

This author's accepted manuscript may be used for non-commercial purposes in accordance with [Wiley Terms and Conditions for Self-Archiving](#).

The full details of the published version of the article are as follows:

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>

1 Abnormal platelet activity in dogs and cats – impact and measurement

2

3 P. Gant\*<sup>1</sup>, D. McBride<sup>1</sup>, K. Humm<sup>1</sup>

4 <sup>1</sup> Queen Mother Hospital for Animals (QMHA), The Royal Veterinary College, Hatfield,

5 Hertfordshire, AL9 7TA, UK

6 \* Corresponding author email: [pgant@rvc.ac.uk](mailto:pgant@rvc.ac.uk)

7

8 **Abstract:**

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

20

21 **Keywords:**

22 Platelet; haemostasis; coagulation; haemorrhage; thrombosis

23

24 **Introduction:**

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

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

44

45 **Normal platelet anatomy and physiology:**

46 Mammalian platelets are anucleate, cytoplasmic fragments that are liberated from megakaryocyte  
47 precursors during thrombopoiesis. The time taken for megakaryocyte maturation and release of  
48 platelets is approximately 3-5 days. Platelet lifespan in laboratory dogs has been reported at 6.0  
49 +/- 1.1 days (van der Meer 2010). No published data is available in cats. Platelet size varies both  
50 inter and intra species, with cats generally having smaller platelets (Sullivan et al. 1993) but with  
51 variation also existing between dog breeds (Lawrence 2013). Most notable is the syndrome of  
52 macrothrombocytopenia in Cavalier King Charles Spaniels (CKCS) and Akitas and the relative  
53 thrombocytopenia in greyhounds (Pederson et al. 2002; Hayakawa 2016; Santoro 2007). These do  
54 not result in a bleeding tendency as an effective total platelet mass is maintained.

55

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

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

68

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

76

#### 77 Adhesion:

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

88

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

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

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

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

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

113

114 Activation and amplification:

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

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

126

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

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

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

139 The phospholipase A<sub>2</sub> (PLA<sub>2</sub>) pathway is activated indirectly by the PLC pathway and by initial  
140 platelet aggregation. This pathway results in the breakdown of phospholipids to arachidonic acid  
141 which are converted to thromboxane A<sub>2</sub> (TXA<sub>2</sub>) by cyclooxygenase (COX) enzymes.  
142 Thromboxane A<sub>2</sub> is a potent platelet agonist, allowing both autocrine and paracrine signalling. This  
143 pathway can be blocked via inhibitors of COX enzymes (Floyd and Ferro 2013; Reviakine 2015).  
144 Some of these intracellular signalling pathways are illustrated in figure 3.

145

#### 146 Aggregation:

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

152

#### 153 **Inhibition of primary haemostasis:**

154 Platelet aggregation must also overcome several inhibitory mechanisms:

- 155 1. Endothelial cells release prostacyclins and adenosine diphosphatase (ADPases). These  
156 either inhibit TXA<sub>2</sub> production via the PLA<sub>2</sub> pathway or activate cAMP pathways to inhibit  
157 platelet activation (Gale 2011).
- 158 2. A layer of negatively charged glycosaminoglycans (GAGs) and proteoglycans, called the  
159 'glycocalyx', lines intact vessels and inhibits thrombin formation. The glycocalyx also



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

162

163 **Abnormalities of platelet function resulting in haemorrhage:**

164 Haemorrhage can occur secondary to quantitative and/or qualitative disorders of platelets, vWF or  
165 vasculopathies. ‘Platelet-type bleeding’, which manifests as ecchymoses and/or petechiae and  
166 intraoperative bleeding, can generally be differentiated from disorders of secondary haemostasis,  
167 in which intra-cavitary bleeding, haematomas and haemarthrosis are more common. Mucosal  
168 bleeding (which can be clinically silent or present as urinary or gastrointestinal bleeding) can be  
169 seen with either primary or secondary haemostatic disorders (Gale 2011; Jandrey 2012; Jandrey  
170 2014).

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

180

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

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

189

#### 190 Congenital thrombocytopathias

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

195

#### 196 Acquired thrombopathia:

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

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

204 information is available in a couple of review articles (Lunsford and Mackin 2007, Thomason et  
205 al., 2016). The interested reader is also referred to a recent consensus statement which summarises  
206 the indications for antithrombotic medication using the current evidence available (Goggs et al.  
207 2018).

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

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

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

224 The effects of non-steroidal anti-inflammatory drugs (NSAIDs) on *in vitro* platelet aggregation are  
225 variable (Gaál et al. 2007; Blois 2010; Mullins 2012). One *in vivo* study demonstrated reduced  
226 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

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

252

253 Von Willebrand Disease (vWD):

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

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

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

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

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

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

286

### 287 **Abnormalities of platelet function resulting in inappropriate thrombosis:**

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

292

#### 293 Endothelial dysfunction:

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

296 and nitric oxide (NO). Endothelial cell activation can also result in increased secretion of large  
297 vWF multimers and concurrent decrease or absence of ADAMTS-13 (Vischer 2006; Luo et al.  
298 2012).

299

### 300 Hypercoagulable states:

301 Inflammatory cytokines can trigger the coagulation cascade, resulting in thrombin production and  
302 platelet activation without adhesion (Esmon 2005). These platelets may also exhibit exaggerated  
303 aggregation in response to a normal stimulus. Activated platelets also provide a procoagulant  
304 membrane and release agonists to further perpetuate aggregation (Brass 2010; Stokes and Granger  
305 2012).

306

### 307 Blood stasis

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

311

### 312 **Clinical signs of thromboembolic disease**

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

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

326

### 327 **Diagnostic approach to disorders of platelet function: Haemorrhage**

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

332

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

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

339

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



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

344

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

346 BMBT is indicated in patients with evidence of bleeding but normal platelet count and PT/APTT.

347 A specific BMBT lancet device must be used. Reference ranges are device specific but are

348 generally considered to be less than 4 minutes in dogs and less than 2 minutes in cats. Anaesthesia

349 and sedation can mildly prolong the BMBT (Sato et al., 2000; Alatsaz et al., 2014). In a patient

350 where anaemia and thrombocytopenia are excluded and PT / APTT are normal, a prolonged BMBT

351 may reflect thrombocytopathia, vWD or more rarely, a vessel wall disorder. It is important to

352 understand the limitations of the BMBT, as it is highly subjective and prone to significant inter-

353 and intraobserver variability (Sato et al 2000; Alatzas et al. 2013). Results should therefore always

354 be interpreted with caution and verified with more specific testing. A BMBT incision is superficial,

355 such as to only stimulate platelet plug formation, and therefore is not recommended as a predictor

356 of surgical haemorrhage.

357

358 4. Von Willebrand Factor Antigen

359 If the BMBT is prolonged, the vWF: Ag assay is indicated to differentiate type I and type III vWD

360 from thrombocytopathia. As mentioned earlier, vWF: Ag cannot diagnose type II vWD as vWF

361 antigen concentration can be normal. A vWF: Ag assay should also be considered in any patient

362 with clinical signs of platelet type bleeding with normal platelet count and BMBT, due to the

363 limitations of BMBT.

