TITLE: Abnormal platelet activity in dogs and cats – impact and measurement

AUTHORS: P. Gant, D. McBride, K. Humm

JOURNAL: Journal of Small Animal Practice

PUBLISHER: Wiley

PUBLICATION DATE: 9 January 2020

DOI: https://doi.org/10.1111/jsap.13092

Abnormal platelet activity in dogs and cats – impact and measurement

P. Gant*[1], D. McBride[1], K. Humm[1]

[1] Queen Mother Hospital for Animals (QMHA), The Royal Veterinary College, Hatfield, Hertfordshire, AL9 7TA, UK

* Corresponding author email: pgant@rvc.ac.uk

Abstract:

Abnormal platelet activity can lead to either bleeding tendencies or inappropriate thrombus formation. This can occur secondary to a wide variety of disease processes, with a range of clinical consequences and severity. This article will discuss the pathophysiology of platelet function abnormalities and consider a logical diagnostic approach in canine and feline patients applicable to veterinary practice. The recent advances in platelet function testing will then be discussed, both with regards to detection of platelet dysfunction but also tailoring of pharmacological manipulation. Although many of these tests are still confined to the research or academia setting, remote techniques for indirectly assessing platelet function are starting to become available. Although we still require further research to develop guidelines for the use of these tests in clinical decision making, the recent advances in this field are an exciting step forward in being able to detect and manage platelet dysfunction in both primary care and referral level practice.

Keywords:

Platelet; haemostasis; coagulation; haemorrhage; thrombosis

Introduction:

Platelets play a vital role in the body's response to vascular injury and clot formation, known as haemostasis. For the purposes of clinical and laboratory assessment, the 'classical model' of haemostasis is most applicable. This identifies 'primary haemostasis' as the interaction between platelets, von Willebrand factor (vWF) and subendothelial collagen that results in the formation of a platelet plug. This plug provides a surface for the assembly of coagulation proteins required for fibrin formation, known as 'secondary haemostasis' (McMichael 2005; Christopherson et al. 2012; Wei et al. 2009). More recently, the 'cell based model' of haemostasis has been developed and better reflects in vivo coagulation and therefore true bleeding tendency. This model emphasises the importance of tissue factor bearing cells in initiating coagulation, but also the requirement for mass platelet activation which provides the negatively charged procoagulant surface required for massive thrombin production (Smith 2009). In both models, depending on the balance between platelet stimulation or inhibition, abnormalities in platelet function can either result in haemorrhagic or thrombotic disorders (McMichael 2005).

With an increase in our current understanding of normal platelet activity has come the development of a plethora of new diagnostic tests that can better localise abnormalities in platelet function. This review article aims to summarise the clinically relevant aspects of platelet anatomy and physiology and identify the potential sources of altered canine and feline platelet function. The investigation of platelet disorders, with reference to both well-established and new tests of platelet function, will then also be discussed.

Normal platelet anatomy and physiology:

Mammalian platelets are anucleate, cytoplasmic fragments that are liberated from megakaryocyte precursors during thrombopoiesis. The time taken for megakaryocyte maturation and release of platelets is approximately 3-5 days. Platelet lifespan in laboratory dogs has been reported at 6.0 +/- 1.1 days (van der Meer 2010). No published data is available in cats. Platelet size varies both inter and intra species, with cats generally having smaller platelets (Sullivan et al. 1993) but with variation also existing between dog breeds (Lawrence 2013). Most notable is the syndrome of macrothrombocytopenia in Cavalier King Charles Spaniels (CKCS) and Akitas and the relative thrombocytopenia in greyhounds (Pederson et al. 2002; Hayakawa 2016; Santoro 2007). These do not result in a bleeding tendency as an effective total platelet mass is maintained.

Platelets have several key anatomical characteristics. These are depicted in figure 1.

- Phospholipid membrane with high density of regulated, adhesive receptors (see table 1).
- Cytoskeleton consisting of abundant contractile proteins (e.g. actin and myosin) to allow shape change.
- A dense tubular system which can sequester or release calcium.
- Secretory granules that allow response to a variety of external stimuli:
    - Alpha granules: release adhesive proteins, such as fibrinogen and P-selectin; prothrombotic factors, such as factor V and XI and growth factors to facilitate vascular healing.
    - Electron dense granules: release platelet agonists required for mass platelet activation, including adenosine diphosphate (ADP), epinephrine, serotonin, histamine and calcium. (Sanford et al. 1981; Jerk and Kehrel 2005).

Primary haemostasis relies on the synergistic activity of multiple receptor interactions to generate a stable aggregation of platelets, called a platelet plug. The three key stages of primary haemostasis are platelet adhesion, activation and aggregation and are discussed below. These stages occur concurrently, with the aim being to generate sufficient platelet aggregation to restore vascular integrity. Abnormalities associated with any of these stages can result in platelet type bleeding or thrombosis (McMichael 2005; Wei et al. 2009). The interested reader is directed to a review of platelet signalling for a more in-depth discussion on this topic (Goggs and Poole 2012).

Adhesion:

In health, haemostasis is only triggered by injury to a vessel wall. Vascular injury exposes subendothelial matrix proteins, primarily collagen, to which platelets can bind. Initial tethering is primarily mediated by direct collagen binding using the GPVI receptor or indirectly via the GP1b-IX-V receptor via von Willebrand's factor (vWF). The latter has increased relevance at sites of high blood velocity in the arterial circulation, where the friction (or 'shear stress') makes direct platelet binding difficult. Both of these interactions are short lasting. However, they also stimulate platelet signalling pathways which result in the conversion of integrin receptors to their high affinity state. This allows more stable adhesion to collagen, either directly via the $\alpha 2\beta 1$ integrin or indirectly via the $\alpha_{IIb}\beta_3$ integrin using vWF (Nieswant 2003; Brass 2010, Auton 2010). These adhesions are depicted in figure 2.

Von Willebrand factor is a large glycoprotein which forms polymers called multimers. These exist in a range of sizes, or 'molecular weights'. Larger multimers have a greater affinity for platelets. The vWF glycoproteins are synthesised in endothelial cells or megakaryocytes and can then be

stored in endothelial Weibel-Palade bodies or platelet alpha granules. Dogs have much less vWF in platelets compared to cats (Waters et al 1989; McCarroll 1998).

Endothelial cells constitutively secrete small multimers into the subendothelial matrix and large multimers into the plasma (Lopes da Silva and Cutler 2016). Here, vWF circulates with factor VIII. Effective secondary haemostasis therefore also requires vWF to prevent the premature degradation of FVIII (Thomas 1996).

Following vascular injury, large vWF multimers are released from endothelial stores. Subsequent binding to collagen results in a conformational change, exposing the binding site for the GPIb/V/IX platelet receptor (Sadler 1998; Ruggeri 1999). Large multimers can also spontaneously bind platelets without collagen binding (Arya 2002). Platelet adhesion and activation releases further vWF from alpha granules. Release of vWF can also be stimulated by other substances, such as the thrombin produced during inflammation (McMichael 2005).

Multimer size is regulated by metalloproteases, specifically *'A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13'* (ADAMTS-13), which is predominantly produced by endothelial cells. This proteolytic enzyme cleaves large multimers into smaller multimers, which have reduced platelet binding potential. When vWF multimers bind to platelets, they also become more susceptible to cleavage by ADAMTS-13, which allows negative feedback inhibition. In the absence of ADAMTS-13 activity, ultra large multimers can accumulate resulting in uncontrolled platelet aggregation and thrombi formation (Arya 2002, Dong 2002). Lower levels of ADAMTS-13 have been reported in people secondary to neoplastic, inflammatory or autoimmune conditions (Banno et al. 2006).

<u>Activation and amplification:</u>

Platelet adhesion triggers intracellular signalling pathways. This results in protein modifications required for successful aggregation such as:

1. Conversion of integrin receptors, most importantly $\alpha_{IIb}\beta_3$, to a high affinity state.
2. Membrane 'flipping' to expose the phospholipid ''phosphatidylserine'' (PS). This creates a negatively charged, procoagulant surface for the assembly of coagulation factors and generation of thrombin (Satta et al. 2010).
3. Contractile protein activity necessary for platelet shape change. This increases surface binding area and allows fibrin-clot retraction.
4. Release of granular contents (McMichael 2005; Wei et al. 2009; Satta et al. 2010). Glycoproteins, such as P-selectin, are transferred to the platelet surface during degranulation and can provide indirect evidence of activation (Moritz et al. 2005).

To restore vascular integrity, the original stimulus for platelet activation must be amplified and sustained using platelet agonists. These are found or produced from a variety of sources, including the subendothelial matrix (e.g. collagen), platelets themselves (e.g. granule contents or products of membrane breakdown: thromboxane), other cells (e.g. epinephrine) or through activation of the coagulation cascade (e.g. thrombin). Each agonist acts at a different receptor and has a variable capacity to stimulate platelet aggregation (Reviakine 2015). Platelet agonists either increase intracellular calcium to trigger intracellular signalling or inhibit cyclic adenosine monophosphate (cAMP) which normally maintains platelets in an inactivated state (Decouture 2015).

Calcium release is triggered via activation of the phospholipase C (PLC) pathway. This enzyme hydrolyses the platelet phospholipid membrane, generating secondary messengers. These

messengers can induce intracellular release of calcium and activate other enzymes required for platelet function (Wei et al. 2009, Brass 2010; Reviakine 2015).

The phospholipase A_2 (PLA_2) pathway is activated indirectly by the PLC pathway and by initial platelet aggregation. This pathway results in the breakdown of phospholipids to arachiodonic acid which are converted to thromboxane A_2 (TXA_2) by cyclooxygenase (COX) enzymes. Thromboxane A_2 is a potent platelet agonist, allowing both autocrine and paracrine signalling. This pathway can be blocked via inhibitors of COX enzymes (Floyd and Ferro 2013; Reviakine 2015). Some of these intracellular signalling pathways are illustrated in figure 3.

Aggregation:

Aggregation of platelets refers to the adhesion of activated platelets to each other using receptor bound fibrinogen as a bridge. The $\alpha_{IIb}\beta_3$ integrin receptor can also bind vWF at sites of high shear stress. Soluble fibrinogen is found free in the plasma but cannot bind to platelets until they have been activated. Ongoing action of platelet agonists is therefore required to provide sufficient aggregation (McMichael 2005; Sangkuhi 2011).

Inhibition of primary haemostasis:

Platelet aggregation must also overcome several inhibitory mechanisms:

1. Endothelial cells release prostacyclins and adenosine diphosphatase (ADPases). These either inhibit TXA_2 production via the PLA_2 pathway or activate cAMP pathways to inhibit platelet activation (Gale 2011).
2. A layer of negatively charged glycosaminoglycans (GAGs) and proteoglycans, called the 'glycocalyx', lines intact vessels and inhibits thrombin formation. The glycocalyx also

releases nitric oxide (NO) at sites of high blood velocity, which inhibits aggregation (van Hinsbergh 2011; Ralph and Brainard 2014).

Abnormalities of platelet function resulting in haemorrhage:

Haemorrhage can occur secondary to quantitative and/or qualitative disorders of platelets, vWF or vasculopathies. 'Platelet-type bleeding', which manifests as ecchymoses and/or petechiae and intraoperative bleeding, can generally be differentiated from disorders of secondary haemostasis, in which intra-cavitary bleeding, haematomas and haemarthosis are more common. Mucosal bleeding (which can be clinically silent or present as urinary or gastrointestinal bleeding) can be seen with either primary or secondary haemostatic disorders (Gale 2011; Jandrey 2012; Jandrey 2014).

Low numbers of platelets (thrombocytopenia) is the most common cause of platelet type bleeding and should always be excluded prior to further investigation of platelet function. A platelet count of 150 x 10^9/L is the generally accepted lower reference limit (Jain 1986). However, it is not well understood what severity of thrombocytopenia leads to spontaneous haemorrhage. Platelet counts less than 30 x 10^9/L have been associated with spontaneous haemorrhage in dogs with idiopathic thrombocytopenia (Williams and Maggio-Price 1984) but concurrent platelet or endothelial dysfunction can result in bleeding at higher platelet counts (Torrent 2005; Ferkau 2013). Thrombocytopenia will not be covered further in this article as it is well described elsewhere (Johnstone et al. 1988; O'Marra et al. 2011; Nakamura et al. 2012).

Qualitative platelet disorders, known as thrombocytopathias, are rare compared to thrombocytopenia. They are classified as either congenital or acquired and the underlying defect can generally be categorised as a defect in:

- Adhesion and signalling
- Platelet aggregation
- Prevention of agonist activation or a defect of secondary signalling
- Deficiency of platelet agonists normally stored within the platelet granules (storage pool deficiencies) (Choi et al. 2014; Paniccia et al. 2015)

Congenital thrombocytopathias

Inherited platelet disorders are classified as either '*extrinsic'*, in which platelets or the vasculature lack a functional protein, or '*intrinsic'* disorders, which are those inherent to the platelet. Some of the best characterised inherited defects of primary haemostasis are summarised in table 2. The reader is referred to other sources for more detail (Jandrey 2014, Callan and Catalfamo 2017).

