REVIEW

Abnormal platelet activity in dogs and cats – impact and measurement

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Abnormal platelet activity can either lead to bleeding tendencies or inappropriate thrombus formation and can occur secondarily 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 applicable to veterinary practice. Recent advances in platelet function testing will then be discussed, with regards to detection of platelet dysfunction and tailoring of pharmacological manipulation. Although many of these tests are still confined to research or academic institutions, techniques for indirectly assessing platelet function are starting to become more widely 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 practice.

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, Wei *et al.* 2009, Christopherson *et al.* 2012). 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 and inhibition, abnormalities in platelet function can result in either 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 aims to summarise the clinically relevant aspects of platelet anatomy and physiology and identify 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 to 5 days. Platelet lifespan in laboratory dogs has been reported at 6.0 ±1.1 days (van der Meer *et al.* 2010). No published data is available for cats. Platelet size varies both within and between species, with cats generally having smaller platelets (Sullivan *et al.* 1993) but with variation also between dog breeds (Lawrence *et al.* 2013). Most notable is the syndrome of macrothrombocytopaenia in cavalier King Charles spaniels and Akitas and the relative thrombocytopenia in greyhounds (Pedersen *et al.* 2002, Santoro *et al.* 2007, Hayakawa

et al. 2016). These do not result in a bleeding tendency because an effective total platelet mass is maintained.

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

1. Phospholipid membrane with high density of regulated adhesive receptors (see Table 1).
2. Cytoskeleton consisting of abundant contractile proteins (*e.g.* actin and myosin) that allow shape change.
3. A dense tubular system which can sequester or release calcium.
4. 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 and can 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 & 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 α2β1 integrin
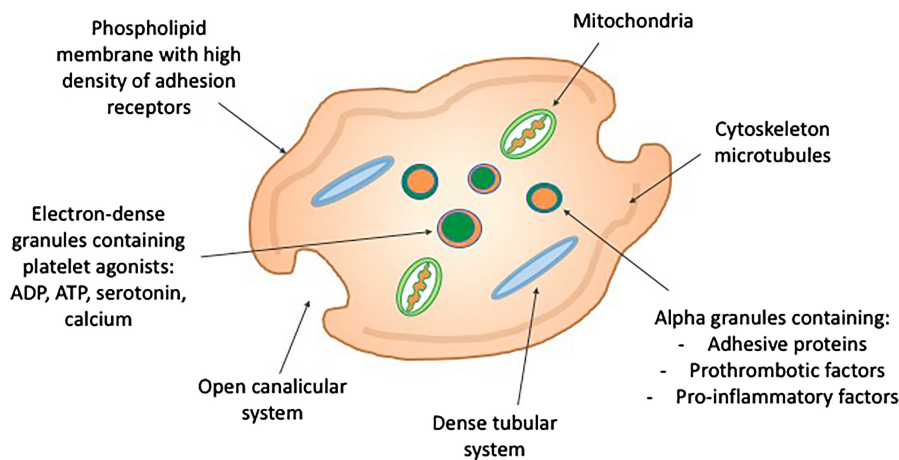

FIG 1. Key anatomical features of platelets

Table 1. Summary of key platelet receptors (McMichael 2005, Brass 2010)			
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	Platelet agonist	Agonist and antagonist
	Prostacyclin	Platelet 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
GP Glycoprotein, vWF Von Willebrands Factor, ADP Adenosine di-Phosphate			

or indirectly via the $α_{IIb}β_3$ integrin using vWF (Nieswandt 2003, Auton *et al.* 2010, Brass 2010). These adhesions are depicted in Fig 2.

vWF is a large glycoprotein which forms polymers called multimers. These exist in a range of 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 (McCarroll *et al.* 1988, Waters *et al.* 1989).