364

365 5. Additional screening for infectious disease:

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

374

375 6. Genetic testing:

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

379

380 7. Platelet function testing:

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

384

385 **Diagnostic approach to disorders of platelet function: Thrombosis**

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

398

#### 399 **Blood Sampling:**

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

408

409 **Specific tests of platelet function:**

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

417

418 Assessment of platelet adhesion under shear stress

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

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

- 432 - The PFA-100 has been shown to detect the anti-platelet effects of aspirin, clopidogrel and  
433 NSAIDs in both healthy dogs and cats. As such, it may have clinical utility for monitoring  
434 response to treatment. However, it is also suggested that, compared to optical  
435 aggregometry, the PFA is less reliable in determining drug responsiveness and can  
436 markedly overestimate the degree of aspirin resistance (Gaal 2007; Dudley 2013; Haines  
437 2016; Ho et al. 2016; Saati et al. 2017, McLewee 2018).
- 438 - The PFA 100 and 200 have also been used to investigate platelet function in dogs with  
439 valvular disease and cats with cardiomyopathy (Jandrey 2008; Clancey 2009; Moesgaard  
440 2009), as well as dogs with chronic kidney disease and endotoxaemia (Dudley 2017;  
441 Yilmaz 2005). The PFA has also been used to diagnose Scott syndrome (Brooks 2009). In  
442 people, the PFA is used as a screening test for vWD (Ardillon 2015). One study reported  
443 increased CTs in 2 dogs with vWD that responded to DDAVP treatment (Burgess 2009).

444

#### 445 Platelet aggregation

446 Light transmission aggregometry (LTA):

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

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

457

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

459 - Heparinised blood is added to platelet agonists including ADP, arachidonic acid and  
460 collagen. This induces platelet aggregates to form on two electrodes. Increased electrical  
461 resistance is detected between the two electrodes which creates a curvilinear graph  
462 (Kalbanter 2010).

463 - In healthy dogs, Multiplate® analysis was able to detect reduced platelet aggregation  
464 following both aspirin and clopidogrel therapy and as such may have clinical utility for  
465 monitoring response to therapy (Saati et al. 2017). In cats, normal variability of platelet  
466 function and inability to detect clinically relevant changes following anti-platelet therapy  
467 currently limits the use of Multiplate analysis, although future studies may further optimise  
468 its use in this species (Ho et al. 2015; Ho et al. 2016).

469 - The Multiplate has also been used to detect reduced platelet aggregation induced in vitro  
470 by the addition of lipopolysaccharide to replicate sepsis (Ferkau et al. 2013, Li and Chan  
471 2016).

472 - This test should be run within 4 hours of sampling.

473

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

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

478 - Although haematology counters are routinely available, the 'Plateletworks' analyser is a  
479 specific point of care assay that uses EDTA and citrate tubes implemented with agonist.  
480 This equipment is more accessible but individual agonist tubes cannot be purchased and  
481 have a relatively short expiry date. Samples should also be analysed within 10 minutes of  
482 collection.

483 - In healthy dogs, the Plateletworks analyser was able to detect reduced platelet aggregation  
484 following both aspirin and clopidogrel therapy and as such may have clinical utility for  
485 monitoring therapy (Saati et al. 2017). In cats, normal variability may again limit clinical  
486 application (Ho et al. 2015). However, studies in both healthy cats and those with  
487 asymptomatic hypertrophic cardiomyopathy have documented clinically detectable  
488 reductions in platelet aggregation following clopidogrel treatment (Hamel-Jolette et al.  
489 2009; Bedard 2009; Ho et al. 2016, (den Toom 2016).

490

491 Global Assessment:

492 Viscoelastic testing: *E.g. Thromboelastography (TEG) +/- platelet mapping and*  
493 *Rotational Thromboelastometry (ROTEM)*

494 - TEG and ROTEM are global coagulation tests. Therefore, although they include platelet  
495 function in the process of clot formation, the presence of contributing factors such as  
496 thrombin and fibrin formation means they are relatively insensitive to abnormalities of  
497 platelet function (Brainard 2010, Brainard 2011).

498 - A citrated blood sample is added to a small cup (where activators of coagulation may or  
499 may not be added depending on the type of analysis to be performed), in which a pin is  
500 suspended. With TEG, the cup rotates which detects changes in torque of blood as a blood

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

503 - Platelet number and function in addition to fibrin formation are major determinants of  
504 overall clot strength (TEG = maximum amplitude or ROTEM = maximum clot firmness)  
505 (Brainard 2010, Brainard 2011).

506 - TEG with 'platelet mapping' can better assess platelet function, which requires two TEG  
507 machines running concurrently with different activators including heparin, which can  
508 exclude the contribution of thrombin to maximum clot strength. (Croft 2004).

509 - Platelet mapping has been validated in healthy dogs (Blois 2013) and has been utilised in  
510 studies investigating clopidogrel efficacy (Brainard 2010).

511 - A novel  $\Delta$  parameter, which aims to differentiate the relative contribution of platelets and  
512 clotting proteases to hypercoagulability, has been retrospectively evaluated in dogs with  
513 immune mediated haemolytic anaemia (Hamzianpour and Chan 2016).

514

## 515 **Tests to assess platelet components and markers of platelet activation:**

### 516 Flow Cytometry :

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



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

536

537 Plasma mean platelet component (MPC) concentration:

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

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

548

549   **Conclusion:**

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

559

560 **References:**

- 561 • Alatzas, D. G., Mylonakis, M. E., Kazakos, G. M., Kostoulas, P., Kritsepi-Konstantinou, M.,  
562 & Polizopoulou, Z. S. (2013). Reference values and repeatability of buccal mucosal bleeding  
563 time in healthy sedated cats. *Journal of Feline Medicine and Surgery*, 16(2), 144–148.
- 564 • Adamantos, S., Waters, S., Boag, A. (2015) Coagulation status in dogs with naturally occurring  
565 *Angiostrongylus vasorum* infection. *Journal of Small Animal Practice* 56, 485–490.
- 566 • Adamik, K. N., Butty, E., Howard, J. (2015) In vitro effects of 3 % hypertonic saline and 20 %  
567 mannitol on canine whole blood coagulation and platelet function. *BMC Veterinary Research*  
568 11, 242.
- 569 • Albrecht, N.A., Howard, J., Kovacevic, A., Adamik, K.N. (2016) In vitro effects of 6 %  
570 hydroxyethyl starch 130/0.42 solution on feline whole blood coagulation measured by  
571 rotational thromboelastometry. *BMC Veterinary Research* 12, 149.
- 572 • Ardillon, L., Ternisien, C., Fouassier, M., Sigaud, M., Lefrançois, A., Pacault, M., Ribeyrol,  
573 O., Fressinaud, E., Boisseau, P., Trossaërt, M. (2015). Platelet function analyser (PFA-100)  
574 results and von Willebrand factor deficiency: a 16-year “real-world” experience. *Haemophilia*,  
575 21(5), 646–652.
- 576 • Arya, M. (2002) Ultralarge multimers of von Willebrand factor form spontaneous high-  
577 strength bonds with the platelet glycoprotein Ib-IX complex: studies using optical tweezers.  
578 *Blood* 17, 3971–7.
- 579 • Auton, M., Zhu, C., Cruz, M.A. (2010) The Mechanism of VWF-Mediated Platelet GPIb $\alpha$   
580 Binding. *Biophysical Journal* 9, 1192–201.

