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INFLAMMATORY RESPONSE TO NATURALLY OCCURRING TIGER SNAKE ENVENOMATION IN DOGS, WITH A SPECIAL EMPHASIS ON IL-6

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Directeur de thèse : Mme Séverine BOULLIER

JURY

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INDEX OF ABBREVIATIONS

APP = Acute Phase Proteins
ACPs = Antigen Presenting Cells
aPTT = activated Partial Thromboplastin Time
ALT = Alanine Amino Transferase
ALKP = Alkaline Phosphatase
APPs = acute phase proteins
BR = Breath Rate
CK = Creatine Kinase
COX = Cyclooxygenases
CRP = C-Reactive Protein
DIC = Disseminated Intravascular Coagulopathy
F. V, VIII, IX, X, XII= Factor V, VIII, IX, X, XII
FPD = Fibrinogen Product Degradation
GLDH = G- Lactate Deshydrogenase
HR = Heart Rate
ICAM-1 = intercellular adhesion molecule 1
IL-1,6,8, etc. = Interleukin 1, 6, 8, etc .
INF-γ = Interferon γ
iNOS = Inducible Nitric Oxide Synthetase
INR = International Normalized Rates
LTE = Leukotriene E
MCP-1 = Monocyte Chemoattractant Protein-1
NK = Natural killer
NO = Nitric Oxide
PECAM-1 = Platelet Endothelial Cell Adhesion Molecule 1
PGE = Prostaglandins E
PLA2 = Phosphatidylcholine 2- Acylhydrolase (phospholipase A2)
PLT = Platelets
PMN = Polymorphonuclear Neutrophils
PT = Prothrombine Time
RANTES = Regulated on Activation Normal T cell Expressed and Secreted
RBC = Red Blood Cell
RT-PCR = Real-Time Reverse Transcriptase-Polymease Chain Reaction
SAA = Serum Amyloid A
SVDK = Snake Venom Detection Kit
SVMP = Snake Venom Metalloproteinases
TNF-α = Tumor Necrosis Factor α
TGF-β = Transforming Growth Factors β
T. snake = Tiger snake
TXA2 = Thromboxane A2
VICC = Venom Induced Consumption Coagulopathy
WBC = White Blood Cells

INTRODUCTION

Venomous snakes are widespread in the world and Australia is home to many of them. The systematic classification of snakes recognises between 11 and 13 distinct families. Venomous snakes are identified in only 5 families: Elapidae, Hydrophiidae, Viperidae, Crotalidae and Colubridae (Order Squamata, class Reptilia) (Harris 1985). The genus *Notechis* belong to the family Elapidae, restricted to the wettest part of Southern and Eastern Australia. The species *Notechus scutatus* (also called tiger snake) presents variable morphs with different sizes and colourations that some authors recognise as several subspecies while others recognise *Notechis* as a monotypic genus (Shea 2000).

Due to their high activity, dogs are particularly subject to be bitten and consequently snake envenomation is a common emergency in this species. It has been estimated that more than 6000 snake envenomed dogs are presented to veterinary clinics in Australia each year (Mirtschin et al. 1998). Tiger snake (*Notechis scutatus*) and eastern brown snake (*Pseudonaja textilis*), both of them from the Elapidae family, were found to be the most commonly implicated (White 1998, Mirtschin et al. 1998). If brown snakes are more often incriminated within Australia, tiger snakes are definitely the most commonly encountered species implicated in dog snake envenomation in the state of Victoria (Mirtschin et al. 1998) and thus represent a major threat to the health of humans and pets in this area.

Snake venoms are complex cocktails of toxins, containing numerous peptides, proteins and salts. These venom components act as neurotoxins, myotoxins, cardiotoxins and modulators of coagulation and inflammation (Fry 1999, Goddard et al. 2011). Tiger snakes are one of the world's most venomous snakes based on their venom LD50 and because of their notexins – including the main neurotoxic phospholipase A2 (PLA2) and snake venom metalloproteinases (SVMP), which are important components of Elapids snake venom (Cher et al. 2003, Birrell et al. 2007).

Dogs envenomed by tiger snakes typically have mydriasis, decreased or absent pupillary light reflexes, depressed mentation, generalised muscle weakness, ataxia, tachypnoea or dyspnoea, vomiting and salivation (Hill 1979; Barr 1984). These clinical abnormalities, associated with the history, as well as laboratory test findings such as prolonged coagulation times, elevated serum creatine kinase and snake venom detection test, lead to the diagnosis of snake envenomation (Heller et al. 2007).