Acquired thrombopathia:

Acquired disorders of primary haemostasis are typically more heterogeneous in their pathophysiology and are therefore more difficult to characterise. A variety of conditions have been reported, with the most well documented summarised in table 3.

Pharmacological manipulation of platelet function can also be used to cause platelet dysfunction and therefore reduce the risk of thrombus formation. These pharmacotherapies can be categorised as: ADP receptor antagonists, thromboxane inhibitors and the newer $\alpha_{IIb}\beta_3$ integrin receptor antagonists. Their mechanisms and duration of action are summarised in table 4 and more in depth

information is available in a couple of review articles (Lunsford and Mackin 2007, Thomason et al., 2016). The interested reader is also referred to a recent consensus statement which summarises the indications for antithrombotic medication using the current evidence available (Goggs et al. 2018).

Multiple studies have demonstrated the inhibitory effects of aspirin and clopidogrel on platelet function in healthy dogs (Blois 2010; Brainard 2010; Sharpe 2010; Dudley 2013; Haines 2016, Saati 2017) and cats, including those with subclinical feline cardiomyopathies (Hogan et al. 2004, Hamel-Jolette et al. 2009; Teuber and Mischke 2016; den Toom et al 2016).

However, as in people, some dogs and cats have been noted to be poor responders to anti-platelet medication and are considered 'resistant' (also known as 'high on-treatment platelet reactivity' [HTPR]). Aspirin 'resistance' in dogs has a reported incidence ranging from 19% to 56% (Dudley et al., 2013; Haines et al., 2016). There are no published incidences of drug resistance in cats. However, high interindividual variability in clopidogrel plasma levels has been reported, although this was considered mostly secondary to differences in metabolism (Lee 2018). The results of the FAT CAT trial (Hogan et al. 2015) did however suggest that clopidogrel is superior when compared to aspirin in reducing the risk of recurrent aortic thromboembolism.

Individual response to platelet inhibitors can be monitored using platelet function tests or by measuring markers of platelet activation. However an understanding of the clinical limitation of each test is required as there is a possibility that reduced platelet activity may not be detected or reduced activity may not correspond to reduced risk of thrombosis.

The effects of non-steroidal anti-inflammatory drugs (NSAIDs) on *in vitro* platelet aggregation are variable (Gaál et al. 2007; Blois 2010; Mullins 2012). One *in vivo* study demonstrated reduced platelet aggregation but no increase in buccal mucosal bleeding time (BMBT) in dogs which

227 received pre-operative ketoprofen prior to ovariohysterectomy (Lemke 2002). A study in cats also
228 documented no decrease in aggregation following 14 days of meloxicam (Cathcart 2012). Data for
229 other NSAIDs is lacking and their peri-operative use in patients, especially those with concurrent
230 primary haemostatic defects, should be carefully considered. Specific guidelines for the peri-
231 operative use of anti-platelet medication is discussed in detail in the previously mentioned
232 consensus statement and depends on the risk of thrombosis and the type of procedure (Goggs et
233 al. 2018).
234
235 Intravenous fluid therapy is also known to cause platelet dysfunction. Some canine studies have
236 suggested that only hypertonic saline, and not hydroxyl starches (HES) (specifically 130/0.4),
237 result in statistically significant changes to platelet function beyond that associated with
238 haemodilution alone (Wurlod et al 2015; McBride et al. 2016). An in vitro study in healthy dogs
239 also reported evidence of platelet dysfunction associated with anaemia secondary to haemodilution
240 but the clinical significance of this was questioned (Clancey 2009). A more recent study
241 investigating resuscitation fluids in a canine haemorrhagic shock model suggested that 20ml/kg of
242 4% succinylated gelatin was associated with reduced platelet function compared to 20ml/kg fresh
243 whole blood or 6% HES (130/0.4) or 80ml/kg crystalloids. Additional significant global
244 coagulation abnormalities were noted in the HES group. Shock alone was associated with a mild
245 increase in platelet function suggestive of hypercoaguability (Claus 2018). In a feline study, HES
246 reduced coagulation to a greater degree than a balanced crystalloids solution alone, but platelet
247 function was not assessed specifically (Albrecht 2016). Mannitol has also been shown to impair
248 platelet aggregation but not at clinically relevant dilutions (Adamik 2015; Yosava 2017). In
249 summary, there is still insufficient data to conclude whether various intravenous fluids will cause

clinically significant abnormalities in platelet function. However, where resuscitation can be achieved with crystalloid therapy alone, the authors would consider this a safer option.

Von Willebrand Disease (vWD):

Although not strictly a disorder of platelet function, qualitative or quantitative defects in vWF are the most commonly recognised congenital bleeding disorders in dogs. Patients with vWD can present with similar clinical signs to platelet function disorders, although generally mucosal bleeding or excessive bleeding following trauma or surgery are more common (Jandrey 2014). Since vWF acts as a carrier for factor VIII, prolongation of activated partial thromboplastin time (APTT) may concurrently occur (Thomas 1996).

Von Willebrand disease is an inherited, autosomal recessive genetic mutation. Three forms of vWD (types 1-3) are well recognised in dogs and are characterised based on the concentration and multimeric size of plasma vWF, as well as bleeding severity (Brooks 1999). Dogs with type 1 disease have variable bleeding tendencies depending on the variable expression of the abnormal gene (Thomas 1996; Venta et al. 2000). Furthermore, although severe bleeding would be expected in dogs with type 3 disease, there are also reports of dogs presenting with mild mucosal bleeding (Pathak 2014; Scuderi 2015). The different types of vWD are summarised in table 5.

In cats, there have been only 2 reported cases of vWD, both of which were considered type 3 (French et al. 1987; Bebar et al. 2014).. Though extremely rare, vWD should therefore still be considered in cats with clinical signs of primary haemostatic dysfunction.

Acquired von Willebrand syndrome (AVWS) occurs when normal vWF is produced but concurrent disease results in increased clearance or its inhibition. In dogs, AVWS has been reported in conjunction with hypothyroidism (Avgeris 1990). Transient AVWS has also been

reported after intravenous colloid administration (Gauthier et al, 2015), secondary to uraemia with acute kidney injury (McBride 2017) and possibly in association with *Angiostrongylus vasorum* infection (Whitley et al. 2005; Hausmann et al. 2016).

Diagnosis of type I and type III vWD is made by measuring low (type I) or minimal (type III) vWF antigen concentration s(vWF:Ag), (Brooks 1999). Dogs with type II vWD may have normal vWF:Ag, however lack large vWF multimers resulting in decreased collagen binding and clinical signs of primary haemostatic dysfunction (Thomas 1996; Favaloro 2010). Type II vWD is diagnosed by measuring vWF collagen binding activity (vWF:CBA) or vWF multimeric pattern, neither of are commercially available to the authors' knowledge. A summary of the diagnostic tests available for vWD are given in table 6. Various factors may interfere with these diagnostic tests (systemic illness and surgery can affect vWF:Ag for example) (Favaloro 2010), therefore diagnosis of congenital vWD should ideally be made with DNA genetic testing, which is available for certain breeds (see table 6).

Abnormalities of platelet function resulting in inappropriate thrombosis:

Hypercoagulability, or thrombophilia, refers to inappropriate thrombus formation. Virchow's triad describes the three broad categories involved in pathological thrombus formation, all of which may impact platelet function. These include endothelial dysfunction, hypercoagulability and blood stasis (Ogedegbe 2002; Gale 2011; Wolberg 2012).

<u>Endothelial dysfunction:</u>

Endothelial dysfunction secondary to inflammation can result in endothelial cell activation and direct platelet adhesion. It can also disturb production of platelet inhibitors such as prostacyclins

and nitric oxide (NO). Endothelial cell activation can also result in increased secretion of large vWF multimers and concurrent decrease or absence of ADAMTS-13 (Vischer 2006; Luo et al. 2012).

Hypercoagulable states:

Inflammatory cytokines can trigger the coagulation cascade, resulting in thrombin production and platelet activation without adhesion (Esmon 2005). These platelets may also exhibit exaggerated aggregation in response to a normal stimulus. Activated platelets also provide a procoagulant membrane and release agonists to further perpetuate aggregation (Brass 2010; Stokes and Granger 2012).

Blood stasis

Areas of blood stasis are thought to result in hypoxia. Hypoxia is thought to upregulate P-selectin, which recruits inflammatory leukocytes. These then act as a source of thrombin generation and further platelet activation (Michiels et al. 2000).

Clinical signs of thromboembolic disease

Clinical signs are related to either a consumptive coagulopathy with microvascular thrombosis or thromboembolic (TE) disease. Consumptive coagulopathy is generally associated with signs of platelet-type bleeding, whereas the site of TE disease will dictate the clinical signs (see table 7) (Ralph and Brainard et al. 2014; Paniccia et al. 2015). Although these signs may be marked, it can still be difficult to document an underlying thrombus and advanced imaging may be required (Goggs et al. 2014).

There are no inherited disorders of thrombophilia reported in veterinary species. Therefore, if clinical signs of thromboembolic disease are present, diagnostic workup for an underlying disease process is warranted. It is also important to consider prophylactic therapy in patients with disease processes known to be predisposed to thromboembolic disease. Disease processes resulting in either overt thromboembolic disease or microvascular thrombosis are summarised in table 8. Although there is evidence of global hypercoagulability with thromboembolic diseases in veterinary medicine, evidence of increased platelet function is currently limited.

Diagnostic approach to disorders of platelet function: Haemorrhage

Once clinical evidence of primary haemostatic dysfunction is suspected (petechiae, ecchymosis, mucosal haemorrhage, bleeding after venepuncture), a diagnostic workup is indicated to differentiate the different causes of primary haemostatic dysfunction. The approach is summarised in figure 4 and outlined below:

1. Perform a Complete Blood Count (CBC) and blood smear assessment (see Figure 5):

A CBC is required to exclude anaemia and thrombocytopenia prior to platelet function testing. True platelet number and size should also be assessed with a good quality, stained blood smear as automated blood cell counters may report pseudothrombocytopenia. In cats this usually occurs because of platelet clumping. There is also considerable overlap between erythrocyte and platelet volumes, resulting in misclassification by impedance analysers (Wang and Brainard 2014).

2. Perform prothrombin time (PT) / Activated partial thromboplastin time (APTT):

Prothrombin time and APTT assess the in vitro intrinsic and extrinsic pathways of the cascade model of secondary haemostasis. This can be particularly useful when physical examination suggests either primary or secondary haemostatic defects e.g. mucosal bleeding.

3. Perform buccal mucosal bleeding time (BMBT) [see figure 8):

BMBT is indicated in patients with evidence of bleeding but normal platelet count and PT/APTT. A specific BMBT lancet device must be used. Reference ranges are device specific but are generally considered to be less than 4 minutes in dogs and less than 2 minutes in cats. Anaesthesia and sedation can mildly prolong the BMBT (Sato et al., 2000; Alatsaz et al., 2014). In a patient where anaemia and thrombocytopenia are excluded and PT / APTT are normal, a prolonged BMBT may reflect thrombocytopathia, vWD or more rarely, a vessel wall disorder. It is important to understand the limitations of the BMBT, as it is highly subjective and prone to significant inter- and intraobserver variability (Sato et al 2000; Alatzas et al. 2013). Results should therefore always be interpreted with caution and verified with more specific testing. A BMBT incision is superficial, such as to only stimulate platelet plug formation, and therefore is not recommended as a predictor of surgical haemorrhage.

4. Von Willebrand Factor Antigen

If the BMBT is prolonged, the vWF: Ag assay is indicated to differentiate type I and type III vWD from thrombocytopathia. As mentioned earlier, vWF: Ag cannot diagnose type II vWD as vWF antigen concentration can be normal. A vWF: Ag assay should also be considered in any patient with clinical signs of platelet type bleeding with normal platelet count and BMBT, due to the limitations of BMBT.

5. Additional screening for infectious disease:

If platelet dysfunction is suspected based on the above diagnostic procedures, infectious disease screening is also warranted in animals living in, or having a travel history to, an area where organisms which affect platelet function are endemic. A common infectious disease which can cause coagulopathies, including platelet dysfunction, is *Angiostrongylus vasorum*. A rapid in-house antigen test has high sensitivity and specificity (94% and 95%) (Schnyder 2011), however a recent study suggests that quantitative PCR of bronchoalveolar lavage may actually have much better sensitivity (Canonne 2018). Leptospirosis can also cause platelet dysfunction and diagnostic testing should be based on clinical suspicion.

6. Genetic testing:

If platelet dysfunction or vWD is suspected in a breed predisposed to congenital platelet function disorders (table 2) or vWD (table 5), genetic testing can be considered. DNA testing for congenital defects can be performed on an EDTA sample, or buccal swab.

7. Platelet function testing:

In patients with platelet type bleeding in which platelet count and vWF: Ag is normal, platelet function tests (see below) may be indicated. Most of these tests are limited to academic institutes at the time of this publication.