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

- 602 • Blois, S.L., Banerjee, A., Wood, R.D., Park, F.M. (2013) Thromboelastography platelet  
603 mapping in healthy dogs using 1 analyzer versus 2 analyzers. *Canadian Journal of Veterinary*  
604 *Research* 77, 231–6.
- 605 • Boccardo, P., Remuzzi, G., Galbusera, M. (2004) Platelet dysfunction in renal failure.  
606 *Seminars in Thrombosis and Hemostasis* 30, 579–589.
- 607 • Borgeat, K. (2013) Arterial Thromboembolism in 250 Cats in General Practice: 2004-2012.  
608 *Journal of Veterinary Internal Medicine* 28, 102–108.
- 609 • Brainard, B.M., Kleine, S.A., Papich, M.G., Budsberg, S.C. (2010) Pharmacodynamic and  
610 pharmacokinetic evaluation of clopidogrel and the carboxylic acid metabolite SR 26334 in  
611 healthy dogs. *American Journal of Veterinary Research* 71, 822–30.
- 612 • Brainard, B.M., Abed, J.M., Koenig, A. (2011) The effects of cytochalasin D and abciximab  
613 on hemostasis in canine whole blood assessed by thromboelastography and the PFA-100®  
614 platelet function analyzer system. *Journal of Veterinary Diagnostic Investigation* 23, 698–703.
- 615 • Brass, L. (2010) Understanding and evaluating platelet function. *Hematology. American*  
616 *Society of Hematology* 1, 387–396
- 617 • Brassard, J.A., Meyers, K. M., Person, M., Dhein C. R. (1994) Experimentally induced renal  
618 failure in the dog as an animal model of uremic bleeding. *The Journal of laboratory and*  
619 *clinical medicine* 124, 48–54
- 620 • Brooks, M., (1999) A review of canine inherited bleeding disorders: biochemical and  
621 molecular strategies for disease characterization and carrier detection. *The Journal of heredity*  
622 90, 112–118.
- 623 • Brooks, M.B. (2002) A hereditary bleeding disorder of dogs caused by a lack of platelet  
624 procoagulant activity. *Blood* 99, 2434–41.

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

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

- 671 • Dong, J.F., Moake, J.L., Nolasco, L., Bernardo, A., Arceneaux, W., Shrimpton, C.N., et al.  
672 (2002) ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor  
673 multimers on the endothelial surface under flowing conditions. *Blood. American Society of*  
674 *Hematology*; 100, 4033–9
- 675 • Dudley, A., Thomason, J., Fritz, S., Grady, J., Stokes, J., Wills, R., Pinchuk, L., Mackin, A.,  
676 Lunsford, K. (2013) Cyclooxygenase Expression and Platelet Function in Healthy Dogs  
677 Receiving Low-Dose Aspirin. *Journal of Veterinary Internal Medicine* 27, 141–149.
- 678 • Dudley, A. (2013) An Investigation of the Multifaceted Platelet Dysfunction in Dogs with  
679 Naturally Occurring Chronic Kidney Disease – thesis. Accessed:  
680 [https://etd.ohiolink.edu/!etd.send\\_file?accession=osu1405012077&disposition=inline](https://etd.ohiolink.edu/!etd.send_file?accession=osu1405012077&disposition=inline)  
681 [Accessed: 17<sup>th</sup> September].
- 682 • Dudley, A., Byron, J.K., Burkhard, M.J., Warry, E., Guillaumin, J. (2017) Comparison of  
683 platelet function and viscoelastic test results between healthy dogs and dogs with naturally  
684 occurring chronic kidney disease. *American Journal of Veterinary Research* 78, 589–600.
- 685 • Dunning, M., May, J., Adamany, J., Heptinstall, S., Fox, S. (2018) A Remote Assay for  
686 Measuring Canine Platelet Activation and the Inhibitory Effects of Antiplatelet Agents.  
687 *Journal of Veterinary Internal Medicine* 32, 119–127.
- 688 • Esmon, C.T. (2005) The interactions between inflammation and coagulation. *British Journal*  
689 *of Haematology* 131, 417–430.
- 690 • Epstein, S.E., Hopper, K., Mellema, M.S., Johnson, L.R. (2013) Diagnostic utility of D-dimer  
691 concentrations in dogs with pulmonary embolism. *Journal of Veterinary Internal Medicine* 27,  
692 1646–9.



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

- 716 • Goggs, R., Chan, D. L., Benigni, L., Hirst, C., Kellett-Gregory, L., Fuentes, V. L. (2014)  
717 Comparison of computed tomography pulmonary angiography and point-of-care tests for  
718 pulmonary thromboembolism diagnosis in dogs. *The Journal of Small Animal Practice* 54,  
719 190–197.
- 720 • Goggs, R., Blais, M. C., Brainard, B. M., Chan, D. L., deLaforcade, A. M., Rozanski, E., &  
721 Sharp, C. R. (2019). American College of Veterinary Emergency and Critical Care (ACVECC)  
722 Consensus on the Rational Use of Antithrombotics in Veterinary Critical Care (CURATIVE)  
723 guidelines: Small animal. *Journal of Veterinary Emergency and Critical Care*, 29(1), 12–36.
- 724 • Gouin, I., Lecompte, T., Morel, M.C., Lebrazi, J., Modderman, P.W., Kaplan, C., et al. (1992)  
725 In vitro effect of plasmin on human platelet function in plasma. Inhibition of aggregation  
726 caused by fibrinogenolysis. *Circulation*. 85, 935–41.
- 727 • Halmay, D., Gaál, T., Kocsis, R. (2008) Influencing factors of ADP-induced, epinephrine-  
728 induced and ristomycin-induced platelet aggregation in dogs. *Blood Coagulation &*  
729 *Fibrinolysis* 19, 14–22
- 730 • Hamel-Jolette, A., Dunn, M., Bédard, C. (2009) Plateletworks: A screening assay for  
731 clopidogrel therapy monitoring in healthy cats. *Canadian Journal of Veterinary Research* 73,  
732 73-76.
- 733 • Hamzianpour, N., Chan, D.L. (2015) Thromboelastographic assessment of the contribution of  
734 platelets and clotting proteases to the hypercoagulable state of dogs with immune-mediated  
735 hemolytic anaemia. *Journal of Veterinary Emergency and Critical Care* 26, 295–9.
- 736 • Haines, J.M., Thomason, J.M., Seage, E.C., Wills, R.W., Bulla, C., Lunsford, K.V., et al.  
737 (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. *Seminars 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. *Tierärztliche 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.

- 760 • Ho, K.K., Abrams-Ogg, A.C., Wood, R.D., O’Sullivan, M.K., Kirby G. M., Blois S. L. (2016)  
761 Assessment of platelet function in healthy cats in response to commonly prescribed antiplatelet  
762 drugs using three point-of-care platelet function tests. *Journal of Feline Medicine and Surgery*  
763 19, 638–647.
- 764 • Hogan, D.F., Andrews, D.A., Green, H.W., Talbott, K.K., Ward, M.P., Calloway, B.M. (2004)  
765 Antiplatelet effects and pharmacodynamics of clopidogrel in cats. *Journal of American*  
766 *Veterinary Medicine Association* 225, 1406-11.
- 767 • Jain, N. C. (1986) Hematologic techniques – counting platelets. In: N. C. Jain (ed.), Schalm’s  
768 *Veterinary Hematology*, Lea & Febiger, Philadelphia, pp. 65–66.
- 769 • Jandrey, K.E., Norris, J. W., MacDonald, K. A., Kittleson, M.D., Tablin, F. (2008) Platelet  
770 function in clinically healthy cats and cats with hypertrophic cardiomyopathy: analysis using  
771 the Platelet Function Analyzer-100 *Veterinary Clinical Pathology* 37, 385–388.
- 772 • Jandrey, K.E. (2012) Assessment of platelet function. *Journal of Veterinary Emergency and*  
773 *Critical Care* 22, 81–98.
- 774 • Jandrey, K. E. (2014) Platelet disorders. In: *Small Animal Critical Care Medicine* 2nd ed.,  
775 Elsevier Health Sciences, Philadelphia.
- 776 • Johnson, L.R., Lappin, M.R. & Baker, D.C. (1999) Pulmonary thromboembolism in 29 dogs:  
777 1985-1995. *Journal of Veterinary Internal Medicine* 13, 338–345.
- 778 • Johnstone, I.B. (1988) Clinical and Laboratory Diagnosis of Bleeding Disorders. *Veterinary*  
779 *Clinics of NA: Small Animal Practice* 18, pp.21–33
- 780 • Kalbantner, K., Baumgarten, A., Mischke, R. (2010) Measurement of platelet function in dogs  
781 using a novel impedance aggregometer. *The Veterinary Journal* 185, 144–51.