The evidence of an inflammatory response to snake envenomation, characterized by oedema, inflammatory cell infiltrate, and changes in inflammatory mediators levels, has been

reported in several studies (Gutiérrez et al. 1995, Picolo et al. 2002, Moreira et al. 2012, Escocard et al. 2006) and represents the major part of the "post envenomation syndrome". It was specifically described for vipers (*Bothrops asper*, *Vipera russelli*, *Ammodytes*, *Meridionalis*, *Aspis*), rattlelsnakes (*Crotalus*) and Elapids (Maria Fernandes et al., 2006, Zamuner et al., 2005, Langhorn et al., 2014, Santhosh et al., 2013, Szold et al., 2001, Tambourgi et al., 1994). Increased pro-inflammatory cytokines levels (e.g. interleukine IL-6, neutrophil attracting chemokine IL-8, the chemokine monocyte chemoattractant protein-1 MCP-1), induced by PLA2 and SVMP are implicated in these inflammatory processes (Rucavado et al. 2002, Moreira et al. 2012).

Out of the pro- inflammatory cytokines, interleukin-6 (IL-6) is one of the major mediators of acute phase proteins (APPs) synthesis in the liver (Murata, Shimada, Yoshioka 2004). IL-6 is induced by macrophages and other various cells, and is further amplified by IL-1 and TNFα. In dogs, its concentration markedly increases after inflammation stimulation (Yamashita et al. 1994) and therefore can be used for quantifying the induced systemic response to inflammation (Cerón, Eckersall, Martínez-Subiela 2005).

Antivenom is formulated from the plasma of horses immunised with the venom of snakes (hyperimmune IgG serum) (CSL, 2014). It is the current standard treatment that efficiently improves the main threatening systemic effects such as coagulopathy resulting from envenoming (MacDonald, White, Currie 2008). Nevertheless, its use seems to be partially inefficient against local manifestation induced by the inflammatory response to Viperidae snake envenomation (Araújo et al. 2007, Picolo et al. 2002, J. M. Gutiérrez 1981, Chaves, Barboza, Gutiérrez 1995). It was even found to further exacerbate the venom induced immune response and brings a second pro-inflammatory hit (León et al. 2013, Stone et al. 2013).
However, even though tiger snakebites hundreds of dogs every year in Australia, its impact on inflammation has not been examined. That is why there is a real need for understanding the mechanism and characteristics of the post-envenomation syndrome that follow after initial obvious crisis. While the relative contribution of inflammatory processes to the overall clinical picture remains insufficiently described at this time, their attenuation may be exploited as an exciting new therapeutic avenue and clinical trials using immune-modulatory drugs could be warranted.

The aim of this prospective study was to examine the systemic inflammatory response associated with snake envenomation and antivenom administration in dogs, with a special emphasis on IL-6.

2. PART 1. BIBLIOGRAPHIC STUDY ON SNAKE ENVENOMATION

1.1 GENERALITIES ON SNAKE ENVENOMATION

1.1.1 DEFINITION OF SNAKE ENVENOMATION AND TIGER SNAKE GROUP OVERVIEW

Definition:

Envenoming is the process where venom is introduced into a human being or an animal, and generally refers to the situation where enough venom is introduced to cause clinical effects. Snake venom can be defined as highly modified saliva able to immobilize and digest the prey by means of the actions of protein-degrading enzymes (Goddard et al. 2011; J. White, 2013).

Taxonomy of tiger snakes

Five venomous snake families are recognized: Elapidea, Hydrophiidae, Viperidae, Crotalidae and Colubridae (Diag. 1). The Hydrophiidae family includes sea snakes and is considered by some as a sub-family or family of the Elapidae. Crotalidae is considered by some as a sub-family of the Viperidae (Harris 1985).