Diagnostic approach to disorders of platelet function: Thrombosis

For patients presenting with clinical signs which may be attributable to thromboembolic disease, a thorough minimum database including CBC with blood smear examination, biochemistry and urinalysis (including urinary protein: creatinine ratio) is indicated. D-dimers (which are a fibrin degradation product), can be measured by some in-house analysers and external laboratories. A low D-dimer concentration has good sensitivity for hypercoaguability but limited ability to specify the underlying cause (Nelson and Andreason 2003; Dewhurst et al. 2008; Epstein et al 2013). Prothrombin time and PTT values below the reference range may also indicate hypercoagulability (Song et al. 2016). Diagnostic imaging may be utilised to both document the presence of a thrombus (Table 7) and also to investigate for underlying diseases which may lead to thrombosis (Table 8). Echocardiography may also be indicated if underlying cardiac disease is suspected. If thrombosis is suspected or detected, platelet function testing can be beneficial in determining if increased platelet activity is contributing to thrombus formation (Song et al. 2016).

Blood Sampling:

When collecting a blood sample for assessment of platelet number or function, atraumatic venepuncture is required to minimise platelet activation and aggregation. For platelet function testing, the blood sample should be collected with a butterfly catheter attached to an anticoagulated vacutainer. The appropriate anticoagulant is determined by the test to be performed. The first sample must be discarded due to risk of platelet activation upon initial venepuncture. Tubes should be gently inverted and rotated to mix blood and anticoagulant. Most samples must be processed within two hours of sample collection. If unexpected results are obtained at any stage, then new sample collection and repeat analysis should be performed.

Specific tests of platelet function:

Tests of platelet function can be used to diagnose both increased and decreased platelet function. Although platelet function involves many in vivo mechanisms, these in vitro tests can still provide important clinical information (Christopherson 2012; Choi et al. 2014). One of the main limitations of the majority of platelet function tests is that immediate sample processing is required for accurate results. Many of the tests discussed below are commonly used as bedside point of care instruments in human hospitals, however at the time of this publication, their availability in veterinary practice is limited to referral institutes.

<u>Assessment of platelet adhesion under shear stress</u>

Aperture closure instruments: *E.g. Platelet function analyser (PFA) 100 or 200*

- The PFA test involves aspirating a citrated whole blood sample through a capillary tube (to generate shear stress) and then an aperture cut in a biologically active membrane. The membrane is coated in a platelet agonist. The aspirated sample, once activated by shear stress and exposure to an agonist, forms a platelet plug over the aperture. The time from aspiration of the sample to closure of the aperture by a platelet plug is measured as the closure time (CT) (Kratzer 1985; Harrison 2009).
- The generation of shear stress means the PFA is the only test able to detect abnormalities of platelet adhesion.
- Three cartridges exist containing different agonists:
    - Collagen and ADP (CADP) cartridge
    - Collagen and epinephrine (CEPI) cartridge to assess response to aspirin
    - Innovance P2Y cartridge measures $P2Y_{12}$ blockade and response to clopidogrel.

- The PFA-100 has been shown to detect the anti-platelet effects of aspirin, clopidogrel and NSAIDs in both healthy dogs and cats. As such, it may have clinical utility for monitoring response to treatment. However, it is also suggested that, compared to optical aggregometry, the PFA is less reliable in determining drug responsiveness and can markedly overestimate the degree of aspirin resistance (Gaal 2007; Dudley 2013; Haines 2016; Ho et al. 2016; Saati et al. 2017, McLewee 2018).
- The PFA 100 and 200 have also been used to investigate platelet function in dogs with valvular disease and cats with cardiomyopathy (Jandrey 2008; Clancey 2009; Moesguaard 2009), as well as dogs with chronic kidney disease and endotoxaemia (Dudley 2017; Yilmaz 2005). The PFA has also been used to diagnose Scott syndrome (Brooks 2009). In people, the PFA is used as a screening test for vWD (Ardillon 2015). One study reported increased CTs in 2 dogs with vWD that responded to DDAVP treatment (Burgess 2009).

Platelet aggregation

Light transmission aggregometry (LTA):

- Platelet rich plasma processed from a citrated sample is added to platelet agonists including ADP, thrombin and collagen separately. As platelet aggregates precipitate out, increased light transmission is detected by photometry creating a curvilinear graph demonstrating platelet function. This is considered the gold standard method. However, the requirement to produce PRP makes it impractical for bed-side testing (Hvar and Favalora 2016).
- Various underlying diseases have been investigated using LTA. One study in dogs showed variable aggregation in chronic kidney disease; increased aggregation in lymphoma Cushing's and diabetes mellitus; and decreased aggregation with ketoprofen but not

carprofen administration (Halmay 2008). More recently LTA has been used alongside the PFA to investigate the optimum dose of aspirin in dogs (McLewee et al. 2018).

Electrical impedance platelet aggregometry: *E.g. Multiplate®*

- Heparinised blood is added to platelet agonists including ADP, arachidonic acid and collagen. This induces platelet aggregates to form on two electrodes. Increased electrical resistance is detected between the two electrodes which creates a curvilinear graph (Kalbanter 2010).
- In healthy dogs, Multiplate® analysis was able to detect reduced platelet aggregation following both aspirin and clopidogrel therapy and as such may have clinical utility for monitoring response to therapy (Saati et al. 2017). In cats, normal variability of platelet function and inability to detect clinically relevant changes following anti-platelet therapy currently limits the use of Multiplate analysis, although future studies may further optimise its use in this species (Ho et al. 2015; Ho et al. 2016).
- The Multiplate has also been used to detect reduced platelet aggregation induced in vitro by the addition of lipopolysaccharide to replicate sepsis (Ferkau et al. 2013, Li and Chan 2016).
- This test should be run within 4 hours of sampling.

Changes in platelet count: *E.g. Plateletworks – an impedance based counter*

- Automated platelet counts in EDTA whole blood and citrated whole blood are compared before and after addition of platelet activators, such as collagen and ADP (Brass, 2010; Jandrey 2012; Choi et al. 2014).

- Although haematology counters are routinely available, the 'Plateletworks' analyser is a specific point of care assay that uses EDTA and citrate tubes implemented with agonist. This equipment is more accessible but individual agonist tubes cannot be purchased and have a relatively short expiry date. Samples should also be analysed within 10 minutes of collection.
- In healthy dogs, the Plateletworks analyser was able to detect reduced platelet aggregation following both aspirin and clopidogrel therapy and as such may have clinical utility for monitoring therapy (Saati et al. 2017). In cats, normal variability may again limit clinical application (Ho et al. 2015). However, studies in both healthy cats and those with asymptomatic hypertrophic cardiomyopathy have documented clinically detectable reductions in platelet aggregation following clopidogrel treatment (Hamel-Jolette et al. 2009; Bedard 2009; Ho et al. 2016, (den Toom 2016).

<u>Global Assessment:</u>

Viscoelastic testing: *E.g. Thromboelastography (TEG) +/- platelet mapping and Rotational **T**hrombo**e**lastometry (ROTEM)*

- TEG and ROTEM are global coagulation tests. Therefore, although they include platelet function in the process of clot formation, the presence of contributing factors such as thrombin and fibrin formation means they are relatively insensitive to abnormalities of platelet function (Brainard 2010, Brainard 2011).
- A citrated blood sample is added to a small cup (where activators of coagulation may or may not be added depending on the type of analysis to be performed), in which a pin is suspended. With TEG, the cup rotates which detects changes in torque of blood as a blood

clot forms in the cup. ROTEM differs in that the pin rotates instead of the cup. Lack of shear stress means these tests are not sensitive to defects in adhesion.

- Platelet number and function in addition to fibrin formation are major determinants of overall clot strength (TEG = maximum amplitude or ROTEM = maximum clot firmness) (Brainard 2010, Brainard 2011).
- TEG with 'platelet mapping' can better assess platelet function, which requires two TEG machines running concurrently with different activators including heparin, which can exclude the contribution of thrombin to maximum clot strength. (Croft 2004).
- Platelet mapping has been validated in healthy dogs (Blois 2013) and has been utilised in studies investigating clopidogrel efficacy (Brainard 2010).
- A novel Δ parameter, which aims to differentiate the relative contribution of platelets and clotting proteases to hypercoagulability, has been retrospectively evaluated in dogs with immune mediated haemolytic anaemia (Hamzianpour and Chan 2016).

Tests to assess platelet components and markers of platelet activation:

Flow Cytometry :

- Specific platelet characteristics, such as receptors and granule contents, are labelled with fluorescent monoclonal antibodies. When passed through a laser beam, conjugated antibodies emit a specific wavelength of light. This can be performed before or after stimulation with various agonists, including ADP, collagen, thrombin and epinephrine. Therefore, although flow cytometry cannot be used to directly assess platelet function, it can be used to detect the presence or absence of normal components or markers of activation.

- Flow cytometry has been used to diagnose inherited canine platelet disorders, such as Scott Syndrome (Brooks 2002) and Glanzmann's thrombasthenia (Bordreaux 1996).
- Markers of activation (such as P-selectin) have been used to investigate platelet activity in conditions such as sepsis (Moritz 2005) and in response to antiplatelet treatment (Sharpe et al. 2010, Dudley et al. 2013).
- Expensive equipment has previously limited clinical application as a bedside test. However, a recent study has shown that the addition of a fixative to blood samples may stabilise platelet activation markers for up to 9 days, allowing remote analysis at a central laboratory (Dunning et al. 2018). General practitioners are able to request test kits from the company 'Platelet Solutions'. This requires no specialist equipment and kits are stable for at least 9 months at ambient temperature. Further information is available at: http://www.plateletsolutions.co.uk/products-2/platelet-function-testing-kits/.

Plasma mean platelet component (MPC) concentration:

- When activated platelets degranulate, there is a decrease in density. This can be assessed using the plasma mean platelet component (MPC) concentration which is derived from the platelet refractive index. This value can be obtained from some automated haematology analysers (Macey et al. 1999).
- Increased platelet P-selectin expression and decreased plasma MPC concentration corresponding to platelet activation can be seen in dogs with septic and non-septic inflammatory conditions (Moritz 2005).

Significantly decreased plasma MPC concentrations have been reported in dogs with IMHA compared to healthy dogs and dogs with other diseases and has been significantly associated with survival (Zoia et al., 2018).

Conclusion:

Our ability to diagnose platelet dysfunction is growing. Although many advanced tests of platelet function are not available in primary care practice, a logical initial work up of these patients can still be performed. Current logistical restrictions for the wide application of these tests centre mainly around the cost of equipment and the requirement to analyse fresh blood samples. With further research, these limitations may be overcome or alternative strategies in assessing platelet dysfunction, such as remotely analysing markers of activation, may become more practical. This could results in clinicians in both primary care and referral level practice having the ability to perform a more in depth diagnostic work up and allow specific tailoring of antiplatelet therapeutics to patient with thromboembolic disorders.

References:

- Alatzas, D. G., Mylonakis, M. E., Kazakos, G. M., Kostoulas, P., Kritsepi-Konstantinou, M., & Polizopoulou, Z. S. (2013). Reference values and repeatability of buccal mucosal bleeding time in healthy sedated cats. Journal of Feline Medicine and Surgery, 16(2), 144–148.
- Adamantos, S., Waters, S., Boag, A. (2015) Coagulation status in dogs with naturally occurring Angiostrongylus vasorum infection. *Journal of Small Animal Practice* 56, 485–490.
- Adamik, K. N., Butty, E., Howard, J. (2015) In vitro effects of 3 % hypertonic saline and 20 % mannitol on canine whole blood coagulation and platelet function. *BMC Veterinary Research* 11, 242.
- Albrecht, N.A., Howard, J., Kovacevic, A., Adamik, K.N. (2016) In vitro effects of 6 % hydroxyethyl starch 130/0.42 solution on feline whole blood coagulation measured by rotational thromboelastometry. *BMC Veterinary Research* 12, 149.
- Ardillon, L., Ternisien, C., Fouassier, M., Sigaud, M., Lefrançois, A., Pacault, M., Ribeyrol, O., Fressinaud, E., Boisseau, P., Trossaërt, M. (2015). Platelet function analyser (PFA-100) results and von Willebrand factor deficiency: a 16-year "real-world" experience. Haemophilia, 21(5), 646–652.
- Arya, M. (2002) Ultralarge multimers of von Willebrand factor form spontaneous high-strength bonds with the platelet glycoprotein Ib-IX complex: studies using optical tweezers. *Blood* 17, 3971–7.
- Auton, M., Zhu, C., Cruz, M.A. (2010) The Mechanism of VWF-Mediated Platelet GPIbα Binding. *Biophysical Journal* 9, 1192–201.