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 *et al.* 2002). Lower levels of ADAMTS-13 have been reported in people secondary to neoplastic, inflammatory or autoimmune conditions (Banno *et al.* 2006).
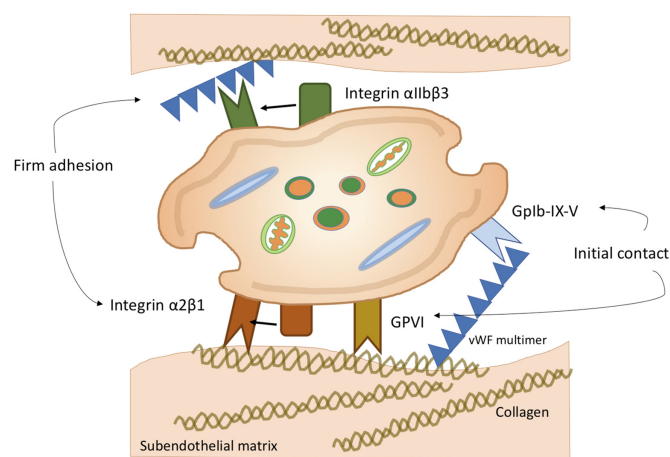

FIG 2. Illustration of key receptors mediating platelet adhesion

Activation and amplification

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

1. Conversion of integrin receptors, most importantly $α_{IIb}β_3$, to a high affinity state.
2. Membrane "flipping" to expose the phospholipid "phosphatidylserine". 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 fibrinclot 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 *et al.* 2015).

Calcium release is triggered by 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 arachidonic acid which are converted to thromboxane A_2 (TXA_2) by cyclooxygenase (COX) enzymes. TXA_2 is a potent platelet agonist, allowing both autocrine and paracrine signalling. This pathway can be blocked by inhibitors of COX enzymes (Floyd & Ferro 2013; Reviakine 2015). Some of these intracellular signalling pathways are illustrated in Fig 3.

Aggregation

Aggregation of platelets refers to the adhesion of activated platelets to each other using receptor bound fibrinogen as a bridge. The $α_{IIb}β_3$ 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, Sangkuhl *et al.* 2011).
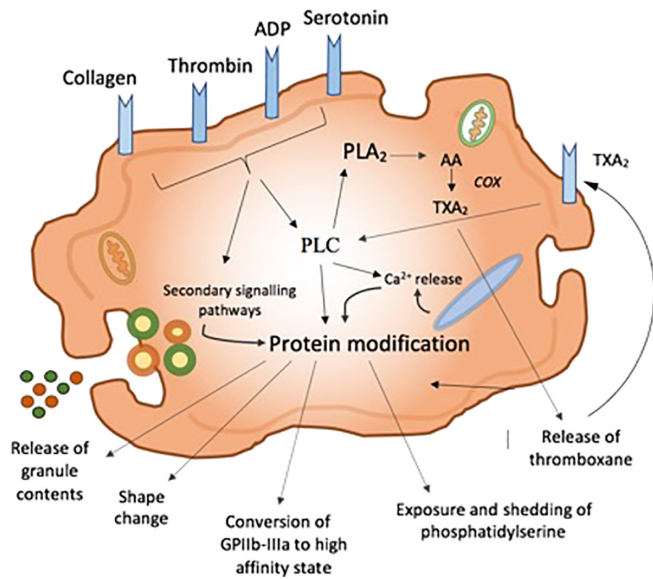
FIG 3. Illustration of intracellular signalling pathways

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 & 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 before further investigation of platelet function. A platelet count of 150×10^9/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 30×10^9/L have been associated with spontaneous haemorrhage in dogs with idiopathic thrombocytopenia (Williams & Maggio-Price 1984) but concurrent platelet or endothelial dysfunction can result in bleeding at higher platelet counts (Torrent *et al.* 2005, Ferkau *et al.* 2013). Thrombocytopenia will not be covered further in this article as it is well described elsewhere (Johnstone 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 lacks 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 & 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 & 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.* 2019).