- 782 • Kerlin, B., Cooley. B.C., Isermann, B.H., Hernandez, I., Sood, R., Zogg, M., et al (2004)  
783 Cause-effect relation between hyperfibrinogenemia and vascular disease. *Blood. American*  
784 *Society of Hematology* 103, 1728–34.
- 785 • Kratzer, M.A., Born, G.V. (1985) Simulation of primary haemostasis in vitro. *Haemostasis* 15,  
786 357–362.
- 787 • Kitrell, D., Berkwitt, L. (2012) Hypercoagulability in dogs: pathophysiology. *Compendium*  
788 *(Yardley, PA)*, 34. Accessed: [https://vetfolio-vetstreet.s3.amazonaws.com/65/7bc1f06ede11e1806d005056ad4734/file/PV0412\\_Kittrell\\_CE.pdf](https://vetfolio-vetstreet.s3.amazonaws.com/65/7bc1f06ede11e1806d005056ad4734/file/PV0412_Kittrell_CE.pdf) [21st September].  
789
- 790 • Klose, T.C., Creevy, K.E., Brainard, B.M.(2011) Evaluation of coagulation status in dogs with  
791 naturally occurring canine hyperadrenocorticism. *Journal of Veterinary Emergency and*  
792 *Critical Care* 21, 625–32.
- 793 • Kristensen, A.T., Weiss, D.J., Klausner, J.S. (1994) Platelet dysfunction associated with  
794 immune-mediated thrombocytopenia in dogs. *Journal of Veterinary Internal Medicine* 8, 323–  
795 327.
- 796 • Laffi, G., Cominelli, F., La Villa, G., et al (1987) Reduced platelet thromboxane A2 production  
797 as a possible cause of defective platelet aggregation in cirrhosis. *Adv Prostaglandin*  
798 *Thromboxane Leukot Res* 17A, 366–9
- 799 • Laffi, G., Marra, F., Gresele, P., et al (1992) Evidence for a storage pool defect in platelets  
800 from cirrhotic patients with defective aggregation. *Gastroenterology* 103, 641–6
- 801 • de Laforcade, A.M., Freeman, L. M., Shaw, S.P., Brooks, M.B., Rozanski, E. A., Rush, J.E.  
802 (2003) Hemostatic changes in dogs with naturally occurring sepsis. *Journal of Veterinary*  
803 *Internal Medicine* 17, 674–679.

- 804 • Lara-García, A., Couto, C.G., Iazbik, M.C., Brooks, M.B. (2008) Postoperative bleeding in  
805 retired racing greyhounds. *Journal of Veterinary Internal Medicine* 22, 525–33.
- 806 • Laurenson, M.P., Hopper, K., Herrera, M.A., Johnson, E.G. (2010) Concurrent Diseases and  
807 Conditions in Dogs with Splenic Vein Thrombosis. *Journal of Veterinary Internal Medicine*  
808 24, 1298–1304.
- 809 • Lawrence, J., Chang, Y-M.R., Szladovits, B., Davison, L.J., Garden, O.A. (2013) Breed-  
810 Specific Hematological Phenotypes in the Dog: A Natural Resource for the Genetic Dissection  
811 of Hematological Parameters in a Mammalian Species. *PLoS ONE* 8, 81288.
- 812 • Lee, P. M., Faus, M. C. L., & Court, M. H. (2019). High interindividual variability in plasma  
813 clopidogrel active metabolite concentrations in healthy cats is associated with sex and  
814 cytochrome P450 2C genetic polymorphism. *Journal of Veterinary Pharmacology and*  
815 *Therapeutics*, 42(1), 16–25.
- 816 • Lemke, K.A., Runyon, C.L., Horney, B.S. (2002) Effects of preoperative administration of  
817 ketoprofen on whole blood platelet aggregation, buccal mucosal bleeding time, and  
818 hematologic indices in dogs undergoing elective ovariohysterectomy. *Journal of American*  
819 *Veterinary Medicine Association* **220**, 1818–1822.
- 820 • Lennon, E.M., Hanel, R.M, Walker, J.M., Vaden S. L. (2013) Hypercoagulability in Dogs with  
821 Protein-Losing Nephropathy as Assessed by Thromboelastography. *Journal of Veterinary*  
822 *Internal Medicine* 27, 462–468.
- 823 • Li, C., Hirsh, J., Xie, C., Johnston, M.A., Eikelboom, J.W. (2012) Reversal of the anti-platelet  
824 effects of aspirin and clopidogrel. *Journal of Thrombosis and Haemostasis* 10, 521–528.

- 825 • Li, R.H.L., Chan, D.L. (2016) Evaluation of platelet function using multiple electrode platelet  
826 aggregometry in dogs with septic peritonitis. *Journal of Veterinary Emergency and Critical*  
827 *Care* 26, 630–638.
- 828 • Luo, G.P., Ni. B., Yang, X., Wu, Y.Z. (2012) von Willebrand factor: more than a regulator of  
829 hemostasis and thrombosis. *Acta Haematologica* 128, 158–169.
- 830 • Lunsford, K. V, & Mackin, A. J. (2007). Thromboembolic therapies in dogs and cats: an  
831 evidence-based approach. *The Veterinary Clinics of North America. Small Animal Practice*,  
832 37(3), 579–609.
- 833 • Macey MG, Carty E, Webb L, et al. Use of mean platelet component to measure platelet  
834 activation on the ADVIA 120 haematology system. *Cytometry* 1999;38:250–255.
- 835 • McBride, D., Hosgood, G., Raisis, A. (2016) Platelet closure time in anesthetized Greyhounds  
836 with hemorrhagic shock treated with hydroxyethyl starch 130/0.4 or 0.9% sodium chloride  
837 infusions. *Journal of Veterinary Emergency and Critical Care* 26, 509–515.
- 838 • McBride, D. (2017) Assessment of primary haemostatic function in dogs with acute kidney  
839 injury. *Proceedings of the International Veterinary Emergency and Critical Care Society*.  
840 September 2017, Washington, USA. pp 56.
- 841 • McCarroll, D.R., Waters, D.C., Steidley, K.R., Clift, R., McDonald, T.P. (1988) Canine  
842 platelet von Willebrand factor: quantification and multimeric analysis. *Experimental*  
843 *Hematology*, 16, 929–37.
- 844 • McLewee N., Archer, T., Wills, R., Mackin, A., Thomason, J. (2017) Effects of aspirin dose  
845 escalation on platelet function and urinary thromboxane and prostacyclin levels in normal  
846 dogs. *Journal of Veterinary Pharmacology* 41, 60–7.