- Banno, F., Kokame, K., Okuda, T., Honda, S., Miyata, S., Kato, H., Tomiyama, Y., Miyata, T., (2006) Complete deficiency in ADAMTS13 is prothrombotic, but it alone is not sufficient to cause thrombotic thrombocytopenic purpura. *Blood* 107, 3161–3166.
- Barthélemy, A., Magnin, M., Pouzot-Nevoret, C., Bonnet-Garin, J. M., Hugonnard, M., Goy-Thollot, I., (2016) Hemorrhagic, Hemostatic, and Thromboelastometric Disorders in 35 Dogs with a Clinical Diagnosis of Leptospirosis: A Prospective Study. *Journal of Veterinary Internal Medicine* 31, 69–80
- Bebar, K.N., Sinnott, V., Brooks, M.B. (2014) Recurrent hemorrhage caused by type 3 von Willebrand disease in a domestic long-haired cat. *Journal of Veterinary Emergency and Critical Care* 24, 326–331
- Bernardo, A., Ball, C., Nolasco, L., Moake, J. F., Dong, J. F. (2004) Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultralarge von Willebrand factor multimers under flow. *Blood* 104, 100–106
- Bick, R. L. (1988) Disseminated intravascular coagulation and related syndromes: A clinical review. *Seminals of Thrombosis and Haemostasis* 14, 299-338.
- Blajchman, M. A., Bordin, J.O., Bardossy. L., Heddle, N.M. (1994) The contribution of the haematocrit to thrombocytopenic bleeding in experimental animals. *British Journal of Haematology* 86, 347–50.
- Blois, S.L., Allen, D.G., Wood, R.D., Conlon, P.D. (2010) Effects of aspirin, carprofen, deracoxib, and meloxicam on platelet function and systemic prostaglandin concentrations in healthy dogs. *American Journal of Veterinary Research* 71, 349–58.

- Blois, S.L., Banerjee, A., Wood, R.D., Park, F.M. (2013) Thromboelastography platelet mapping in healthy dogs using 1 analyzer versus 2 analyzers. *Canadian Journal of Veterinary Research* 77, 231–6.
- Boccardo, P., Remuzzi, G., Galbusera, M. (2004) Platelet dysfunction in renal failure. *Seminars in Thrombosis and Hemostasis* 30, 579–589.
- Borgeat, K. (2013) Arterial Thromboembolism in 250 Cats in General Practice: 2004-2012. *Journal of Veterinary Internal Medicine* 28, 102–108.
- Brainard, B.M., Kleine, S.A., Papich, M.G., Budsberg, S.C. (2010) Pharmacodynamic and pharmacokinetic evaluation of clopidogrel and the carboxylic acid metabolite SR 26334 in healthy dogs. *American Journal of Veterinary Research* 71, 822–30.
- Brainard, B.M., Abed, J.M., Koenig, A. (2011) The effects of cytochalasin D and abciximab on hemostasis in canine whole blood assessed by thromboelastography and the PFA-100® platelet function analyzer system. *Journal of Veterinary Diagnostic Investigation* 23, 698–703.
- Brass, L. (2010) Understanding and evaluating platelet function. *Hematology. American Society of Hematology* 1, 387–396
- Brassard, J.A., Meyers, K. M., Person, M., Dhein C. R. (1994) Experimentally induced renal failure in the dog as an animal model of uremic bleeding. *The Journal of laboratory and clinical medicine* 124, 48–54
- Brooks, M., (1999) A review of canine inherited bleeding disorders: biochemical and molecular strategies for disease characterization and carrier detection. *The Journal of heredity* 90, 112–118.
- Brooks, M.B. (2002) A hereditary bleeding disorder of dogs caused by a lack of platelet procoagulant activity. *Blood* 99, 2434–41.

- Brooks, M.B., Randolph, J., Warner, K. (2009) Evaluation of platelet function screening tests to detect platelet procoagulant deficiency in dogs with Scott syndrome. *Veterinary Clinical Pathology* 38, 306–315.
- Burgess, H.J., Woods, P.J., Abrams-Ogg, A.C.G., Wood, R.D. (2009) Evaluation of laboratory methods to improve characterisation of dogs with von Willebrand disease. Canadian Journal of Veterinary Research 73(4): 252-259.
- Callan, M.B., Giger, U. (2002) Effect of desmopressin acetate administration on primary hemostasis in Doberman Pinschers with type-1 von Willebrand disease as assessed by a point-of-care instrument. *American Journal of Veterinary Research* 63, 1700–170.
- Callan, M.B., Catalfamo, J.L. (2017) Immune-mediated Thrombocytopenia, von Willebrand Disease, and Other Platelet Disorders. In: Textbook of Veterinary Internal Medicine. 4th edn. Eds S. J. Ettinger and E. C. Feldman. W. B. Saunders, Philadelphia. pp 1414-1419.
- Carr, A.P., Panciera, D.L., Kidd, L. (2002) Prognostic Factors for Mortality and Thromboembolism in Canine Immune-Mediated Hemolytic Anaemia: A Retrospective Study of 72 Dogs. *Journal of Veterinary Internal Medicine* 16, 504–509.
- Cathcart, C.J., Brainard, B.M., Reynolds, L.R., Al-Nadaf, S., Budsberg, S.C. (2012) Lack of inhibitory effect of acetylsalicylic acid and meloxicam on whole blood platelet aggregation in cats. Journal of Veterinary Emergency and Critical Care 22, 99–106.
- Chen, G., Liu, H., Liu, F. (2013) A glimpse of the glomerular milieu: From endothelial cell to thrombotic disease in nephrotic syndrome. *Microvascular Research* 89, 1–6.
- Cheng, T., Mathews, K., Abrams-Ogg, A., Wood, D. (2011) The Link Between Inflammation and Coagulation: Influence on the Interpretation of Diagnostic Laboratory Tests. Accessed on: http://vetfolio-vetstreet.s3.amazonaws.com [accessed 2018 Sep 28].

- Choi, J.L., Li, S., Han, J.Y. (2014) Platelet Function Tests: A Review of Progresses in Clinical Application. *BioMed Research International* 3, 1–7.
- Christopherson, P.W., Spangler, E.A., Boudreaux, M.K. (2012) Evaluation and Clinical Application of Platelet Function Testing in Small Animal Practice. *Veterinary Clinics of NA: Small Animal Practice* 42, 173–188.
- Clancey, N., Burton, S., Horney, B., MacKenzie, A., Nicastro, A., Côté, E. (2009) Evaluation of platelet function in dogs with cardiac disease using the PFA-100 platelet function analyzer. *Veterinary Clinical Pathology* 38, 299–305.
- Clancey, N., Burton, S., Horney, B., MacKenzie, A., Nicastro, A. (2009) Effects of in vitro haemodilution of canine blood on platelet function analysis using the PFA-100. *Veterinary Clinical Pathology* 38, 467–70
- Claus, M. A., Boyd, C. J., Hosgood, G., Smart, L., Raisis, A. L., & Sharp, C. R. (2018). Hypocoagulability and Platelet Dysfunction Are Exacerbated by Synthetic Colloids in a Canine Hemorrhagic Shock Model. *Frontiers in Veterinary Science*, *5*(November), 1–11
- Craft, R.M., Chavez, J.J., Bresee, S.J., Wortham, D.C., Cohen, E., Carroll, R.C. (2004) A novel modification of the Thrombelastograph assay, isolating platelet function, correlates with optical platelet aggregation. *Journal of Laboratory and Clinical Medicine.* 143, 301–9
- Decouture, B., Dreano, E., Belleville-Rolland, T., Kuci, O., Dizier, B., Bazaa, A., et al. (2015) Impaired platelet activation and cAMP homeostasis in MRP4-deficient mice. *Blood. American Society of Hematology* 126, 1823–30
- Dewhurst, E., Cue, S., Crawford, E., Papasouliotis, K. (2008) A retrospective study of canine D-dimer concentrations measured using an immunometric “Point-of-Care” test Journal of Small Animal Practice 49, 344–8

- Dong, J.F., Moake, J.L., Nolasco, L., Bernardo, A., Arceneaux,W., Shrimpton, C.N., et al. (2002) ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. *Blood. American Society of Hematology*; 100, 4033–9
- Dudley, A., Thomason, J., Fritz, S., Grady, J., Stokes, J., Wills, R., Pinchuk, L., Mackin, A., Lunsford, K. (2013) Cyclooxygenase Expression and Platelet Function in Healthy Dogs Receiving Low-Dose Aspirin. *Journal of Veterinary Internal Medicine* 27, 141–149.
- Dudley, A. (2013) An Investigation of the Multifaceted Platelet Dysfunction in Dogs with Naturally Occurring Chronic Kidney Disease – thesis. Accessed: https://etd.ohiolink.edu/!etd.send_file?accession=osu1405012077&disposition=inline [Accessed: 17th September].
- Dudley, A., Byron, J.K., Burkhard, M.J., Warry, E., Guillaumin, J. (2017) Comparison of platelet function and viscoelastic test results between healthy dogs and dogs with naturally occurring chronic kidney disease. *American Journal of Veterinary Research* 78, 589–600.
- Dunning, M., May, J., Adamany, J., Heptinstall, S., Fox, S. (2018) A Remote Assay for Measuring Canine Platelet Activation and the Inhibitory Effects of Antiplatelet Agents. *Journal of Veterinary Internal Medicine* 32, 119–127.
- Esmon, C.T. (2005) The interactions between inflammation and coagulation. *British Journal of Haematology* 131, 417–430.
- Epstein, S.E., Hopper, K., Mellema, M.S., Johnson, L.R. (2013) Diagnostic utility of D-dimer concentrations in dogs with pulmonary embolism. *Journal of Veterinary Internal Medicine* 27, 1646–9.

- Favaloro, E.J. (2010) Genetic testing for von Willebrand disease: the case against. *Journal of Thrombosis and Haemostasis* 8, 6–12.
- Ferkau, A., Gillman, H-J., Mischke, R., Calmer, S., Ecklebe, S., Abid, M., Minde, J-W., Echtermeyer, F., Theilmeier, G. (2013) Infection-associated platelet dysfunction of canine platelets detected in a flow chamber model *BMC Veterinary Research* 9, 112.
- Floyd, C.N., Ferro, A. (2013) Mechanisms of aspirin resistance. *Pharmacology and Therapeutics* 141, 1–10
- French, T.W., Fox, L.E., Randolph, J. F., Dodds, W.J. (1987) A bleeding disorder (von Willebrand's disease) in a Himalayan cat. *Journal of the American Veterinary Medical Association* 190, 437–439.
- Gaál, T., Halmay, D., Kocsis, R., Abonyi-Tóth, Z. Evaluation of the effect of ketoprofen and carprofen on platelet function in dogs studied by PFA-100 point-of-care analyser (2007) Acta Veterinaria Hungarica 55, 287–94.
- Gale, A.J. (2011) Current Understanding of Hemostasis. *Toxicologic Pathology* 39, 273–280
- Garosi, L.M. (2010) Cerebrovascular Disease in Dogs and Cats. *Veterinary Clinics of North America: Small Animal Practice* 40, 65–79.
- Glaspy, J.A. (1992) Hemostatic abnormalities in multiple myeloma and related disorders. *Hematology/oncology clinics of North America.* 6, 1301–1314.
- Goggs, R. and Poole, A. W. (2012) Platelet signalling – A primer. Journal of Veterinary Emergency Critical Care. 22(1):5-29.
- Goggs, R., Wiinberg, B., Kjelgaard-Hansen, M., Chan, D.L (2012) Serial assessment of the coagulation status of dogs with immune-mediated haemolytic anaemia using thromboelastography Veterinary Journal 191, 347–53.