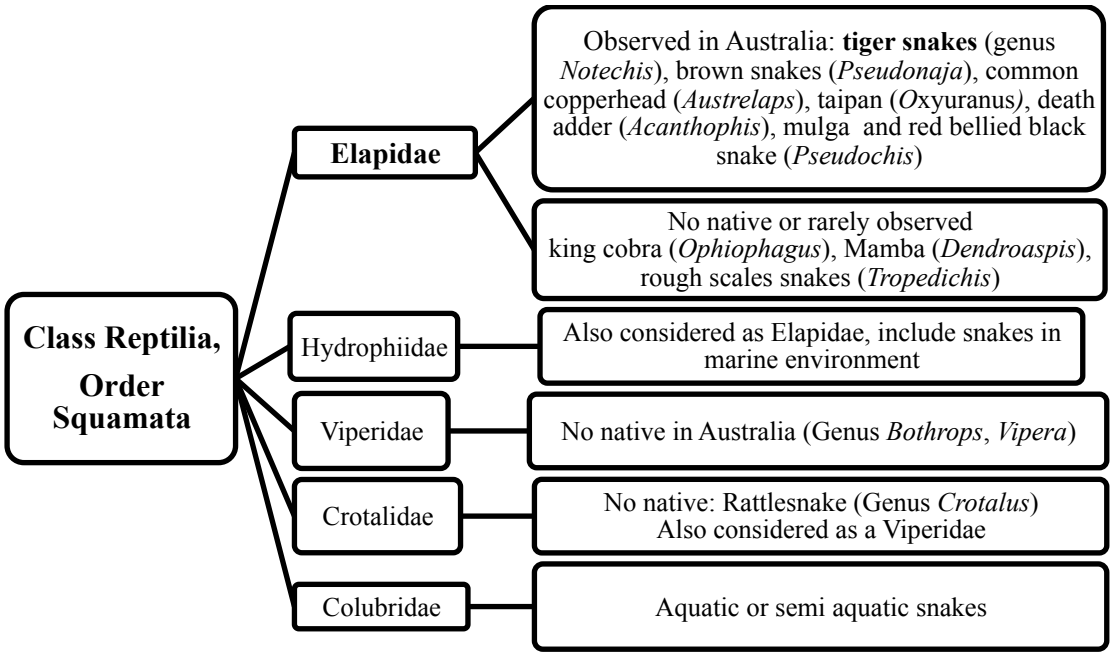

Diagram 1. Taxonomy of most venomous snake snakes. Focus on snakes observed in Australia (modified from Harris 1985; White, 2013).

Most of the research on the Elapidae family was focused on five major groups: brown snakes (genus *Pseudonaja*), tiger snakes (genus *Notechis*), black snakes (genus *Pseudechis*), death adders (genus *Acanthophis*) and taipans (genus *Oxyuranus*) (Hodgson and Wickramaratna 2006). Tiger snakes (*Notechis scutatus*) belong to the Elapidae family.

Description and identification of tiger snakes

Venomous snakes use fangs to inject their venom (Fig.I).

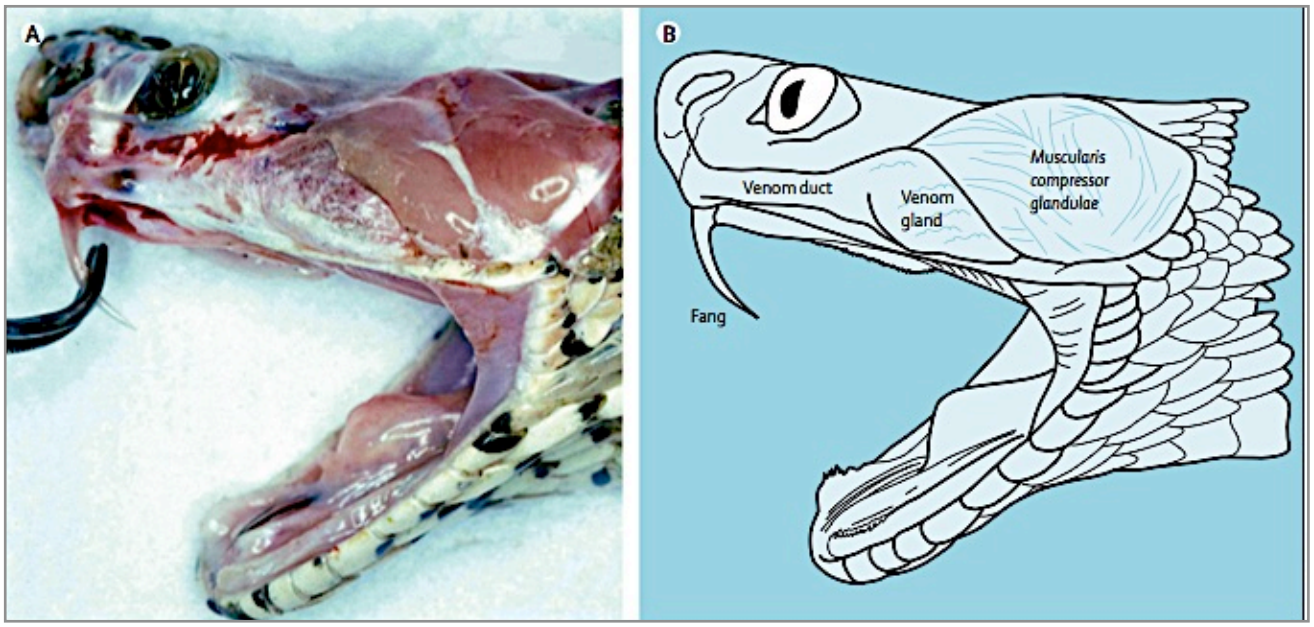

Figure I. Venom apparatus of Russell's viper: dissected snake (A), annotated diagram (B) (Warrell 2010)

The venom is stored in the venom gland; the activation of muscularis compressor glandulae allows venom ejection through the venom duct until the fangs.

Tiger snakes are, as all Elapid snakes, proteroglyphous carnivorous snakes, ie. snakes with permanently erect poisonous fangs at the front of the maxilla (Meier J. et al. 1995). Fangs of adult tiger snakes are about 3.5mm long and produce a moderate amount of very toxic venom.

Tiger snakes have an average length between 1.2m to 1.5m according to the authors. Colouration can vary from the classical banded dark grey/black and yellow skin to unbanded types with a range of colors from black to light grey (Geoffrey K. Isbister et al. 2012). Most of tiger snake species show some bands but unbanded species exist too (Shea 2000). As colouration and banding vary, brown snakes can be easily confused with tiger snakes (Photographies 1, 2).

