de estudo a integrar na tese de Mestrado Integrado em Medicina Veterinária

4. **O paciente tem acesso ao exterior? Existe possibilidade de haver contacto com substâncias tóxicas ou algum tipo de veneno? Se sim, responda qual/quais:**
   R:

5. **Viajou recentemente com o seu animal para fora do país? Se sim, refira o país/países e duração da viagem:**
   R:

6. **Idade ao primeiro episódio:**
   R:

7. **Duração da primeira convulsão:**

Menos de 1 minuto ☐ Entre 1 a 2 minutos ☐

Entre 2 minutos a 3 minutos ☐ Entre 3 minutos a 5 minutos ☐

Mais de 5 minutos ☐

8. **Frequência dos episódios convulsivos:**

Uma vez por semana ☐ Uma vez por mês ☐

Uma vez a cada 6 meses ☐ Uma vez por ano ☐

Outro:

9. **Denotou um aumento no número de episódios convulsivos ou na sua duração desde o primeiro evento?**

Sim ☐ Não ☐

10. **Distribuição das convulsões:**

Apenas um episódio/dia ☐ Dois episódios por dia ☐

Múltiplos episódios/dia (mais do que 2 episódios) ☐

11. **Episódios convulsivos relatados mais durante certos períodos do dia como:**

De manhã ☐ Durante o sono ☐ Após acordar ☐

Para efeitos de estudo a integrar na tese de Mestrado Integrado em Medicina Veterinária

De tarde ☐ De noite ☐ Durante exercício físico ☐

Em repouso ☐

12. Fatores prévios ao episódio convulsivo que possam ter despoletado o episódio tais como:

Mudança de casa ☐ Exercício físico ☐ Trauma físico ☐

Novo animal em casa ☐ Mudança alimentar ☐

Mudança em hábitos diários (número de vezes que animal vai à rua ☐

Outro:

13. Existência de doenças de foro endócrino/hormonal

Sim ☐ Não ☐

Se sim, qual/quais?

R:

14. Denota que há perda de consciência durante a convulsão?

(Sinais de perda de consciência <u>podem ser</u>: falta de reação ao chamamento por parte do dono, incapacidade de redirecionar o olhar para o dono, etc..)

Sim ☐ Não ☐

15. Como se encontrava o tónus muscular durante a convulsão?

Flácido ☐ Rígido ☐

16. Denotou alguma alteração de comportamento?

a. Se sim, refira qual/quais:
R:

17. Descrição breve e sucinta pelo proprietário sobre o episódio epilético:

(Pontos-chave: Tonicidade/Flacidez muscular, Postura, Consciência, Duração, Momento, Movimentos, Frequência, etc..)

R:

Para efeitos de estudo a integrar na tese de Mestrado Integrado em Medicina Veterinária