- Goggs, R., Chan, D. L., Benigni, L., Hirst, C., Kellett-Gregory, L., Fuentes, V. L. (2014) Comparison of computed tomography pulmonary angiography and point-of-care tests for pulmonary thromboembolism diagnosis in dogs. *The Journal of Small Animal Practice* 54, 190–197.
- Goggs, R., Blais, M. C., Brainard, B. M., Chan, D. L., deLaforcade, A. M., Rozanski, E., & Sharp, C. R. (2019). American College of Veterinary Emergency and Critical Care (ACVECC) Consensus on the Rational Use of Antithrombotics in Veterinary Critical Care (CURATIVE) guidelines: Small animal. *Journal of Veterinary Emergency and Critical Care*, *29*(1), 12–36.
- Gouin, I., Lecompte, T., Morel, M.C., Lebrazi, J., Modderman, P.W., Kaplan, C., et al. (1992) In vitro effect of plasmin on human platelet function in plasma. Inhibition of aggregation caused by fibrinogenolysis. *Circulation*. 85, 935–41.
- Halmay, D., Gaál, T., Kocsis, R. (2008) Influencing factors of ADP-induced, epinephrine-induced and ristomycin-induced platelet aggregation in dogs. *Blood Coagulation & Fibrinolysis* 19, 14–22
- Hamel-Jolette, A., Dunn, M., Bédard, C. (2009) Plateletworks: A screening assay for clopidogrel therapy monitoring in healthy cats. *Canadian Journal of Veterinary Research* 73, 73-76.
- Hamzianpour, N., Chan, D.L. (2015) Thromboelastographic assessment of the contribution of platelets and clotting proteases to the hypercoagulable state of dogs with immune-mediated hemolytic anaemia. *Journal of Veterinary Emergency and Critical Care* 26, 295–9.
- Haines, J.M., Thomason, J.M., Seage, E.C., Wills, R.W., Bulla, C., Lunsford, K.V., et al. (2016) In vitro and in vivo assessment of platelet function in healthy dogs during

738 administration of a low-dose aspirin regimen. *American Journal of Veterinary Research*. 77,
739 174–85.
740 • Harrison, P., Mumford, A. (2009) Screening tests of platelet function: update on their
741 appropriate uses for diagnostic testing. Seminals of Thrombosis and Hemostasis 35, 150–157
742 • Hausmann, L., Pack A., Hausmann, S., Neiger, R. (2016) Acquired Von Willebrand Factor
743 and Factor VIII deficiency due to angiostrongylosis in a dog**.** *Tierarztliche Praxis. Ausgabe K,*
744 *Kleintiere/Heimtiere* 44, 189–193.
745 • Hayakawa, S., Spangler, E.A., Christopherson, P.W., Boudreaux, M.K. (2016) Veterinary
746 Clinical Pathology. 45(1), 103-5.
747 • Helms, C.C., Marvel, M., Zhao , W., Stahle, M., Vest, R., Kato, G.J., et al. (2013) Mechanisms
748 of hemolysis-associated platelet activation. Journal of Thrombosis and Haemostasis 11, 2148–
749 54.
750 • Hershko, K., Simhadri, V. L., Blaisdell A., Hunt, R. C., Newell, J., Tseng, S. C., Hershko, A.
751 Y., Choi, J.W., Sauna, Z. E., Wu,A., Bram, R. J., Komar, A. A., Kimchi-Sarfaty, C. (2012)
752 Cyclosporin A impairs the secretion and activity of ADAMTS13 (a disintegrin and
753 metalloprotease with thrombospondin type 1 repeat) *The Journal of biological chemistry* 287,
754 44361–44371.
755 • van Hinsbergh, V.W.M. (2011) Endothelium—role in regulation of coagulation and
756 inflammation. *Seminars in Immunopathology* 34, 93–106
757 • Ho, K.K., Abrams-Ogg, A.C., Wood, R.D., O'Sullivan, M.K., Kirby G. M., Blois S. L (2015)
758 Assessment of platelet function in healthy sedated cats using three whole blood platelet
759 function tests *Journal of Veterinary Diagnostic Investigation* 27, 352–360.

- Ho, K.K., Abrams-Ogg, A.C., Wood, R.D., O'Sullivan, M.K., Kirby G. M., Blois S. L. (2016) Assessment of platelet function in healthy cats in response to commonly prescribed antiplatelet drugs using three point-of-care platelet function tests. *Journal of Feline Medicine and Surgery* 19, 638–647.
- Hogan, D.F., Andrews, D.A., Green, H.W., Talbott, K.K., Ward, M.P., Calloway, B.M. (2004) Antiplatelet effects and pharmacodynamics of clopidogrel in cats. Journal of American Veterinary Medicine Association 225, 1406-11.
- Jain, N. C. (1986) Hematologic techniques – counting platelets. In: N. C. Jain (ed.), Schalm's Veterinary Hematology, Lea & Febiger, Philadelphia, pp. 65–66.
- Jandrey, K.E., Norris, J. W., MacDonald, K. A., Kittleson, M.D., Tablin, F. (2008) Platelet function in clinically healthy cats and cats with hypertrophic cardiomyopathy: analysis using the Platelet Function Analyzer-100 *Veterinary Clinical Pathology* 37, 385–388.
- Jandrey, K.E. (2012) Assessment of platelet function. *Journal of Veterinary Emergency and Critical Care* 22, 81–98.
- Jandrey, K. E. (2014) Platelet disorders. In: *Small Animal Critical Care Medicine* 2nd ed., Elsevier Health Sciences, Philadelphia.
- Johnson, L.R., Lappin, M.R. & Baker, D.C. (1999) Pulmonary thromboembolism in 29 dogs: 1985-1995. *Journal of Veterinary Internal Medicine* 13, 338–345.
- Johnstone, I.B. (1988) Clinical and Laboratory Diagnosis of Bleeding Disorders. *Veterinary Clinics of NA: Small Animal Practice* 18, pp.21–33
- Kalbantner, K., Baumgarten, A., Mischke, R. (2010) Measurement of platelet function in dogs using a novel impedance aggregometer. *The Veterinary Journal* 185, 144–51.

- Kerlin, B., Cooley. B.C., Isermann, B.H., Hernandez, I., Sood, R., Zogg, M., et al (2004) Cause-effect relation between hyperfibrinogenemia and vascular disease. *Blood. American Society of Hematology* 103, 1728–34.
- Kratzer, M.A., Born, G.V. (1985) Simulation of primary haemostasis in vitro. *Haemostasis* 15, 357–362.
- Kitrell, D., Berkwitt, L. (2012) Hypercoagulability in dogs: pathophysiology. *Compendium (Yardley, PA)*, 34. Accessed: https://vetfolio-vetstreet.s3.amazonaws.com/65/7bc1f06ede11e1806d005056ad4734/file/PV0412_Kittrell_CE.pdf [21st September].
- Klose, T.C., Creevy, K.E., Brainard, B.M.(2011) Evaluation of coagulation status in dogs with naturally occurring canine hyperadrenocorticism. Journal of Veterinary Emergency and Critical Care 21, 625–32.
- Kristensen, A.T., Weiss, D.J., Klausner, J.S. (1994) Platelet dysfunction associated with immune-mediated thrombocytopenia in dogs. *Journal of Veterinary Internal Medicine* 8, 323–327.
- Laffi, G., Cominelli, F., La Villa, G., et al (1987) Reduced platelet thromboxane A2 production as a possible cause of defective platelet aggregation in cirrhosis. *Adv Prostaglandin Thromboxane Leukot Res* 17A, 366–9
- Laffi, G., Marra, F., Gresele, P., et al (1992) Evidence for a storage pool defect in platelets from cirrhotic patients with defective aggregation. *Gastroenterology* 103, 641–6
- de Laforcade, A.M., Freeman, L. M., Shaw, S.P., Brooks, M.B., Rozanski, E. A., Rush, J.E. (2003) Hemostatic changes in dogs with naturally occurring sepsis. *Journal of Veterinary Internal Medicine* 17, 674–679.

- Lara-García, A., Couto, C.G., Iazbik, M.C., Brooks, M.B. (2008) Postoperative bleeding in retired racing greyhounds. J*ournal of Veterinary Internal Medicine* 22, 525–33.
- Laurenson, M.P., Hopper, K., Herrera, M.A., Johnson, E.G. (2010) Concurrent Diseases and Conditions in Dogs with Splenic Vein Thrombosis. *Journal of Veterinary Internal Medicine* 24, 1298–1304.
- Lawrence, J., Chang, Y-M.R., Szladovits, B., Davison, L.J., Garden, O.A. (2013) Breed-Specific Hematological Phenotypes in the Dog: A Natural Resource for the Genetic Dissection of Hematological Parameters in a Mammalian Species. PLoS ONE 8, 81288.
- Lee, P. M., Faus, M. C. L., & Court, M. H. (2019). High interindividual variability in plasma clopidogrel active metabolite concentrations in healthy cats is associated with sex and cytochrome P450 2C genetic polymorphism. Journal of Veterinary Pharmacology and Therapeutics, 42(1), 16–25.
- Lemke, K.A., Runyon, C.L., Horney, B.S. (2002) Effects of preoperative administration of ketoprofen on whole blood platelet aggregation, buccal mucosal bleeding time, and hematologic indices in dogs undergoing elective ovariohysterectomy. *Journal of American Veterinary Medicine Association* **220**, 1818–1822.
- Lennon, E.M., Hanel, R.M, Walker, J.M., Vaden S. L. (2013) Hypercoagulability in Dogs with Protein-Losing Nephropathy as Assessed by Thromboelastography. *Journal of Veterinary Internal Medicine* 27, 462–468.
- Li, C., Hirsh, J., Xie, C., Johnston, M.A., Eikelboom, J.W. (2012) Reversal of the anti-platelet effects of aspirin and clopidogrel. *Journal of Thrombosis and Haemostasis* 10, 521–528.

- Li, R.H.L., Chan, D.L. (2016) Evaluation of platelet function using multiple electrode platelet aggregometry in dogs with septic peritonitis. *Journal of Veterinary Emergency and Critical Care* 26, 630–638.
- Luo, G.P., Ni. B., Yang, X., Wu, Y.Z. (2012) von Willebrand factor: more than a regulator of hemostasis and thrombosis. *Acta Haematologica* 128, 158–169.
- Lunsford, K. V, & Mackin, A. J. (2007). Thromboembolic therapies in dogs and cats: an evidence-based approach. The Veterinary Clinics of North America. Small Animal Practice, 37(3), 579–609.
- Macey MG, Carty E, Webb L, et al. Use of mean platelet component to measure platelet activation on the ADVIA 120 haematology system. Cytometry 1999;38:250–255.
- McBride, D., Hosgood, G., Raisis, A. (2016) Platelet closure time in anesthetized Greyhounds with hemorrhagic shock treated with hydroxyethyl starch 130/0.4 or 0.9% sodium chloride infusions. *Journal of Veterinary Emergency and Critical Care* 26, 509–515.
- McBride, D. (2017) Assessment of primary haemostatic function in dogs with acute kidney injury. Proceedings of the International Veterinary Emergency and Critical Care Society. September 2017, Washington, USA. pp 56.
- McCarroll, D.R., Waters, D.C., Steidley, K.R., Clift, R., McDonald, T.P. (1988) Canine platelet von Willebrand factor: quantification and multimeric analysis. *Experimental Hematology*, 16, 929–37.
- McLewee N., Archer, T., Wills, R., Mackin, A., Thomason, J. (2017) Effects of aspirin dose escalation on platelet function and urinary thromboxane and prostacyclin levels in normal dogs. *Journal of Veterinary Pharmacology* 41, 60–7.

- McMichael, M.A. (2005) Primary hemostasis. *Journal of Veterinary Emergency and Critical Care* 15, 1-8.
- McMichael, M.A., Smith, S.A., Galligan, A., Swanson, K.S. (2014) In vitro hypercoagulability on whole blood thromboelastometry associated with in vivo reduction of circulating red cell mass in dogs. *Veterinary Clinical Pathology* 43, 154–63
- van der Meer, P.F., Tomson, B., Brand, A. (2010) In vivo tracking of transfused platelets for recovery and survival studies: An appraisal of labelling methods. Transfusion and Apheresis Science. 42(1):53-61.
- Michiels, C., Arnould, T., Remacle, J. (2000) Endothelial cell responses to hypoxia: initiation of a cascade of cellular interactions. *Biochimica et biophysica acta* 1497, 1–10
- Mischke, R., Keidel, A. (2003) Influence of Platelet Count, Acetylsalicylic Acid, von Willebrand's Disease, Coagulopathies, and Haematocrit on Results Obtained Using a Platelet Function Analyser in Dogs. *The Veterinary Journal* 165, 43–52.
- Mischke, R., Schulze, U. (2004) Studies on platelet aggregation using the Born method in normal and uraemic dogs. *The Veterinary Journal* 168, 270–5.
- Moesgaard, S.G., Sørensen, T.M., Sterup, A., Tarnow, I., Kristensen, A.T., Jensen, A.L., et al. (2009) Changes in platelet function in Dachshunds with early stages of myxomatous mitral valve disease. *Respiratory Veterinary Science* 86, 320–4
- Moise, N.S. (2007) Presentation and management of thromboembolism in cats. *In Practice* 29, 2–8.
- Moritz, A., Walcheck, B.K., Weiss, D.J. (2005) Evaluation of flow cytometric and automated methods for detection of activated platelets in dogs with inflammatory disease. *American Journal of Veterinary Research* 66, 325–9

- Nakamura, R.K., Tompkins, E., Bianco, D. (2012) Therapeutic options for immune-mediated thrombocytopenia. *Journal of Veterinary Emergency and Critical Care* 10, 59-72
- Nelson, O.L., Andreasen, C. (2003) The Utility of Plasma D-dimer to Identify Thromboembolic Disease in Dogs. *Journal of Veterinary Internal Medicine* 17, 830–4.
- Nieswandt, B. (2003) Platelet-collagen interaction: is GPVI the central receptor? *Blood* 102, 449–61.
- O'Marra, S.K., de Laforcade, A.M., Shaw, S.P. (2011) Treatment and predictors of outcome in dogs with immune-mediated thrombocytopenia. *Journal of the American Veterinary Medical Association* 238, 346–352.
- H.O., Ogedegbe. (2002). An Overview of Hemostasis. *Toxicologic Pathology*, *21*(2), 170–179.
- Ordinas, A., Maragall, S., Castillo, R., et al (1978) A glycoprotein I defect in the platelets of three patients with severe cirrhosis of the liver. Thrombosis Research 13, 297–302.
- Paniccia, R., Priora, R., Liotta, A.A., Abbate, R. (2015) Platelet function tests: a comparative review. *Vascular Health and Risk Management* 11, 133–16.
- Park, F.M., Blois, S.L., Abrams-Ogg, A.C., Wood, R.D., Allen D. G., Nykamp, S.G., Downie, A., (2013) Hypercoagulability and ACTH-Dependent Hyperadrenocorticism in Dogs. *Journal of Veterinary Internal Medicine* 27, 1136–1142.
- Pathak, E.J. (2004) Type 3 von Willebrand's disease in Shetland sheepdog. Canadian Veterinary Journal. 45(8):685-687.
- Pedersen, H. D., Haggstro, M.J., Olsen, L.H., Christiansen, K., Selin, A., Burmeister, M.M.K., Larsen, H. (2002) Idiopathic asymptomatic thrombocytopenia in cavalier King Charles spaniels is an autosomal recessive trait. *Journal of Veterinary Internal Medicine* 16, 169-173.