Photograph 1. Common tiger snake (*Notechis scutatus*) (personal picture)

Photograph 2. Eastern Brown Snake (*Pseudonaja textilis*) (from Queensland museum website)

A study on the ability of Australians to recognize snake species has shown that identification is highly inaccurate (Morrison, Pearn, Covacevich, et al. 1983). In this study composed of 558 volunteers, only 19% of snakes were correctly identified, which includes the identification of 18% of tiger snakes only. Subjects who grew up in rural areas seem to be better at identifying snakes. It seems that the misidentification is due to a combination of

factors, including inconsistent common nomenclature and similarities in appearance for different snake species.

The most reliable method of snake species' identification is scale counting but it requires the snake to be dead at the time of the examination. Tiger snakes can be most readily differentiated from other snakes by the combination of a broad parietal scale - as wide as long, but shorter than the temporolabial scale and unpaired subcaudal scales (Shea 2000). Some authors have used a combination of detailed appearance and scalation for classification (Heller et al. 2007).

Geographic range, habitat and ecology:

The three most commonly encountered snakes causing envenomation of veterinary importance in Australia are the brown snake, the tiger snake, and the red-bellied black snake, from the Elapidae family (Hardy, Cochrane, Allavena 2014).

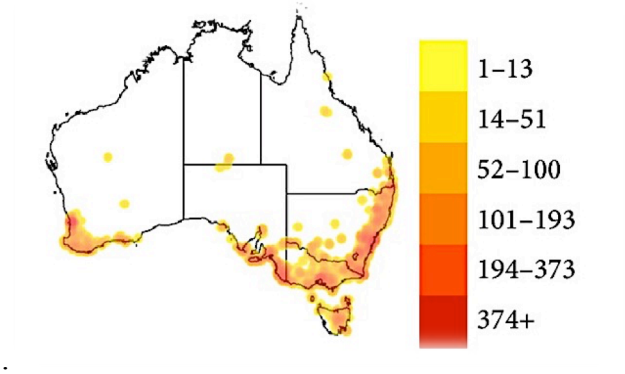

Figure II. Map of the distribution of the tiger snake, 2,366 records (Hardy, Cochrane, Allavena 2014).

It indicates the relative density of this species in Australia: tiger snakes are mainly concentrated along the South coast, especially along the South - East coast.

Tiger snakes are especially distributed near the coast, in Eastern, Southern and Western Australian waters. Fig.II indicates the relative density of this species in Australia. They are also found in Bass Strait Islands and Tasmania. This species is found near water areas such as riparian woodlands and watercourses, and eat amphibians and birds (IUCN 2009).

Epidemiology aspects of snake envenomation in dogs

The snakebite in domestic animals is frequent, with an average of four cases per year presented to each veterinary clinic and a total of about 6,240 cases reported annually. Moreover these numbers are probably underestimated because of the number of animals bitten but not treated, like those who survived alone and those who died before having been brought to a

clinic (Mirtschin et al. 1998). Tiger snakes, genus *Notechis*, are a common cause of snakebites and the second most common cause of snakebite deaths in humans in Australia (J. White, 2013).

A study describes some epidemiology aspects of 125 dogs envenomation enrolled in the Melbourne university hospital in Werribee. In this study, 77% of the dogs (n=125) were sporting breeds, around 4.1 years old with 40% being 2 years old or younger. 48% of the dogs were seen bitten by a snake, almost all by a tiger snake (only one case was a brown snake identified). The highest number of snakebite' occurences in dogs occurred in December and January (50%), when the temperature rises to around 20°C or above (Barr 1984).

To determine the extent of the snakebite problem in domestic animals, a study was made based on a questionnaire sent to 10% of veterinary surgeons, selected randomly throughout Australia. Queensland was the region where most of snakebite cases were recorded (Tabl.I) over a 5-year period (413 dogs), followed by Victoria (88 dogs) and New South Wales (87dogs) (Mirtschin et al. 1998).

Table I. Geographical distribution of snakebites (Mirtschin et al. 1998)

Animal	Qld	NSW	SA	Vic	WA	Tas	ACT	NT	Not known[a]
Cat	232	157	186	115	103	13	11	0	4
Dog	413	87	60	88	37	11	4	0	2
Cattle	14	17	1	3	0	0	0	1	0
Horse	8	4	5	8	0	0	0	0	2
Other[b]	0	4	0	0	0	0	0	0	0
Total	667	269	252	214	140	24	15	1	8

Most of snakebites in dogs occurred in Queensland (413 dogs), Victoria (88 dogs) and New South Wales (87 dogs). Considering all animals, the total number of snakebites that occurred in Queensland is three times more important than in any other states.

QLD= Queensland, NSW= New South Whales, SA= South Australia, Vic= Victoria, WA= Western Australia, Tas= Tasmania, ACT Australian Capital territory, NT= Northern territory. Not known= address non-indicated by respondents, Other= Wallaby, Kangaroo and two unidentified animals.

In Victoria and Tasmania, more than half of dogs were bitten by tiger snakes (121 dogs), while in the other states, brown snakes were more often incriminated (Tabl. II).