- 847 • McMichael, M.A. (2005) Primary hemostasis. *Journal of Veterinary Emergency and Critical*  
848 *Care* 15, 1-8.
- 849 • McMichael, M.A., Smith, S.A., Galligan, A., Swanson, K.S. (2014) In vitro hypercoagulability  
850 on whole blood thromboelastometry associated with in vivo reduction of circulating red cell  
851 mass in dogs. *Veterinary Clinical Pathology* 43, 154–63
- 852 • van der Meer, P.F., Tomson, B., Brand, A. (2010) In vivo tracking of transfused platelets for  
853 recovery and survival studies: An appraisal of labelling methods. *Transfusion and Apheresis*  
854 *Science*. 42(1):53-61.
- 855 • Michiels, C., Arnould, T., Remacle, J. (2000) Endothelial cell responses to hypoxia: initiation  
856 of a cascade of cellular interactions. *Biochimica et biophysica acta* 1497, 1–10
- 857 • Mischke, R., Keidel, A. (2003) Influence of Platelet Count, Acetylsalicylic Acid, von  
858 Willebrand's Disease, Coagulopathies, and Haematocrit on Results Obtained Using a Platelet  
859 Function Analyser in Dogs. *The Veterinary Journal* 165, 43–52.
- 860 • Mischke, R., Schulze, U. (2004) Studies on platelet aggregation using the Born method in  
861 normal and uraemic dogs. *The Veterinary Journal* 168, 270–5.
- 862 • Moesgaard, S.G., Sørensen, T.M., Sterup, A., Tarnow, I., Kristensen, A.T., Jensen, A.L., et al.  
863 (2009) Changes in platelet function in Dachshunds with early stages of myxomatous mitral  
864 valve disease. *Respiratory Veterinary Science* 86, 320–4
- 865 • Moise, N.S. (2007) Presentation and management of thromboembolism in cats. *In Practice* 29,  
866 2–8.
- 867 • Moritz, A., Walcheck, B.K., Weiss, D.J. (2005) Evaluation of flow cytometric and automated  
868 methods for detection of activated platelets in dogs with inflammatory disease. *American*  
869 *Journal of Veterinary Research* 66, 325–9



- 870 • Nakamura, R.K., Tompkins, E., Bianco, D. (2012) Therapeutic options for immune-mediated  
871 thrombocytopenia. *Journal of Veterinary Emergency and Critical Care* 10, 59-72
- 872 • Nelson, O.L., Andreasen, C. (2003) The Utility of Plasma D-dimer to Identify  
873 Thromboembolic Disease in Dogs. *Journal of Veterinary Internal Medicine* 17, 830–4.
- 874 • Nieswandt, B. (2003) Platelet-collagen interaction: is GPVI the central receptor? *Blood* 102,  
875 449–61.
- 876 • O'Marra, S.K., de Laforcade, A.M., Shaw, S.P. (2011) Treatment and predictors of outcome in  
877 dogs with immune-mediated thrombocytopenia. *Journal of the American Veterinary Medical*  
878 *Association* 238, 346–352.
- 879 • H.O., Ogedegbe. (2002). An Overview of Hemostasis. *Toxicologic Pathology*, 21(2), 170–179.
- 880 • Ordinas, A., Maragall, S., Castillo, R., et al (1978) A glycoprotein I defect in the platelets of  
881 three patients with severe cirrhosis of the liver. *Thrombosis Research* 13, 297–302.
- 882 • Paniccia, R., Priora, R., Liotta, A.A., Abbate, R. (2015) Platelet function tests: a comparative  
883 review. *Vascular Health and Risk Management* 11, 133–16.
- 884 • Park, F.M., Blois, S.L., Abrams-Ogg, A.C., Wood, R.D., Allen D. G., Nykamp, S.G., Downie,  
885 A., (2013) Hypercoagulability and ACTH-Dependent Hyperadrenocorticism in Dogs. *Journal*  
886 *of Veterinary Internal Medicine* 27, 1136–1142.
- 887 • Pathak, E.J. (2004) Type 3 von Willebrand's disease in Shetland sheepdog. *Canadian*  
888 *Veterinary Journal*. 45(8):685-687.
- 889 • Pedersen, H. D., Haggstro, M.J., Olsen, L.H., Christiansen, K., Selin, A., Burmeister, M.M.K.,  
890 Larsen, H. (2002) Idiopathic asymptomatic thrombocytopenia in cavalier King Charles  
891 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 • Respass, 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

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

- 937 Thromboelastometry in Dogs Naturally Infected With *Angiostrongylus vasorum*. *Journal of*  
938 *Veterinary Internal Medicine* 31, 1091–9.
- 939 • Slauson, D. O, Gribble, D. H. (1971) Thrombosis complicating renal amyloidosis in dogs.  
940 *Veterinary Pathology* 8, 352–363.
- 941 • Smith, S.A., The cell-based model of coagulation. *Journal of Veterinary Emergency and*  
942 *Critical Care* (San Antonio). 2009;19(1):3-10.
- 943 • Smith, S.A., McMichael, M.A., Gilor, S., Galligan, A.J., Hoh, C.M. (2012) Correlation of  
944 hematocrit, platelet concentration, and plasma coagulation factors with results of  
945 thromboelastometry in canine whole blood samples. *American Journal of Veterinary Research*  
946 73, 789–98.
- 947 • Smith, J.R., Smith, K.F., Brainard, B.M. (2014) Platelet parameters from an automated  
948 hematology analyzer in dogs with inflammatory clinical diseases. *The Veterinary Journal* 201,  
949 406–11.
- 950 • Song, J., Drobatz, K.J. & Silverstein, D.C. (2016) Retrospective evaluation of shortened  
951 prothrombin time or activated partial thromboplastin time for the diagnosis of  
952 hypercoagulability in dogs: 25 cases (2006-2011). *Journal of Veterinary Emergency and*  
953 *Critical Care* 26, 398–405.
- 954 • Stokes, K.Y., Granger, D.N. (2012) Platelets: a critical link between inflammation and  
955 microvascular dysfunction. *The Journal of Physiology* 590, 1023–1034
- 956 • Sullivan. P., Gompf. R., Schmeitzel. L., Clift. R., Cottrell. M., McDonald. T.P. (1993) Altered  
957 platelet indices in dogs with hypothyroidism and cats with hyperthyroidism. *American Journal*  
958 *of Veterinary Research* 54, 2004–9

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

- 981 bleeding complications in patients with probable leptospirosis. *PLOS Neglected Tropical*  
982 *Diseases* 11, 15–18.
- 983 • Turitto, V.T., Weiss, H.J. (1980) Red blood cells: their dual role in thrombus formation.  
984 *Science* 207, 541-543.
- 985 • Varela, F., Font, X., Valladares, J. E., Alberola, J. (1997) Thrombocytopathia and light-chain  
986 proteinuria in a dog naturally infected with Ehrlichia canis. *Journal of Veterinary Internal*  
987 *Medicine* 11, 309–311.
- 988 • Venta, P.J., Li, J., Yuzbasiyan-Gurkan, V., Brewer, G.J., Schall, W.D. (2000) Mutation  
989 Causing von Willebrand's Disease in Scottish Terriers. *Journal of Veterinary Internal*  
990 *Medicine* 14, 10–19.
- 991 • VETgirl. (2016) How to perform a How to Perform a BMBT in a Dog. VetGirl Veterinary CE  
992 Blog. Accessed 10<sup>th</sup> November 2018. [https://vetgirlontherun.com/perform-buccal-mucosal-](https://vetgirlontherun.com/perform-buccal-mucosal-bleeding-time-bmbt-vetgirl-veterinary-ce-videos-blog/)  
993 [bleeding-time-bmbt-vetgirl-veterinary-ce-videos-blog/](https://vetgirlontherun.com/perform-buccal-mucosal-bleeding-time-bmbt-vetgirl-veterinary-ce-videos-blog/).
- 994 • Vischer, U.M. (2006) von Willebrand factor, endothelial dysfunction, and cardiovascular  
995 disease. *Journal of thrombosis and haemostasis* 4, 1186–1193.
- 996 • Wang, A., Brainard, B. M. Thrombocytopenia. In: *Small Animal Critical Care Medicine* 2nd  
997 ed., Elsevier Health Sciences, Philadelphia pp
- 998 • Waters, D.C., Eaton, A.H., Steidley, K.R., McCarroll, D.R. (1989) Expression of von  
999 Willebrand factor in plasma and platelets of cats. *American Journal of Veterinary Research*  
1000 50, 201–4
- 1001 • Wei, A.H., Schoenwaelder, S.M., Andrews, R.K., Jackson, S.P. (2009) New insights into the  
1002 haemostatic function of platelets. *British Journal of Haematology* 147, 415–430