892 • Peng, J., Friese, P., Wolf, R.F., Harrison, P., Downs, T., Lok, S., et al. (1996) Relative
893 reactivity of platelets from thrombopoietin- and interleukin-6-treated dogs. Blood 87, 4158–
894 63.
895 • Ralph, A. G., Brainard, B. M. (2014) Hypercoagulable states. In: *Small Animal Critical Care*
896 *Medicine* 2nd ed., Elsevier Health Sciences, Philadelphia.
897 • Respess, M., O'Toole, T.E., Taeymans, O., Rogers, C.L., Johnston, A., Webster, C.R. (2012)
898 Portal Vein Thrombosis in 33 Dogs: 1998-2011. *Journal of Veterinary Internal Medicine* 26,
899 230–237.
900 • Rose, L.J., Dunn, M.E., Allegret, V., Bédard, C. (2011) Effect of prednisone administration on
901 coagulation variables in healthy Beagle dogs. *Veterinary Clinical Pathology* 40, 426–34.
902 • Rose, L.J., Dunn, M.E., Bédard, C. (2012) Effect of canine hyperadrenocorticism on
903 coagulation parameters. *Journal of Veterinary Internal Medicine* 27, 207–11.
904 • Ridyard, A.E., Shaw, D.J., Milne, E.M. (2010) Evaluation of platelet activation in canine
905 immune-mediated haemolytic anaemia. *Journal of Small Animal Practice* 51, 296–304.
906 • Ruggeri, Z.M. (1999) Structure and function of von Willebrand factor. *Thrombosis and*
907 *haemostasis* 82, 576–584.
908 • Saati, S., Abrams-Ogg, A.C.G, Blois, S.L., Wood, R.D. (2017) Comparison of Multiplate,
909 Platelet Function Analyzer-200, and Plateletworks in Healthy Dogs Treated with Aspirin and
910 Clopidogrel. *Journal of Veterinary Internal Medicine* 32, 111–118
911 • Sadler, J.E. (1998) Biochemistry and genetics of von Willebrand factor. *Annual review of*
912 *biochemistry* 67, 395–424
913 • Sato, I., Anderson, G. A., Parry, B. W. (2000). An interobserver and intraobserver study of
914 buccal mucosal bleeding time in Greyhounds. Research in Veterinary Science, 68(1), 41–45

- Satta, N., Toti, F, Fressinaud, E., Meyer, D., Freyssinet, J.M. (2010) Scott syndrome: an inherited defect of the procoagulant activity of platelets. *Platelets* 8, 117–124.
- Sanford, J., Shattil, M. D., Bennett, J.S. (1981) Platelets and Their Membranes in Hemostasis: Physiology and Pathophysiology. *Annals of Internal Medicine* 94, 108-119
- Sanchez-Roig, M.J., Rivera, J., Moraleda, J.M., et al (1994) Quantitative defect of glycoprotein Ib in severe cirrhotic patients. *American Journal of Hematology* 45, 10–5.
- Sangkuhl, K., Shuldiner, A.R., Klein, T.E., Altman, R.B. (2011) Platelet aggregation pathway. *Pharmacogenetics and Genomics* 21, 516–21.
- Santoro, S.K., Garrett, L.D., Wilkerson, M. (2007) Platelet concentrations and platelet-associated IgG in greyhounds. *Journal of Veterinary Internal Medicine* 21, 107–12.
- Schneider, D.J. (2009) Factors Contributing to Increased Platelet Reactivity in People with Diabetes. *Diabetes care* 32, 525–527
- Scuderi, M., Bessey, L., Snead, E., Burgess, H., Carr, A. (2015) Congenital Type III von Willebrand's disease unmasked by hypothyroidism in a Shetland sheepdog. 65(9):937-41.
- Shahar, R., Harrus, S., Yakobson, B. (1998) Mesenteric vein thrombosis in a dog. *The Journal of the American Animal Hospital Association* 34, 431–433.
- Sharpe, K., Center, S., Randolph, J., Brooks, M., Warner, L. K., Stokol, T., et al. (2010) Influence of treatment with ultralow-dose aspirin on platelet aggregation as measured by whole blood impedance aggregometry and platelet P-selectin expression in clinically normal dogs. *American Journal of Veterinary Research* 71, 1294–304.
- Sigrist, N.E., Hofer-Inteeworn, N., Jud Schefer, R., Kuemmerle-Fraune, C., Schnyder, M., Kutter, A.P.N. (2017) Hyperfibrinolysis and Hypofibrinogenemia Diagnosed with Rotational

Thromboelastometry in Dogs Naturally Infected With Angiostrongylus vasorum. *Journal of Veterinary Internal Medicine* 31, 1091–9.

- Slauson, D. O, Gribble, D. H. (1971) Thrombosis complicating renal amyloidosis in dogs. *Veterinary Pathology* 8, 352–363.
- Smith, S.A., The cell-based model of coagulation. *Journal of Veterinary Emergency and Critical Care* (San Antonio). 2009;19(1):3-10.
- Smith, S.A., McMichael, M.A., Gilor, S., Galligan, A.J., Hoh, C.M. (2012) Correlation of hematocrit, platelet concentration, and plasma coagulation factors with results of thromboelastometry in canine whole blood samples. *American Journal of Veterinary Research* 73, 789–98.
- Smith, J.R., Smith, K.F., Brainard, B.M. (2014) Platelet parameters from an automated hematology analyzer in dogs with inflammatory clinical diseases. *The Veterinary Journal* 201, 406–11.
- Song, J., Drobatz, K.J. & Silverstein, D.C. (2016) Retrospective evaluation of shortened prothrombin time or activated partial thromboplastin time for the diagnosis of hypercoagulability in dogs: 25 cases (2006-2011). *Journal of Veterinary Emergency and Critical Care* 26, 398–405.
- Stokes, K.Y., Granger, D.N. (2012) Platelets: a critical link between inflammation and microvascular dysfunction. *The Journal of Physiology* 590, 1023–1034
- Sullivan. P., Gompf. R., Schmeitzel. L., Clift. R., Cottrell. M., McDonald. T.P. (1993) Altered platelet indices in dogs with hypothyroidism and cats with hyperthyroidism. *American Journal of Veterinary Research* 54, 2004–9

- Schnyder M, Tanner M, Webster P, Barutzki D, Deplazes P: An ELISA for sensitive and specific detection of circulating antigen of Angiostrongylus vasorum in serum samples of naturally infected dogs. Vet Parasitol 2011, 179:152–158.
- Tablin, F., Schumacher, T., Pombo, M., Marion, C.T., Huang, K., Norris, J.W., et al. (2014) Platelet activation in cats with hypertrophic cardiomyopathy. *Journal of Veterinary Internal Medicine* 28, 411–8.
- Teuber, M., Mischke, R. (2016) Influence of a low dosage of clopidogrel on platelet function in cats as measured by the platelet function analyser PFA-100 and the multiplate analyser. *Research Veterinary Science* 109; 149–56.
- Torrent, E., Leiva, M., Segales, J., Franch, J., Pena, T., Cabrera, B., et al. (2005) Myocarditis and generalised vasculitis associated with leishmaniasis in a dog. Journal of Small Animal Practice. 46, 549–52.
- Thomas, J.S. (1996) Von Willebrand's Disease in the Dog and Cat. *Veterinary Clinics of NA: Small Animal Practice* 26, 1089–1110.
- Thomason, J., Lunsford, K., & Mackin, A. (2016). Anti-platelet therapy in small animal medicine. *Journal of Veterinary Pharmacology and Therapeutics*, *39*(4), 318–335.
- Toom den, M.L., van Leeuwen, M.W., Szatmári, V., Teske, E. (2017) Effects of clopidogrel therapy on whole blood platelet aggregation, the Plateletworks® assay and coagulation parameters in cats with asymptomatic hypertrophic cardiomyopathy: a pilot study. Veterinary Quarterly 37, 8–15.
- Tunjungputri, R.N., Gasem, M.H., van der Does, W., Sasongko, P.H., Isbandrio, B., Urbanus, R. T., de Groot, P. G., van der Ven, A., de Mast, Q. (2017) Platelet dysfunction contributes to

bleeding complications in patients with probable leptospirosis. *PLOS Neglected Tropical Diseases* 11, 15–18.

- Turitto, V.T., Weiss, H.J. (1980) Red blood cells: their dual role in thrombus formation. *Science* 207, 541-543.
- Varela, F., Font, X., Valladares, J. E., Alberola, J. (1997) Thrombocytopathia and light-chain proteinuria in a dog naturally infected with Ehrlichia canis. *Journal of Veterinary Internal Medicine* 11, 309–311.
- Venta, P.J., Li, J., Yuzbasiyan-Gurkan, V., Brewer, G.J., Schall, W.D. (2000) Mutation Causing von Willebrand's Disease in Scottish Terriers. *Journal of Veterinary Internal Medicine* 14, 10–19.
- VETgirl. (2016) How to perform a How to Perform a BMBT in a Dog. VetGirl Veterinary CE Blog. Accessed 10th November 2018. https://vetgirlontherun.com/perform-buccal-mucosal-bleeding-time-bmbt-vetgirl-veterinary-ce-videos-blog/.
- Vischer, U.M. (2006) von Willebrand factor, endothelial dysfunction, and cardiovascular disease. *Journal of thrombosis and haemostasis* 4, 1186–1193.
- Wang, A., Brainard, B. M. Thrombocytopenia. In: *Small Animal Critical Care Medicine* 2nd ed., Elsevier Health Sciences, Philadelphia pp
- Waters, D.C., Eaton, A.H., Steidley, K.R., McCarroll, D.R. (1989) Expression of von Willebrand factor in plasma and platelets of cats. *American Journal of Veterinary Research* 50, 201–4
- Wei, A.H., Schoenwaelder, S.M., Andrews, R.K., Jackson, S.P. (2009) New insights into the haemostatic function of platelets. *British Journal of Haematology* 147, 415–430

- Weiss, D.J., Brazzell, J.L. (2006) Detection of Activated Platelets in Dogs with Primary Immune-Mediated Hemolytic Anemia. *Journal of Veterinary Internal Medicine* 20, 682
- Whitley, N.T., Corzo-Menendez, N., Carmichael, N.G., McGarry, J.W. (2005) Cerebral and conjunctival haemorrhages associated with von Willebrand factor deficiency and canine angiostrongylosis. *Journal of Small Animal Practice* 46, 75–78.
- Williams, D.A., Maggio-Price, L. (1984) Canine idiopathic thrombocytopenia: clinical observations and long-term follow-up in 54 cases. *Journal of American Veterinary Medicine Association* 185, 660–663.
- Williams, T.P., Shaw, S., Porter, A., Berkwitt, L. (2017) Aortic thrombosis in dogs. *Journal of veterinary emergency and critical care* 27, 9–22.
- Willis, S.E., Jackson, M.L., Meric, S.M., Rousseaux, C.G. (1989) Whole blood platelet aggregation in dogs with liver disease. *American Journal of Veterinary Research*. 50, 1893–7.
- Wolberg, A.S., Aleman, M.M, Leiderman, K., Machlus, K, R. (2012) Procoagulant Activity in Hemostasis and Thrombosis. *Anaesthesia & Analgesia* 114, 275–285.
- Wurlod, V.A., Howard, J., Francey, T., Schweighauser, A., Adamik, K.N. (2015) Comparison of the in vitro effects of saline, hypertonic hydroxyethyl starch, hypertonic saline, and two forms of hydroxyethyl starch on whole blood coagulation and platelet function in dogs. *Journal of Veterinary Emergency and Critical Care* 25, 474–487.
- Yilmaz, Z., Ilcol, Y.O., Ulus, I.H. (2005) Investigation of diagnostic importance of platelet closure times measured by Platelet Function Analyzer--PFA 100 in dogs with endotoxemia. Berl Munch Tierarztl Wochenschr 118, 341-8.