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

However, as in people, some dogs and cats have been noted to be poor responders to anti-platelet medication and are con-

Table 2. 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

vWD Von Willebrand Disease, ADP Adenosine di-Phosphate, PS Phosphatidylserine, GP Glycoprotein
Readers are referred to the following book chapters for more detail (Jandrey 2014, Callan & Catalfamo 2017)

Table 3. Acquired disorders of platelet dysfunction

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; Gouin *et al*. 1992; de Laforcade *et al*. 2003).	Clinical relevance of platelet dysfunction not known.
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)

Hct haematocrit, BMBT, buccal mucosal bleeding time, TXA$_2$ Thromboxane A$_2$, CKD Chronic kidney disease, vWD Von Willebrand's disease, IMT Immune-mediated thrombocytopenia, DIC Disseminated intravascular coagulation, FDPs Fibrinogen degradation products, vWF Von Willebrand's factor, AKI Acute kidney injury
*Near wall excess: phenomenon in which platelets are displaced by red blood cells to the lateral margin of flowing column of blood

sidered "resistant" [also known as "high on-treatment platelet reactivity" (HTPR)]. Aspirin "resistance" in dogs has a reported prevalence ranging from 19 to 56% (Dudley *et al*. 2013, Haines *et al*. 2016). There is no published prevalence of drug resistance in cats. High interindividual variability in clopidogrel plasma levels has been reported, although this was considered mostly secondary to differences in metabolism (Lee *et al*. 2019). The results of the FAT CAT trial (Hogan et al. 2015) did suggest

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

ADP Adenosine diphosphate, COX Cyclooxygenase, ECM Extracellular matrix proteins, GP Glycoprotein, NSAIDs Non-steroidal anti-inflammatory drugs, TXA2 Thromboxane

that clopidogrel is superior 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 *et al*. 2010; Mullins *et al*. 2012). One *in vivo* study demonstrated reduced platelet aggregation but no increase in buccal mucosal bleeding time (BMBT) in dogs which received pre-operative ketoprofen prior to ovariohysterectomy (Lemke *et al*. 2002). A study in cats also documented no decrease in aggregation following 14 days of meloxicam (Cathcart *et al*. 2012). Data for other NSAIDs is lacking and their peri-operative use in patients, especially those with concurrent primary haemostatic defects, should be carefully considered. Specific guidelines for the peri-operative use of anti-platelet medication are discussed in detail in the previously mentioned consensus statement and depends on the risk of thrombosis and the type of procedure (Goggs *et al*. 2019).

Intravenous fluid therapy is also known to cause platelet dysfunction. Some canine studies have suggested that only hypertonic saline, and not hydroxyl starches (HES) (specifically 130/0.4), result in statistically significant changes to platelet function beyond that associated with haemodilution alone (Wurlod *et al*. 2015, McBride *et al*. 2016). An *in vitro* study in healthy dogs also reported evidence of platelet dysfunction associated with anaemia secondary to haemodilution but its clinical importance was questioned (Clancey *et al*. 2009a, 2009b). A more recent study investigating resuscitation fluids in a canine haemorrhagic shock model suggested that 20 mL/kg of 4% succinylated gelatin was associated with reduced platelet

function compared to 20 mL/kg fresh whole blood or 6% HES (130/0.4) or 80 mL/kg crystalloids. Additional significant global coagulation abnormalities were noted in the HES group. Shock alone was associated with a mild increase in platelet function suggestive of hypercoaguability (Claus *et al*. 2018). In a feline study, HES reduced coagulation to a greater degree than a balanced crystalloid solution alone, but platelet function was not assessed specifically (Albrecht *et al*. 2016). Mannitol has also been shown to impair platelet aggregation but not at clinically relevant dilutions (Adamik *et al*. 2015, Yozova *et al*. 2017). In summary, there are 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, we 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 to 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 pre-

senting with mild mucosal bleeding (Pathak 2004, Scuderi *et al*. 2015). The different types of vWD are summarised in Table 5.

In cats, there have been only two 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 concentrations(vWF:Ag), (Brooks 1999). Dogs with type II vWD may have normal vWF:Ag but 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 which are commercially available to our knowledge. A summary of the diagnostic tests available for vWD are given in Table 6. Various factors may interfere with these diagnostic tests (*e.g.* systemic illness and surgery can affect vWF:Ag) (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 *et al*. 2012).

Endothelial dysfunction

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 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 & 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).