Table II. Distribution of snakebites in Australia in relation to species (Mirtschin et al. 1998).

Type of snake	Qld	NSW	SA	Vic	WA	Tas	ACT	NT	Not known[a]
Brown	549	223	236	71	108	0	14	1	3
Tiger	11	12	0	121	31	24	0	0	1
Taipan	14	1	0	0	0	0	0	0	0
Black	35	29	1	2	0	0	1	0	0
Unknown	58	4	15	20	1	0	0	0	4
Total	667	269	252	214	140	24	15	1	8

[a]Respondents did not give their address.

Different proportion of snake species are implied in snakebites depending on the state considered. Dogs have been bitten in majority by tiger snakes in Victoria whereas brown snakes are more frequently involved in other states.

QLD= Queensland, NSW= New South Whales, SA= South Australia, Vic= Victoria, WA= Western Australia, Tas= Tasmania, ACT Australian Capital territory, NT= Northern territory. Not known= address non-indicated by respondents

Bites have also been shown to be more prominent in rural areas (78%) compared to urban areas (22%) with brown, tiger and black snakes accounting for 76%, 13% and 6% of cases, respectively (Mirtschin et al. 1998).

Venom toxicity

The tiger snake is one of the world's most venomous snakes based on its venom Lethal Dose 50% (LD50= 0,118mg/Kg) (4th venomous species with the lower LD50) (Broad, Sutherland, and Coulter 1979). Nevertheless, to assess the relative danger of each snake species, many other factors such as the relative toxicity should be taken into consideration. The amount of venom injected into the prey and the 'efficiency' of the bite (i.e. the masse of venom actually injected into tissues) are of great importance. They have been shown to depend on the number of consecutive bites, fang length and oral morphology, posture and ferocity in attack or defense. The age of the snake may influence the relative toxicity: for example, decreased coagulant activity has been reported with increasing age (Chippaux, Williams, and White 1991; Heller et al. 2007; Morrison et al. 1983). Dogs seem to be particularly sensitive, with lethal doses reported of 0,03mg/Kg, making them three times more sensitive than cats (Barr 1984).

1.1.2 PRINCIPAL COMPONENTS OF SNAKE VENOM

Snake venoms are the most complex of all natural venoms and poisons. They contain a large number of toxic and non-toxic proteins and peptides, non-protein toxins, carbohydrates, lipids, amines, and other small molecules. These components allow to digest the prey, to paralyse it or to kill it. Under evolutionary pressures, venoms have evolved to take full advantage of ecological niches and prey species. Venom toxins have been selected to be specific for many targets in animal tissue (Warrell 2010; Goddard et al. 2011).

The tiger snake venom contains potent neurotoxins (nerve damaging, pre- and post-synaptic toxins), myotoxins and modulators of coagulation and inflammation. Neurotoxic components affect the central nervous system by blocking neurotransmission. Myotoxins lead to severe muscle damage. Venom constituents are also able to disrupt the haemostatic system by affecting blood coagulation (procoagulant and anticoagulant components, fibrino(geno)lytic components), by acting on vessel walls and by acting on platelets (aggregating agents and aggregating inhibitors agents) (Tabl.III).

Three main types of venom components have been characterised from Australian Elapids, family in which tiger snake belongs: powerful peptidic neurotoxins, proteins acting on the hemostatic system and lipases (Fry 1999; Goddard et al. 2011).

1.1.2.1 Venom neurotoxins

The neurotoxic components of Elapid snake venom may act either pre or post synaptic at the neuromuscular junction to disrupt nervous function.

Pre-synaptic action (β-neurotoxins)

Presynaptic neurotoxins have been identified in the venoms of four major families of venomous snakes (i.e. Crotalidae, Elapidae, Hydrophiidae and Viperidae). Some of the most well characterised β-neurotoxins have been identified in Australian snake venoms, including notexin (isolated from *Notechis scutatus*) the principle neurotoxic and myotoxic phospholipase A_2 (PLA_2) in Elapids (Cull-Candy et al. 1976; Hodgson and Wickramaratna 2006). These PLA_2 bind selectively and irreversibly to specific sites with the membrane of the nerve ending and block neurotransmitter Acetycholine (ACh) release (Heller et al. 2007). Thus, they interfere with the formation of synaptic vesicles by arresting vesicle membrane recycling

(Fig.III). When the stores of neurotransmitter vesicles are depleted, a neuromuscular block results, leading to paralysis (Cull-Candy et al. 1976). Other less potent pre-synaptic neurotoxic PLA2 enzymes such as scutatoxin and notechis II-5 are also found in tiger snake venom (Fry 1999).

Post-synaptic action (α-neurotoxins)

The alpha neurotoxins found in Australian Elapids are reversible postsynaptic blocking neurotoxins. These toxins are present in small amounts and act in a similar way: they bind with high affinity to skeletal nicotinic acetylcholine receptors (Fig.III) and thus prevent the binding between ACh and its receptors (Fry 1999). Tiger snake antivenom counteracted the postsynaptic, but not the presynaptic effects of tiger snake venom when they had developed (Fig. III) (Datyner and Gage 1973).