- 1003 • Weiss, D.J., Brazzell, J.L. (2006) Detection of Activated Platelets in Dogs with Primary  
1004 Immune-Mediated Hemolytic Anemia. *Journal of Veterinary Internal Medicine* 20, 682
- 1005 • Whitley, N.T., Corzo-Menendez, N., Carmichael, N.G., McGarry, J.W. (2005) Cerebral and  
1006 conjunctival haemorrhages associated with von Willebrand factor deficiency and canine  
1007 angiostrongylosis. *Journal of Small Animal Practice* 46, 75–78.
- 1008 • Williams, D.A., Maggio-Price, L. (1984) Canine idiopathic thrombocytopenia: clinical  
1009 observations and long-term follow-up in 54 cases. *Journal of American Veterinary Medicine*  
1010 *Association* 185, 660–663.
- 1011 • Williams, T.P., Shaw, S., Porter, A., Berkwitz, L. (2017) Aortic thrombosis in dogs. *Journal of*  
1012 *veterinary emergency and critical care* 27, 9–22.
- 1013 • Willis, S.E., Jackson, M.L., Meric, S.M., Rousseaux, C.G. (1989) Whole blood platelet  
1014 aggregation in dogs with liver disease. *American Journal of Veterinary Research*. 50, 1893–  
1015 7.
- 1016 • Wolberg, A.S., Aleman, M.M, Leiderman, K., Machlus, K, R. (2012) Procoagulant Activity in  
1017 Hemostasis and Thrombosis. *Anaesthesia & Analgesia* 114, 275–285.
- 1018 • Wurlod, V.A., Howard, J., Francey, T., Schweighauser, A., Adamik, K.N. (2015) Comparison  
1019 of the in vitro effects of saline, hypertonic hydroxyethyl starch, hypertonic saline, and two  
1020 forms of hydroxyethyl starch on whole blood coagulation and platelet function in dogs. *Journal*  
1021 *of Veterinary Emergency and Critical Care* 25, 474–487.
- 1022 • Yilmaz, Z., Ilcol, Y.O., Ulus, I.H. (2005) Investigation of diagnostic importance of platelet  
1023 closure times measured by Platelet Function Analyzer--PFA 100 in dogs with endotoxemia.  
1024 *Berl Munch Tierarztl Wochenschr* 118, 341-8.

- 1025 • Yozova, I.D., Howard, J., Henke, D., Dirkmann, D., Adamik, K.N. (2017) Comparison of the  
1026 effects of 7.2% hypertonic saline and 20% mannitol on whole blood coagulation and platelet  
1027 function in dogs with suspected intracranial hypertension - a pilot study. *BMC Veterinary*  
1028 *Research* 13, 402.
- 1029 • Yu, D., Noh, D., Park, J. (2015) Flow cytometric evaluation of disseminated intravascular  
1030 coagulation in a canine endotoxemia model. *Canadian Journal of Veterinary Research* 79, 52.
- 1031 • Zoia, A., Gerou-Ferriani, M., Drigo, M., & Caldin, M. (2018). Case-control study of plasma  
1032 mean platelet component concentration and survival analysis for dogs with immune-mediated  
1033 hemolytic anemia. *Journal of the American Veterinary Medical Association*, 252(11), 1384–  
1034 1392
- 1035
- 1036



1037 Table Legend:

1038 Table 1: Summary of key platelet receptors

Receptors	Main ligand	Function	Comments
$\alpha_2\beta_1$ integrin (previously GPIa-IIa)	Collagen	Platelet adhesion	Firm adhesion after activation
GP VI	Collagen	Platelet adhesion	Initial tethering triggers intracellular 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 <i>Site of clopidogrel action</i>
5HT2	Serotonin	Platelet agonist	Serotonin=Weak platelet agonist
Prostaglandin receptors	Thromboxane Prostacyclin	Platelet agonist Platelet antagonist	Agonist and antagonist <i>Site of aspirin action</i>
Protease-activated receptors (PAR)	Thrombin	Platelet agonist	Thrombin=Strong agonist
$\alpha$ adrenergic receptor	Epinephrine	Platelet agonist	Enhances stimulation by other agonists

1039

1040

1041 Table 2: Summary of congenital disorders of platelet function

<b>Congenital disorders</b>	<b>Type of defect</b>	<b>Specific mechanism</b>	<b>Breed affected</b>	<b>Clinical relevance</b>
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 <sub>12</sub> 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 <i>in vivo</i> , <i>i.e.</i> , reduction in “near wall excess” (Turitto & Weiss 1980)	Hct < 35 g/L associated with hypocoagulability (Clancey <i>et al.</i> 2009a, 2009b). BMBT improves with blood transfusion (Brassard <i>et al.</i> 1994). An artificial hypercoagulability may be seen with thromboelastography in anaemic patients (McMichael <i>et al.</i> , 2014).
Uraemia	Storage pool deficiencies; decreased response to agonists; abnormal calcium mobilisation; decreased TXA <sub>2</sub> synthesis and receptor deficiencies; effects of concurrent anaemia (Boccardo <i>et al.</i> 2004)	Induced uraemia in healthy dogs lead to increased BMBT but no clinical bleeding (Brassard <i>et al.</i> 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 vWF <sub>Ag</sub> :CBA which correlated with creatinine (McBride 2017).
Hepatopathy	Storage pool deficiencies (Laffi <i>et al.</i> 1992); decreased TXA <sub>2</sub> synthesis (Laffi <i>et al.</i> 1987); adhesion receptor deficiencies (Ordinas <i>et al.</i> 1978, Sanchez-Roig <i>et al.</i> 1994).	Dogs with hepatopathies have been shown to have reduced platelet aggregation (Willis <i>et al.</i> 1989)
IMT	Antibody against fibrinogen receptor likely causes additional platelet dysfunction (Kristensen <i>et al.</i> 1994)	Haemorrhage does not always correlate with degree of thrombocytopenia and additional platelet dysfunction may be involved Kristensen <i>et al.</i> (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 <i>et al.</i> 1992; de Laforcade <i>et al.</i> 2003).	
Monoclonal gammopathy	Coating of platelets with monoclonal or polyclonal proteins suspected to cause reduced aggregation. (Glaspy 1992; Varela <i>et al.</i> 1997).	Recurrent epistaxis in Ehrlichia-infected dog with normal platelet count, prolonged BMBT and abnormal aggregation (Varela <i>et al.</i> 1997)
Leptospirosis	Circulation of inappropriately activated platelets. Decreased response to platelet agonists. Increased vWF-platelet binding (Barthélemy <i>et al.</i> 2016; Tunjungputri <i>et al.</i> 2017).	Haematuria, melena, petechiae and epistaxis seen clinically. Hypocoagulability, as measured by TEG, associated with mortality; however, no platelet function test performed (Barthélemy <i>et al.</i> 2016)
Angiostrongylus vasorum	Hyperfibrinolysis (Adamantos <i>et al.</i> 2015; Sigrist <i>et al.</i> 2017). Increased FDPs suggested to compete at platelet fibrinogen receptors, limiting aggregation (Bick 1988; Gouin <i>et al.</i> 1992).	Spontaneous bleeding reported in one third of dogs. Currently no studies investigating platelet function specifically (Adamantos <i>et al.</i> 2015; Sigrist <i>et al.</i> 2017)