- Yozova, I.D., Howard, J., Henke, D., Dirkmann, D., Adamik, K.N. (2017) Comparison of the effects of 7.2% hypertonic saline and 20% mannitol on whole blood coagulation and platelet function in dogs with suspected intracranial hypertension - a pilot study. *BMC Veterinary Research* 13, 402.
- Yu, D., Noh, D., Park, J. (2015) Flow cytometric evaluation of disseminated intravascular coagulation in a canine endotoxemia model. *Canadian Journal of Veterinary Research* 79, 52.
- Zoia, A., Gerou-Ferriani, M., Drigo, M., & Caldin, M. (2018). Case-control study of plasma mean platelet component concentration and survival analysis for dogs with immune-mediated hemolytic anemia. *Journal of the American Veterinary Medical Association*, *252*(11), 1384–1392

Table Legend:

Table 1: Summary of key platelet receptors

Receptors	Main ligand	Function	Comments
α2β1 integrin *(previously GP1a-IIa)*	Collagen	Platelet adhesion	Firm adhesion after activation
GP VI	Collagen	Platelet adhesion	Initial tethering triggers intra-cellular signalling and activation of integrins
GPIb-IX-V	vWF	Platelet adhesion	Important in arterial circulation
$\alpha_{IIb}\beta_3$ integrin *(previously GPIIb-IIIa)*	Fibrinogen and vWF	Platelet aggregation	Allows fibrinogen binding and aggregation after activation
P2Y1 and P2Y12	ADP	Platelet agonist	ADP=Weak platelet agonist *Site of clopidogrel action*
5HT2	Serotonin	Platelet agonist	Serotonin=Weak platelet agonist
Prostaglandin receptors	Thromboxane Prostacyclin	Platelet agonist Platelet antagonist	Agonist and antagonist *Site of aspirin action*
Protease-activated receptors (PAR)	Thrombin	Platelet agonist	Thrombin=Strong agonist
αadrenergic receptor	Epinephrine	Platelet agonist	Enhances stimulation by other agonists

1041 Table 2: Summary of congenital disorders of platelet function

Congenital disorders	Type of defect	Specific mechanism	Breed affected	Clinical relevance
vWD	Extrinsic Adhesion	Absence or deficiency of GP1b-IX-V	See Table 5	
Bernard–Soulier syndrome	Extrinsic Adhesion	GPIb/V/IX deficiency	Cocker spaniel	Severe bleeding
Glanzmann thrombasthenia	Intrinsic Aggregation	Absence or deficiency of GPIIb-IIIa	Great Pyrenees and Otterhounds	Spontaneous mucosal haemorrhage
Scott Syndrome	Intrinsic Procoagulant deficiency	Impaired PS externalisation ⇓ prothrombinase	German shepherd dog	Postoperative haemorrhage and epistaxis
$P2Y_{12}$ receptor disorder	Intrinsic Prevention of agonist action	Impaired binding of ADP → reduced fibrinogen binding	Greater Swiss Mountain dog	Postoperative haemorrhage
Ca1DAG-GEFI thrombopathia	Intrinsic Signalling	Prevents GPIIb-IIIa conformation change for fibrinogen binding	Basset hound, Landseer and spitz	Spontaneous mucosal haemorrhage
Chediak-Higashi	Intrinsic Granular storage pool deficiency	Agonist deficiency. Absent aggregation response to collagen	Persian cats	Prolonged bleeding times
Delta-storage pool disease	Intrinsic Granular storage pool deficiency	Dense granule deficiency of ADP	American cocker spaniel	Postoperative haemorrhage

1042

1043

1044 Table 3: Summary of acquired disorders of platelet function

Acquired disorders	Reported mechanisms of platelet dysfunction	Evidence in veterinary species and relevance
Anaemia	Considered a rheological change *in vivo*, *i.e*., reduction in "near wall excess" (Turitto & Weiss 1980)	Hct < 35 g/L associated with hypocoagulability (Clancey *et al*. 2009a, 2009b). BMBT improves with blood transfusion (Brassard *et al*. 1994).An artificial hypercoagulability may be seen with thromboelastography in anaemic patients (McMichael et al., 2014).
Uraemia	Storage pool deficiencies; decreased response to agonists; abnormal calcium mobilisation; decreased TXA_2 synthesis and receptor deficiencies; effects of concurrent anaemia (Boccardo *et al*. 2004)	Induced uraemia in healthy dogs lead to increased BMBT but no clinical bleeding (Brassard *et al*. 1994). Dogs with clinical CKD had platelet dysfunction but were hypercoagulable on global viscoelastic testing (Dudley 2013). Dogs with AKI had decreased aggregation and type II vWD phenotype with high vWFAg:CBA which correlated with creatinine (McBride 2017).
Hepatopathy	Storage pool deficiencies (Laffi *et al*. 1992); decreased TXA_2 synthesis (Laffi *et al*. 1987); adhesion receptor deficiencies (Ordinas *et al*. 1978, Sanchez-Roig *et al*. 1994).	Dogs with hepatopathies have been shown to have reduced platelet aggregation (Willis *et al*. 1989)
IMT	Antibody against fibrinogen receptor likely causes additional platelet dysfunction (Kristensen *et al*. 1994)	Haemorrhage does not always correlate with degree of thrombocytopenia and additional platelet dysfunction may be involved Kristensen *et al*. (1994)
DIC	Decreased response to agonists (Li & Chan 2016); increased FDPs suggested to compete at platelet fibrinogen receptors, limiting aggregation **(**Bick 1988;	Clinical relevance of platelet dysfunction not known.

Acquired disorders	Reported mechanisms of platelet dysfunction	Evidence in veterinary species and relevance
	Gouin *et al*. 1992; de Laforcade *et al*. 2003).	
Monoclonal gammopathy	Coating of platelets with monoclonal or polyclonal proteins suspected to cause reduced aggregation. (Glaspy 1992; Varela *et al*. 1997).	Recurrent epistaxis in Ehrlichia-infected dog with normal platelet count, prolonged BMBT and abnormal aggregation (Varela *et al*. 1997)
Leptospirosis	Circulation of inappropriately activated platelets. Decreased response to platelet agonists. Increased vWF-platelet binding (Barthélemy *et al*. 2016; Tunjungputri *et al*. 2017).	Haematuria, melena, petechiae and epistaxis seen clinically. Hypocoagulability, as measured by TEG, associated with mortality; however, no platelet function test performed (Barthélemy *et al*. 2016)
Angiostrongylus vasorum	Hyperfibrinolysis (Adamantos *et al*. 2015; Sigrist *et al*. 2017). Increased FDPs suggested to compete at platelet fibrinogen receptors, limiting aggregation (Bick 1988; Gouin *et al*. 1992).	Spontaneous bleeding reported in one third of dogs. Currently no studies investigating platelet function specifically (Adamantos *et al*. 2015; Sigrist *et al*. 2017)

1045

1046

1047 Table 4: Drugs causing platelet dysfunction

Medication	Mechanism of platelet dysfunction	Duration of action	Comments
ADP receptor antagonists			
Clopidogrel	Irreversible P2Y12 inhibitor	Life time of platelet. Normal function returns 5 to 10 days after single dose	Active metabolite requires cytochrome P450 pathway
Ticagrelor	Reversible P2Y12 inhibitor	Reversible action results in shorter duration compared to clopidogrel	Usage only reported in people
Thromboxane inhibitors			
Non-selective COX inhibitor *e.g*. aspirin	Irreversible inhibition of TXA^2 formation via action on the COX-1 enzyme	Life time of platelet. Normal function returns 7 to 10 days after single dose	Not all dogs show response to empirical therapy
Relatively COX-1 selective NSAIDs (*e.g*. ketoprofen)	Reversible inhibition of TXA2 formation via action primarily on the COX-1 enzyme	Dependent on specific drug, serum levels and half-life.	Inhibition of platelet function seen at clinical doses. No association with bleeding tendency in healthy animals
Relatively COX-2 selective NSAIDs (*e.g*. carprofen, meloxicam)	Reversible inhibition of TXA2 formation via some action on the COX-1 enzyme	Dependent on specific drug, serum levels and half-life.	Clinically relevant inhibition of platelet aggregation not expected
αIIbβ3 integrin *(GP IIb/IIIa)* receptor antagonists			
Abciximab, tirofiban, and eptifibatide	Blockage of receptor prevents fibrinogen and other ECM binding	Unknown	Only reported in experimental studies of dogs/cats

1048

1049

1050 Table 5: Types of von Willebrand's disease

Type	Breeds affected	Multimer concentration	Multimeric size	Clinical importance
1	Doberman, Corgis, Airedale Terriers, and various others breeds	Low	Full spectrum of sizes	Mild to moderate bleeding tendency
2	German Short Haired pointer	Variable	Absence of large multimers	Moderate to severe bleeding tendency
3	Dutch kooiker, Scottish terrier, Shetland sheepdog	Marked reduction or absence of all multimers	N/A	Mild to severe bleeding

1051

1052 Table 6: Summary of diagnostic options for dogs with suspected von Willebrand's factor

Diagnostic test	Clinical utility	Result interpretation	Comments
BMBT	Indicates presence of primary haemostatic disorder	Consider further testing if: Dog: >4 minutes Cat: >2.5 minutes	High inter- and intraobserver variability Not specific for vWD
Plasma vWF antigen ELISA (VWF:Ag)	Diagnosis of type I and III vWD	Normal: 70 to 180% Borderline: 50 to 69% Abnormal:0 to 49%	Influenced by a variety of other physiological and pathological factors
Plasma vWF CBA ELISA (vWF:CBA)	Detects decreased CBA relative to vWF:Ag	VWF: CBA in normal/type 1 dogs=50 to 170% Type 2 dogs typically >2.0	Not routinely available.
Genetic testing	Detection of causative mutation	Positive or negative	Mode of inheritance not completely understood in Type 1 disease

Diagnostic test	Clinical utility	Result interpretation	Comments
Platelet function analyser	Aperture closure time prolonged with severe vWF deficiency	References ranges not available and non-specific	Used in humans as a screening test for vWD

1053

1054 Table 7: Potential sites of thromboembolic disease

Site of thromboembolism	Clinical signs	Diagnostic tests available
Pulmonary thromboembolism (Johnson *et al*. 1999).	Respiratory changes ranging from tachypnoea to dyspnoea and potentially cyanosis.	CT angiography
Cerebrovascular accidents (Garosi 2010)	Acute onset neurological signs *e.g*. lateralised motor deficits or seizures.	MRI
Occlusions of peripheral or central veins (Williams *et al*. 2017; Moise 2007	Ischaemic damage to the limbs: Acute onset limb paralysis with cold extremities, firm painful muscles, and non-palpable pulse distal to the thromboembolism. Swelling of the face.	Ultrasound
Occlusion of abdominal veins (Slauson & Gribble 1971; Shahar *et al*. 1998; Laurenson *et al*. 2010; Respess *et al*. 2012)	Ischaemic damage to abdominal organs (hepatic, splenic, renal and mesenteric vessels). Acute abdominal pain and signs related to specific organ dysfunction such as acute kidney injury, hepatopathy.	Ultrasound

1055

1056

1057 Table 8: Summary of acquired platelet disorders with proposed mechanism

Acquired disorders	Reported mechanisms of platelet dysfunction in people and dogs	Veterinary evidence and clinical relevance
Systemic Inflammation - septic and non-septic, *e.g*. pancreatitis	Increased thrombin formation leads to platelet activation; cytokines enhance endothelial adhesion; EC dysfunction decreases NO and prostacyclin production (Cheng *et al*. 2011).	Cytokines shown to increase reactivity to thrombin (Peng *et al*. 1996); Endotoxin administration or SIRS increases P-selectin expression (Yu *et al*. 2015). Clinical relevance is not known and there is insufficient data to support routine anticoagulation (Goggs *et al*. 2019).
Glomerulopathies	Loss of inhibitors including antithrombin III; glomerular EC dysfunction and reduced NO (Chen *et al*. 2013).	Incidence of thromboembolism in dogs with PLNs reported as high as 25%; albumin nor antithrombin levels can be used to predict risk of thromboembolic disease (Lennon *et al*. 2013). Antithrombotic medication is recommended (Goggs *et al*. 2019).
IMHA	Release of intraerythrocytic ADP activates platelets; free haemoglobin scavenges NO (Helms *et al*. 2013).	Thromboembolism reported in 46 to 80% of dogs at *post mortem* (Carr *et al*. 2002); usually hypercoagulable but relative hypocoagulability as measured by global viscoelastic testing is a negative prognostic indicator (Goggs *et al*. 2012); circulating activated platelets shown by presence of P-selectin or low plasma mean platelet component (MPC) concentration (Weiss & Brazzell 2006, Ridyard *et al*. 2010, Zoia *et al*. 2018). Antithrombotic medication is recommended (Goggs *et al*. 2019).
Hypercortisolaemia: iatrogenic or hyperadrenocorticism	Increased fibrinogen and thrombin-antithrombin complexes promote aggregation (Kerlin *et al*.	Both hypercoagulability (Halmay et al., 2008; Park 2013; Rose et al., 2011; Rose et al., 2013) and hypocoagulability (Klose 2011) as

Acquired disorders	Reported mechanisms of platelet dysfunction in people and dogs	Veterinary evidence and clinical relevance
	2004,Klose *et al*. 2011 , Park *et al*. 2013).	measured by global viscoelastic testing has been reported but these studies are not specific for platelet function. TEG measurement did not normalise in well-controlled dogs, therefore increased plasma glucocorticoid concentration may not be solely responsible (Klose *et al*. 2011, Park *et al*. 2013). Routine antithrombotic medication is not recommended unless other risk factors for thrombosis are present (Goggs *et al*. 2019).
Diabetes mellitus	Hyperglycaemia activates platelets and promotes expression of fibrinogen receptors, in addition to systemic inflammation and EC dysfunction (Schneider 2009).	Prevalence in dogs has not been documented.
Cardiomyopathy	Platelets circulate in an activated state likely secondary to EC dysfunction (Tablin *et al*. 2014).	Prevalence reported at 0.3% (Borgeat 2013); platelet function testing does not differ between healthy and subclinical cats (Jandrey *et al*. 2008); clopidogrel and aspirin reduce the likelihood of recurrent ATE (Hogan 2015). Routine antithrombotic medication is recommended in cats, particularly those with left atrial dilation, spontaneous echocontrast, or reduced left atrial appendage flow velocity (Goggs *et al*. 2019). Canine cardiac diseases are not associated with a high risk for development of thrombosis and routine anti-thrombotics are not recommended (Goggs *et al*. 2019).