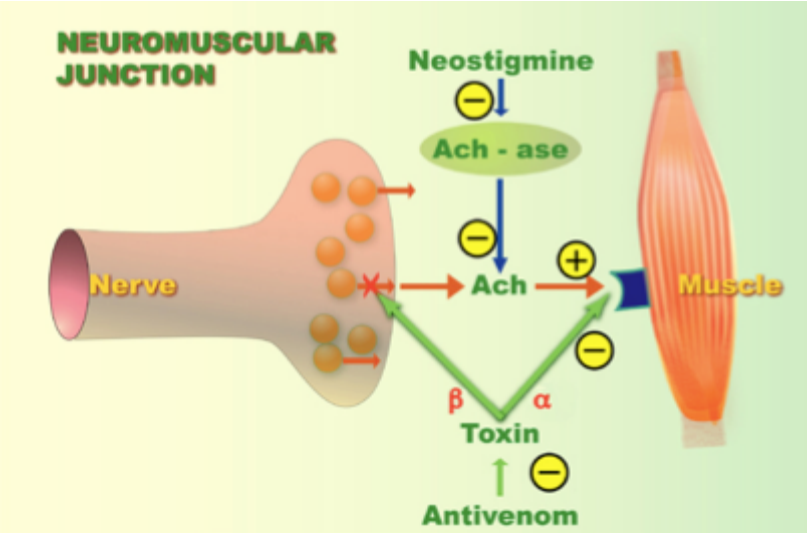

Figure III. Site of action of presynaptic and postsynaptic snake venom neurotoxins (Van den Enden MD, Erwin. 2015).

Presynaptic (β-neurotoxin) and post synaptic (α-neurotoxin) toxins are represented with a green cross. Antivenoms are able to counteract the post synaptic action. These mecanisms are the main one involved in Elapids snake.

Metabolic changes

Alvarez et al. investigated the effects of several snake venoms on the Ca2+-dependent K+ channels of human red cells. Venom of the snake *Notechis scutatus* is able to irreversibly inhibit Ca2+ dependant K+ transport. Thus, the neuromuscular block is further potentiated by venom-induced metabolic changes that interfere with ions (Alvarez and García-Sancho 1989).

1.1.2.2 Venom components acting on the hemostatic system

1.1.2.2.1 Venom proteins acting on blood coagulation factors

Haemorrhaging and incoagulable blood are common findings in Australian Elapids snakebites. Many species have the prothrombin activating enzymes in their venoms and the vast majority contains phospholipaseA2s (PLA2s). Elapids can be divided into two main classes: species with procoagagulant venom including *Notechis scutatus* (tiger snake) and species with no procoagulant venom (Fry 1999).

Venom proteins acting as coagulant factors

Venom proteins include metalloproteinases such as factors FV activators, FX activators, thrombin-like enzymes and prothrombin activators. Metalloproteinases affect coagulation by activating coagulation factors in the cascade of hemostasis (Fig.IV).

Prothrombin activators are snake proteins known in *Notechis scutatus* and many other species, in the form of factor Xa-like which function is to catalyse the formation of thrombin, resulting in a disturbance of the coagulation cascade (Heller et al. 2007). Prothrombin activators are able to convert prothrombin into meizothrombin or thrombin (Birrell et al. 2007). According to (Rosing and Tans 1992), based on their structure, venoms can be divided into four groups depending on what prothrombin can be converted and depending on the cofactors needed. Venoms from Australian Elapids fall into either group II or group III (serine proteinases), able to convert prothrombin to thrombin. More precisely, tiger snake belonged to group II, in which activators are Ca^{2+} dependant and their activity increased by the presence of Ca^{2+}, factor Va, and phospholipid (Fry 1999). Then, thrombin act as a positive feedback in the coagulation cascade, leading to the activation of other coagulation factors, subsequent fibrin formation and almost complete consumption of Factor V, Factor VIII, Factor XII and fibrinogen (G. K. Isbister et al. 2010). The latter consumption of coagulation factors results in the situation of venom-induced consumptive coagulopathy (VICC) that will be discussed further.

FV and FX activators have been isolated from Viperidae, Colubridae and a few snakes from Elapid family, and act as procoagulant in the coagulation cascade (Lu, Clemetson, and Clemetson 2005). Thrombin like enzymes, mainly found in Colubridae and Viperidae, do not activate the same coagulation factor than thrombin (which activates FXIII). Thus, the fibrin formed is easily removed from the circulation and thus can act as a defibrinogenating agent (Fig. IV) (Tans and Rosing 2001; Lu, Clemetson, and Clemetson 2005).