1045

1046

1047 Table 4: Drugs causing platelet dysfunction

Medication	Mechanism of platelet dysfunction	Duration of action	Comments
ADP receptor antagonists			
Clopidogrel	Irreversible P2Y <sub>12</sub> inhibitor	Life time of platelet. Normal function returns 5 to 10 days after single dose	Active metabolite requires cytochrome P450 pathway
Ticagrelor	Reversible P2Y <sub>12</sub> inhibitor	Reversible action results in shorter duration compared to clopidogrel	Usage only reported in people
Thromboxane inhibitors			
Non-selective COX inhibitor <i>e.g.</i> aspirin	Irreversible inhibition of TXA <sub>2</sub> 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 <i>(e.g.</i> ketoprofen)	Reversible inhibition of TXA <sub>2</sub> 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 <i>(e.g.</i> carprofen, meloxicam)	Reversible inhibition of TXA <sub>2</sub> 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
$\alpha$ IIB $\beta$ 3 integrin ( <i>GP IIb/IIIa</i> ) 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 <i>et al.</i> 1999).	Respiratory changes ranging from tachypnoea to dyspnoea and potentially cyanosis.	CT angiography
Cerebrovascular accidents (Garosi 2010)	Acute onset neurological signs <i>e.g.</i> lateralised motor deficits or seizures.	MRI
Occlusions of peripheral or central veins (Williams <i>et al.</i> 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 <i>et al.</i> 1998; Laurenson <i>et al.</i> 2010; Respass <i>et al.</i> 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

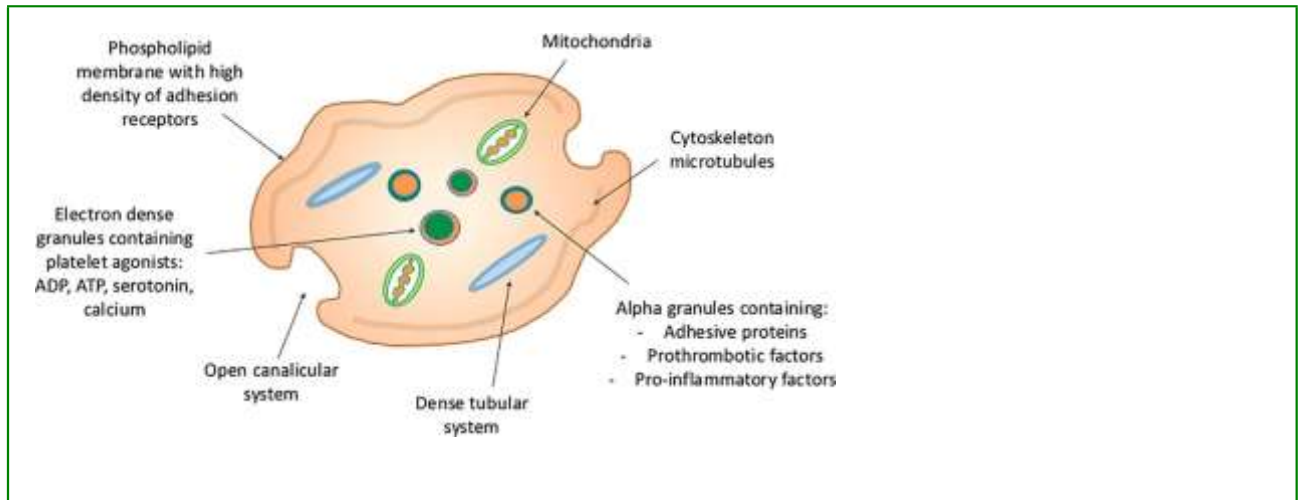
Acquired disorders	Reported mechanisms of platelet dysfunction in people and dogs	Veterinary evidence and clinical relevance
Systemic Inflammation - septic and non-septic, <i>e.g.</i> pancreatitis	Increased thrombin formation leads to platelet activation; cytokines enhance endothelial adhesion; EC dysfunction decreases NO and prostacyclin production (Cheng <i>et al.</i> 2011).	Cytokines shown to increase reactivity to thrombin (Peng <i>et al.</i> 1996); Endotoxin administration or SIRS increases P-selectin expression (Yu <i>et al.</i> 2015). Clinical relevance is not known and there is insufficient data to support routine anticoagulation (Goggs <i>et al.</i> 2019).
Glomerulopathies	Loss of inhibitors including antithrombin III; glomerular EC dysfunction and reduced NO (Chen <i>et al.</i> 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 <i>et al.</i> 2013). Antithrombotic medication is recommended (Goggs <i>et al.</i> 2019).
IMHA	Release of intraerythrocytic ADP activates platelets; free haemoglobin scavenges NO (Helms <i>et al.</i> 2013).	Thromboembolism reported in 46 to 80% of dogs at <i>post mortem</i> (Carr <i>et al.</i> 2002); usually hypercoagulable but relative hypocoagulability as measured by global viscoelastic testing is a negative prognostic indicator (Goggs <i>et al.</i> 2012); circulating activated platelets shown by presence of P-selectin or low plasma mean platelet component (MPC) concentration (Weiss & Brazzell 2006, Ridyard <i>et al.</i> 2010, Zoia <i>et al.</i> 2018). Antithrombotic medication is recommended (Goggs <i>et al.</i> 2019).
Hypercortisolaemia: iatrogenic or hyperadrenocorticism	Increased fibrinogen and thrombin-antithrombin complexes promote aggregation (Kerlin <i>et al.</i>	Both hypercoagulability (Halmay <i>et al.</i> , 2008; Park 2013; Rose <i>et al.</i> , 2011; Rose <i>et al.</i> , 2013) and hypocoagulability (Klose 2011) as



Acquired disorders	Reported mechanisms of platelet dysfunction in people and dogs	Veterinary evidence and clinical relevance
	2004, Klose <i>et al.</i> 2011, Park <i>et al.</i> 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 <i>et al.</i> 2011, Park <i>et al.</i> 2013). Routine antithrombotic medication is not recommended unless other risk factors for thrombosis are present (Goggs <i>et al.</i> 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 <i>et al.</i> 2014).	Prevalence reported at 0.3% (Borgeat 2013); platelet function testing does not differ between healthy and subclinical cats (Jandrey <i>et al.</i> 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 <i>et al.</i> 2019). Canine cardiac diseases are not associated with a high risk for development of thrombosis and routine anti-thrombotics are not recommended (Goggs <i>et al.</i> 2019).