1058

Figure Legends:

Figure 1. Key anatomical features of platelets

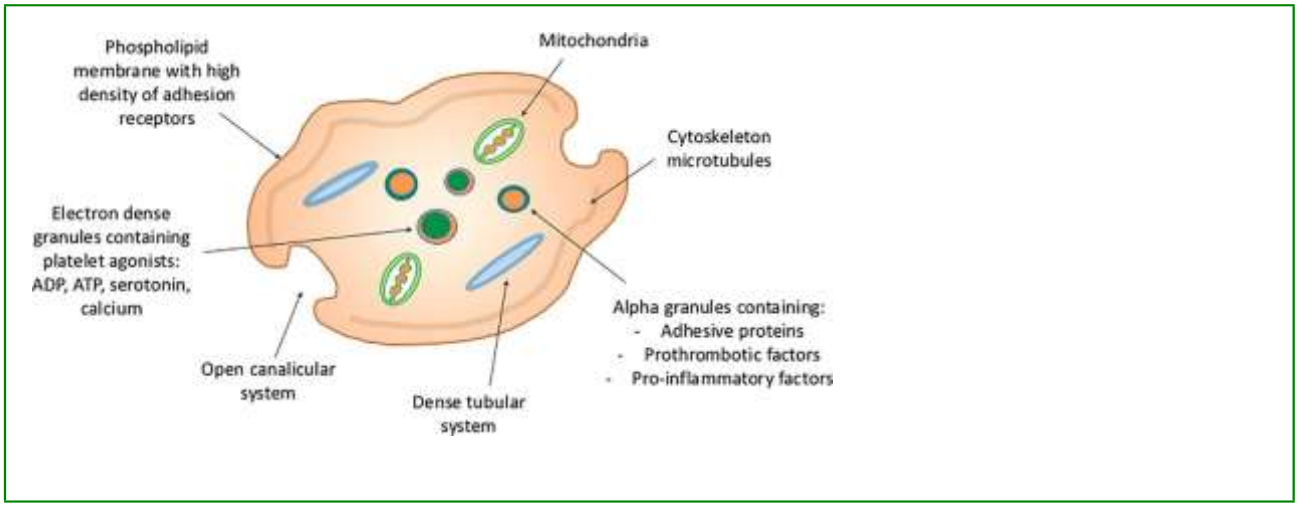


Figure 2. Illustration of key receptors mediating platelet adhesion

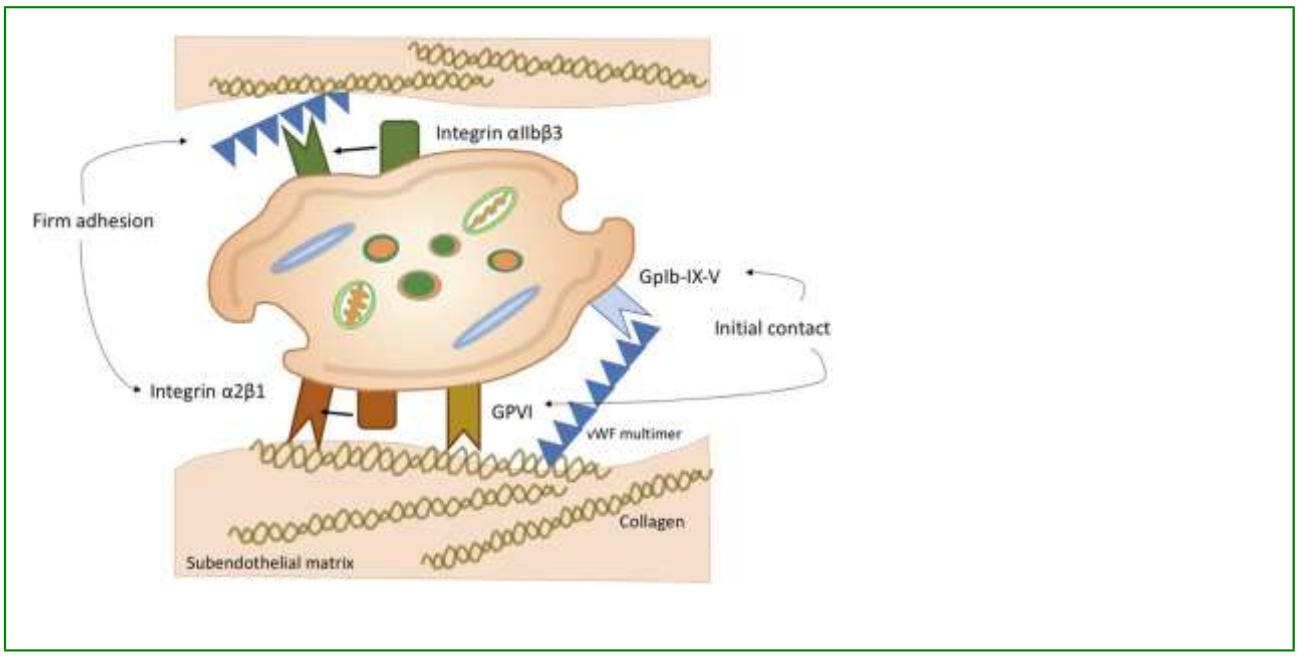


Figure 3. Illustration of intracellular signalling pathways

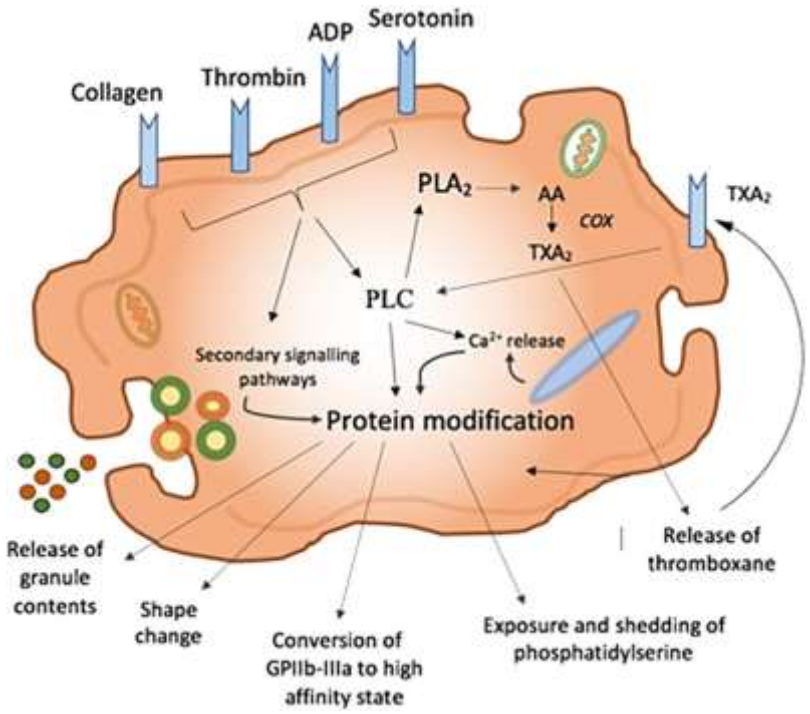


Figure 4. Diagnostic steps for patients presenting with platelet type bleeding disorders

1. Perform complete blood count and exclude thrombocytopenia with blood smear (Figure 5)
2. Perform prothrombin time and activated partial thromboplastin time
3. Perform buccal mucosal bleeding time (Figure 8)
4. Submit vWF antigen assay
5. Perform infectious antibody, PCR or antigen screening depending on clinical suspicion
6. Genetic testing
7. If all of the above are normal consider platelet function testing

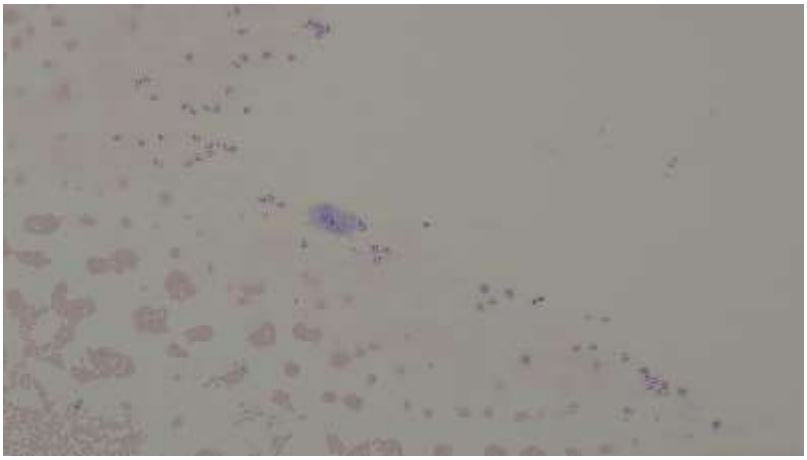
Figure 5. Exclusion of thrombocytopenia as a cause of platelet type bleeding using a blood smear

1. Using an appropriately filled and well mixed EDTA blood sample, make a blood smear. Ensure adequate staining of slide.
2. Assess the sides and the feathered edge of the smear for platelet clumping at 10 x magnification (see figures 6 and 7). Presence of clumps will falsely decrease the platelet count.
3. Using the 100 x magnification with oil immersion, count the number of platelets per high power field in the monolayer. Assess for macroplatelets.
4. Repeat over 10 fields to calculate an average platelet count.
5. Multiply the average (P) by 15 to estimate the platelet count: (Px15) x10^9/L

1075

1076

1077 Figure 6. Canine platelet clump (Modified-Wrights stain x 50 magnification)



1078

1079

Figure 7. Feline platelet clump (Modified-Wrights stain x100 magnification)

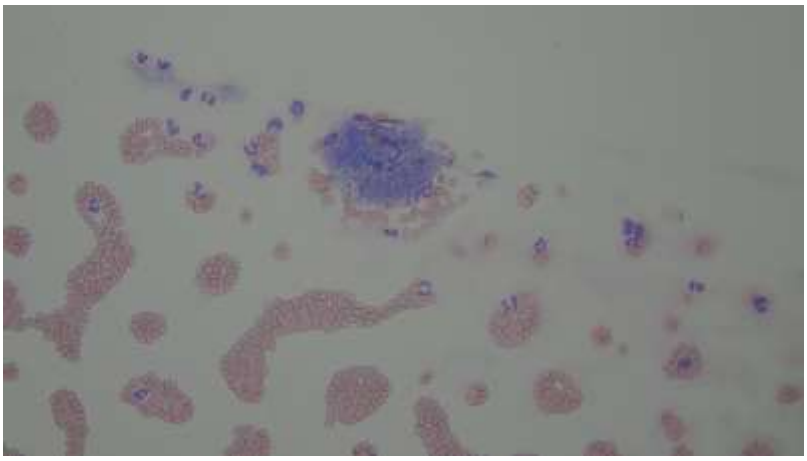


Figure 8. Performing a buccal mucosal bleeding time

See video on VETgirl website: https://vetgirlontherun.com/perform-buccal-mucosal-bleeding-time-bmbt-vetgirl-veterinary-ce-videos-blog/

1. Restrain patient in lateral or sternal recumbency.
2. Gently fold up the upper lip to expose the mucosal surface using a gauze strip. Do not tie the gauze strip excessively as it can occlude venous blood flow.
3. Select an area that is free of visible blood vessels.
4. Remove the guard from a buccal mucosal bleeding time lancet device and apply gentle pressure to the mucosal surface.
5. Firing the lancet causes the blade to make a controlled incision on the mucosa.
6. Commence timing as the incision starts to bleed. Use filter paper to blot excess blood from the mucosa, with care not touch the incision directly as this will disturb clot formation.
7. Buccal mucosal bleeding time is complete at the time when bleeding discontinues.