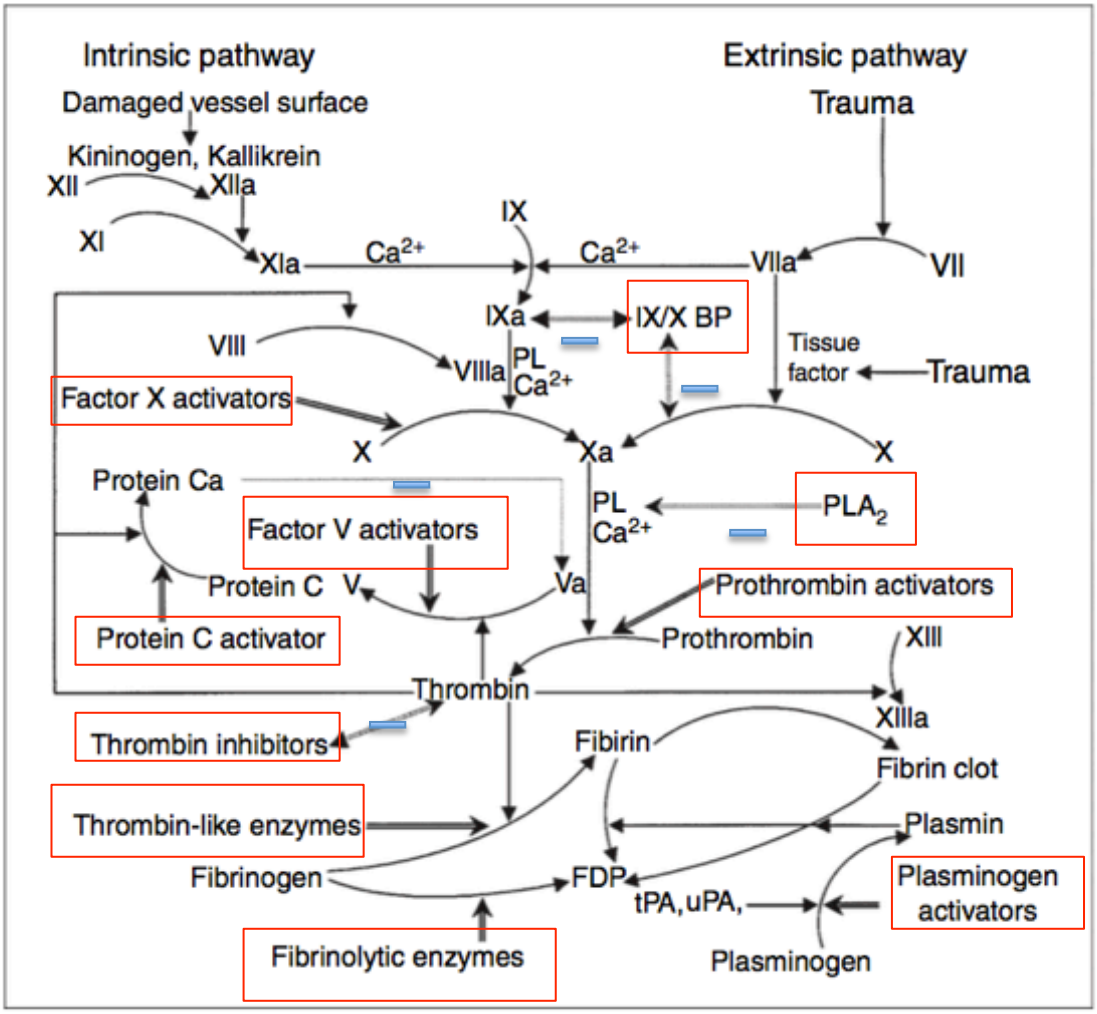

Figure IV. Site of action of venom proteins on the cascade of haemostasis (Lu, Clemetson, Clemetson 2005, p1792).

Venom proteins are surrouned in red, straight arrows represent the activation of factor. Straight arrow = activation, curved arrow = conversion.

: *Inhibiton* : *Venom component*

PLA_2= Phospholipase A2; IX, X BP= IX, X binding protein, tPA= tissue type Plasminogene activators; uPA= Urokinase plasminogene activators

Venom proteins acting as anticoagulant factors

While procoagulant effects are widely described, some venom proteins inhibit FIX and FX and thus act as anticoagulant (Morita 2004). Furthermore, Protein C activators are anticoagulant purified from some venoms, which degrade FVa and FVIIIa (Fig.IV) (Lu, Clemetson, and Clemetson 2005). Thrombin inhibitors have been found in *Bothrops* venom (Viperidae family). It has several roles including prolonged fibrinogen-clotting time by inhibiting competitively the binding of thrombin to fibrinogen (Zingali et al. 1993). PLA2s can

also be classified as strong anticoagulant due to inhibiton of FX and inhibition of the prothrombinase complexe (FVa, FXa, phospholipids and calcium ions) but it is not reported in tiger snake venom (Francis S. Markland 1998).

Venom proteins acting on fibrinolysis

Fibrinolytic and fibrinogenolytic activity has been described in the venoms of members of the Crotalidae, Viperidae and Elapidae families. They act by lysing fibrinogen in FPD (fibrinogen product degradation). Plasminogen activators (Fig.IV) act by activating plasminogen to plasmin, which can transform fibrin clot into FPD (F. S. Markland 1998; Lu, Clemetson, and Clemetson 2005).