Table 5. Types of von Willebrand disease (Brooks 1999)

Type	Breeds affected	Multimer concentration	Multimeric size	Clinical importance
1	Doberman, corgi, Airedale terrier, 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

Table 6. Summary of diagnostic options for dogs with suspected vWF

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
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

BMBT Buccal mucosal bleeding time, vWF Von Willebrands factor, ELISA Enzyme-linked immunosorbent assay, CBA Collagen binding activity, Ag Antigen

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 & Brainard 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 TE 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 predispose for TE disease. Disease processes resulting in either overt TE disease or microvascular thrombosis are summarised in Table 8. Although there is evidence of global

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 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

Table 8. Summary of acquired platelet disorders with proposed mechanisms

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.* 2004, Klose *et al.* 2011, Park *et al.* 2013).	Both hypercoagulability (Halmay *et al.* 2008; Park 2013, Rose *et al.* 2011; Rose *et al.* 2013) and hypercoagulability (Klose *et al.* 2011) as 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 *et al.* 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).

EC Endothelial cell, NO Nitric oxide, PLN Protein losing nephropathy, IMHA Immune-mediated haemolytic anaemia, ADP Adenosine diphosphate, SIRS Systemic inflammatory response syndrome, ATE Arterial thromboembolism

hypercoagulability with TE 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 Fig 4 and outlined below:

PERFORM A COMPLETE BLOOD COUNT (CBC) AND BLOOD SMEAR ASSESSMENT

A CBC is required to exclude anaemia and thrombocytopenia before platelet function testing (see Fig 5). True platelet number and size should also be assessed with a good quality, stained blood smear as automated blood cell counters may report pseudothrombocytopaenia. In cats this usually occurs because of platelet clumping (Fig 6). There is also considerable overlap between erythrocyte and platelet volumes, resulting in misclassification by impedance analysers (Wang & Brainard 2014).

1. **Complete blood count and exclude thrombocytopenia with blood smear (Figure 5)**
2. **Prothrombin time and activated partial thromboplastin time**
3. **Buccal mucosal bleeding time (Figure 8)**
4. **VWF antigen assay**
5. **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**

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

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

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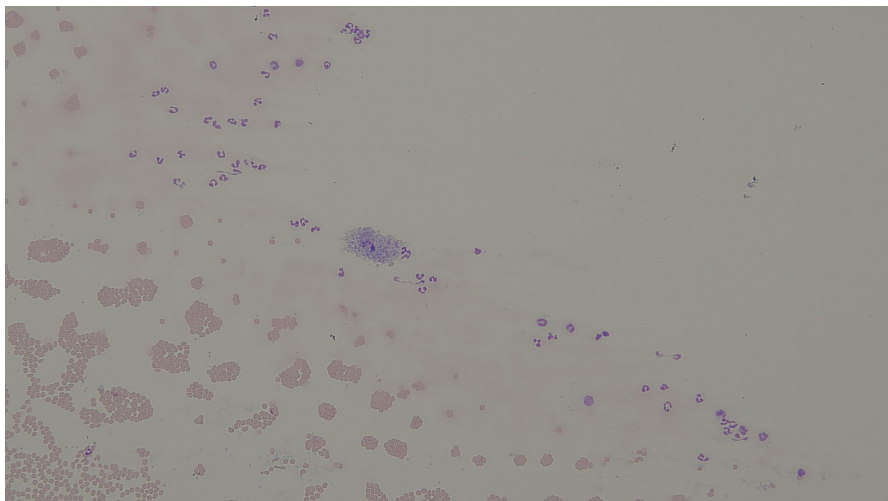

FIG 6. Canine platelet clump (Modified-Wrights stain x50 magnification)

PERFORM PT/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 (Fig 7).

PERFORM BMBT

BMBT is indicated in patients with evidence of bleeding but normal platelet count and PT/APTT (Fig 8). A specific BMBT lancet device must be used. Reference intervals 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; Alatzas *et al.* 2014). In a patient in which 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 interobserver and intraobserver variability (Sato *et al.* 2000, Alatzas *et al.* 2014). 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.

VWF 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 because 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.