1059 Figure Legends:

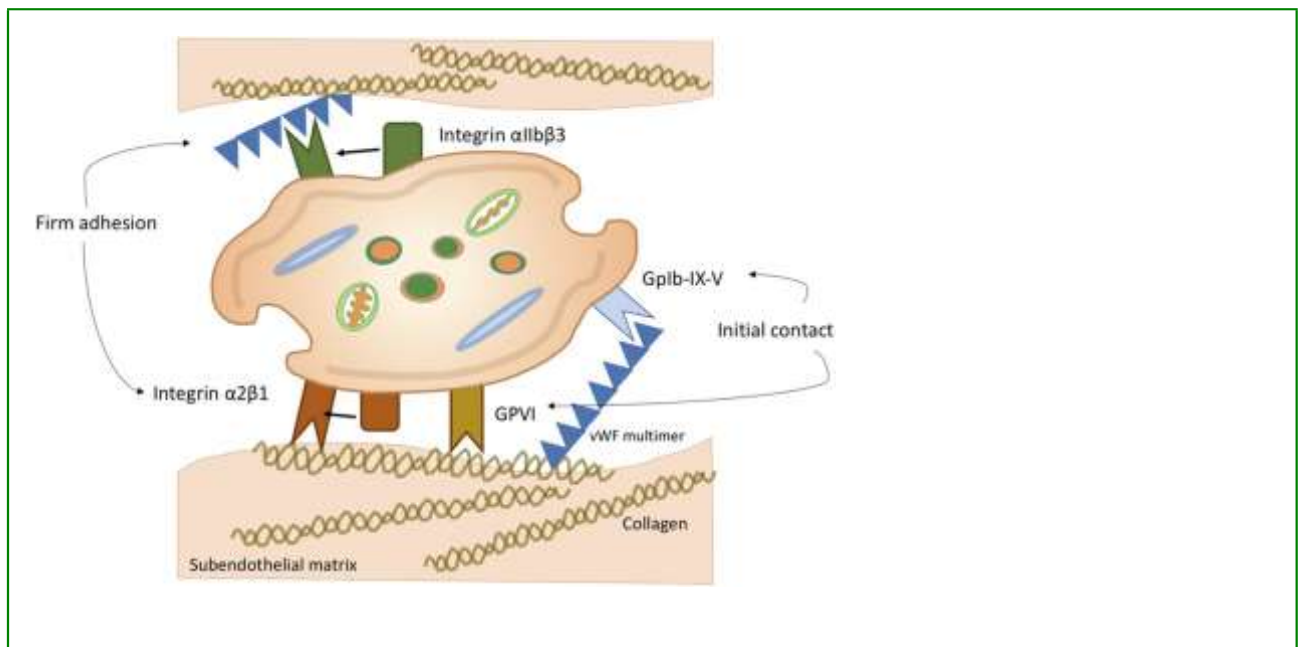
1060 Figure 1. Key anatomical features of platelets



1061

1062

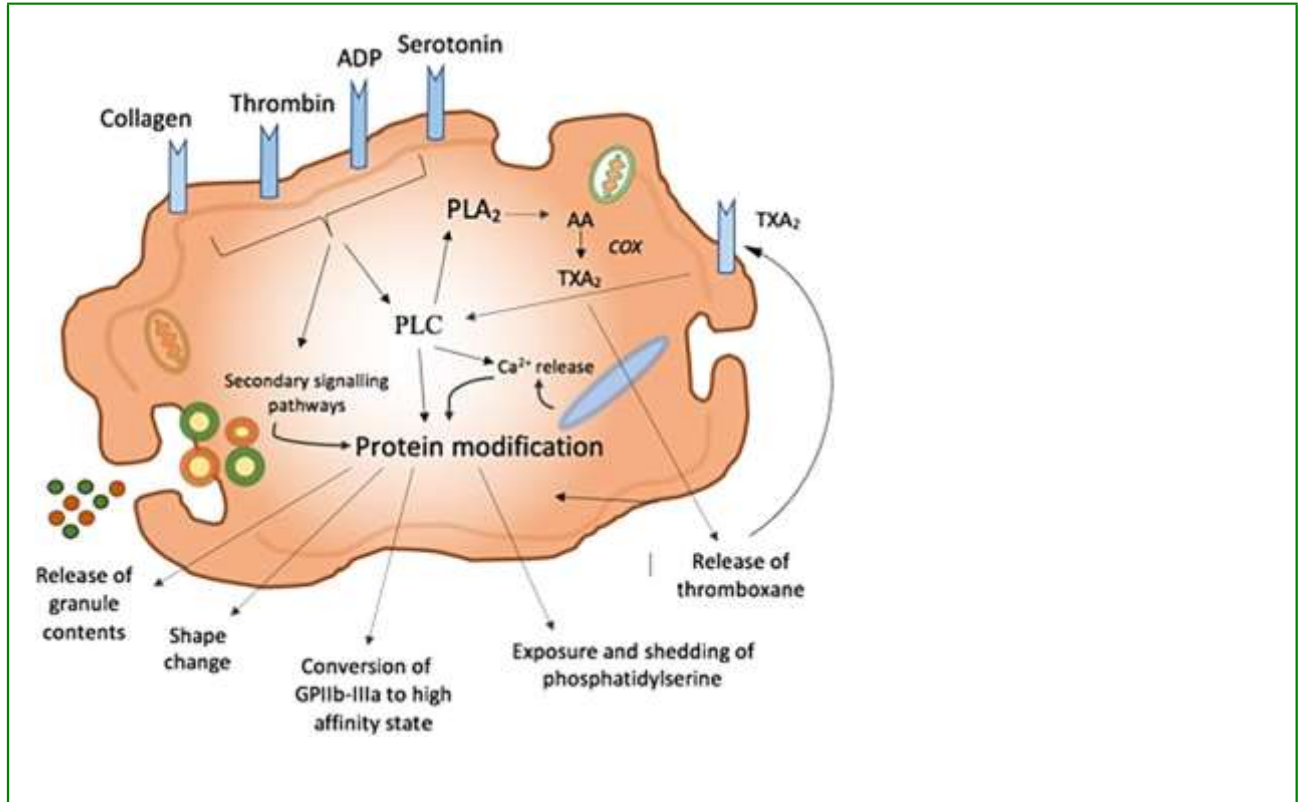
1063 Figure 2. Illustration of key receptors mediating platelet adhesion



1064

1065

1066 Figure 3. Illustration of intracellular signalling pathways



1067

1068

1069 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

1070

1071

1072

1073

1074 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:  $(P \times 15) \times 10^9/L$

1075

1076

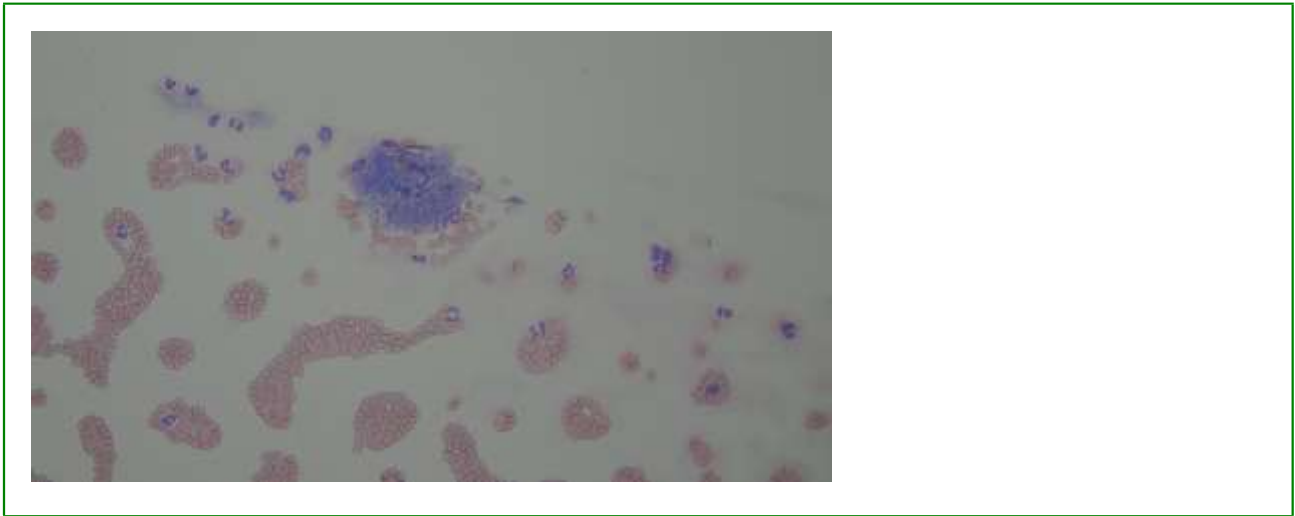
1077 Figure 6. Canine platelet clump (Modified-Wrights stain x 50 magnification)



1078

1079

1080 Figure 7. Feline platelet clump (Modified-Wrights stain x100 magnification)



1081

1082

1083 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.

1084

1085

1086