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 com-
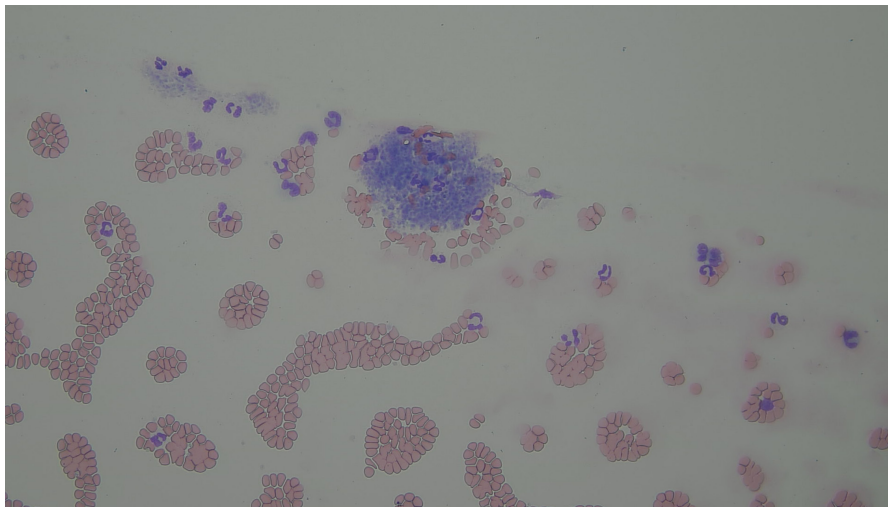

FIG 7. Feline platelet clump (Modified-Wrights stain x100 magnification)

See video on VETgirl website: (VETgirl 2006)
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.

FIG 8. Performing a buccal mucosal bleeding time

mon 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 *et al.* 2011); however, a recent study suggests that quantitative PCR of bronchoalveolar lavage may have much better sensitivity (Canonne *et al.* 2016). Leptospirosis can also cause platelet dysfunction and diagnostic testing should be based on clinical suspicion.

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.

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 TE disease, a thorough 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 & Andreasen 2003, Dewhurst *et al.* 2008, Epstein *et al.* 2013). Prothrombin time and PTT values below the reference interval 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 cath-

eter 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 2 hours of sample collection. If unexpected results are obtained at any stage, then new samples should be collected and analysed.

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 *et al.* 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, although at the time of this publication, their availability in veterinary practice is limited to referral institutes.

Assessment of platelet adhesion under shear stress
Aperture closure instruments: *e.g. Platelet function analyser (PFA) 100 or 200*

1. 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 & Born 1985, Harrison & Mumford 2009).
2. The generation of shear stress means the PFA is the only test able to detect abnormalities of platelet adhesion.
3. 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.
4. 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 (Gaál *et al.* 2007, Dudley 2013, Haines *et al.* 2016, Ho *et al.* 2016, McLewee *et al.* 2017, Saati *et al.* 2017).
5. The PFA 100 and 200 have also been used to investigate platelet function in dogs with valvular disease and cats with cardiomyopathy (Jandrey *et al.* 2008, Moesgaard *et al.* 2009,

Clancey *et al.* 2009a, 2009b), as well as dogs with chronic kidney disease and endotoxaemia (Yilmaz *et al.* 2005, Dudley *et al.* 2017). The PFA has also been used to diagnose Scott syndrome (Brooks *et al.* 2009). In people, the PFA is used as a screening test for vWD (Ardillon *et al.* 2015). One study reported increased CTs in 2 dogs with vWD that responded to DDAVP treatment (Burgess *et al.* 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 (Hvas and Favalora 2017).
- 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 *et al.* 2008). More recently LTA has been used alongside the PFA to investigate the optimum dose of aspirin in dogs (McLewee *et al.* 2017).

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 (Kalbantner *et al.* 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 & 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; Ho *et al.* 2016, (Toom den *et al.* 2017).

Global assessment

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 *et al.* 2010, Brainard *et al.* 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 *et al.* 2010, Brainard *et al.* 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. (Craft *et al.* 2004).
- Platelet mapping has been validated in healthy dogs (Blois *et al.* 2013) and has been utilised in studies investigating clopidogrel efficacy (Brainard *et al.* 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 & Chan 2015).

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 (Boudreaux *et al.* 1996).
- Markers of activation (such as P-selectin) have been used to investigate platelet activity in conditions such as sepsis (Moritz *et al.* 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 *et al.* 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).

In 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 TE disorders.

Conflict of interest

No conflicts of interest have been declared.

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