1.1.2.2.2 Venom proteins acting on vessel walls

Many metalloproteinases described in Crotalidae, Viperidae and Elapidae venoms are haemorragic toxins that act in synergy to degrade the blood vessel extracellular matrix, and to play key roles in the development of such symptoms as haemorrhage, edema, hypotension, hypovolemia, inflammation, and necrosis (Hite et al. 1994; Birrell et al. 2007).

C-type lectins belong to the composition of some Elapidae venoms and can cause oedema, increased vascular permeability and agglutinate erythrocytes although mechanisms remain unclear (Lu, Clemetson, and Clemetson 2005; Birrell et al. 2007).

1.1.2.2.3 Venom proteins acting on platelets

Snake venoms contain proteins acting on platelets such as PLA2s, C-type lectins, disintegrins and metalloproteinases binding to collagen or collagen receptors on platelets. PLA2s affect platelets function by several mechanisms (for example: cleaving platelet membrane phospholipids). Tough in many cases PLA2s induce platelet aggregations initially, in high concentration they will often inhibit it (Hutton and Warrell 1993).

Table III. Summary of principal types of toxin effects on the haemostatic system (modified from Francis S. Markland 1998).

Toxin type	Effect
Procoagulants	Prothrombin activators FV activators FX activators Thrombin like enzymes
Anticoagulants	Protein C activators Factor IX, X inhibitors Thrombin inhibitors
Fibrinolytic	Fibrinolytic enzymes Plasminogen activators
Vessel walls interactive	Haemorragin toxins C-type lectins
Platelets aggregating agents	PLA2s C-type lectins Desintegrins Metalloproteinases
Platelets aggregation inhibitors	PLA2s

Examples of different venom toxins acting as procoagulant, anticoagulant, fibrinolytic agent, acting on vessel walls and on platelets. For example, FV activators have been described in Naja naja oxiana and Naja nigricollis nigricollis venoms, prothrombin activators in Pseudonaja textilis.

1.1.2.3 Lipases

The vast majority of lipases found in Australian snake venoms are phospholipases. There are four main species of phospholipases (A1, A2, C, D). Most venom phospholipases are Phosphatidylcholine 2 - Acylhydrolase (PLA2): they are the most frequently encountered proteins identified across the species (Fry 1999; Birrell et al. 2007). The PLA2s found in Elapid venom may be classified into five types: neurotoxic, haemotoxic, myotoxic, non-toxic enzymatically active and non-toxic non-enzymatically active (Heller et al. 2007).

Neurotoxic PLA2s produce neurological effects not through the enzymatic esterase activity but rather through direct blockage (presynaptic acethylcholine release blocker) (see part 1.1.2.1). Notexin is the principle neurotoxic and myonecrotic phospholipase A2 in *Notechis* species (Fry 1999).

Haemotoxic PLA2s produce haemorrhage through blockage of factors in the coagulation cascade. It doesn't produce net anticoagulation through fibrinolysis but rather bind to specific factors essential in the coagulation cascade (see previous part 1.1.2.2). Hemotoxic PLA2s isolated from the tiger snake are responsible for hypotension and haemorrhage (Fry 1999).

Myotoxins, defined as venom components acting on skeletal muscle, act by causing damage to the integrity of the sarcolemma, resulting in local haemorrhage and necrosis to systemic skeletal rhabdomyolysis producing severe myoglobinuria (Heller et al. 2007; Mebs, Ehrenfeld, and Samejima 1983). The degeneration of muscle induced by the inoculation of either venom or pure myotoxins is rapid and followed by regeneration of the affected tissue (Harris et al. 2003; Heller et al. 2007). This myotoxin PLA2s has been well described in a particular species of Elapidae (Pseudechis australis), which differs from the other species with the primary toxins being myotoxic rather than neurotoxic (Fry 1999).

Non-toxic PLA2s have also been isolated from the tiger snake and do not present any toxic activity (Fry 1999).

Moreover, PLA2s are known to play a role in the hyperalgesia induced by *Bothrops asper* venom (Chacur et al. 2001) and may play a role in the inflammatory process (Fry 1999; Cher et al. 2003), which will be further detailed (see part 1.2.3.2).

Other lipases such as lyophospholipases and phospholipases also exist and are responsible for haemolysis and myolysis (Heller et al. 2007).

1.1.2.4 Venom component specific to Elapid snakes

The venom protein components of 18 Elapid snake species were separated and identified using mass spectrometry and peptide sequencing. Three groups of proteins account for a large proportion of the total venom proteins, confirming their high abundance: neurotoxins, prothrombin activators, and PLA_2s (Tabl. IV).

SVMPs, known in Viperidae venoms has been identified in 7 out of the 18 Elapid species (species non precised). A limited number of C-type lectins have also been identified in this study in some Elapids.

Table IV. Venom protein components of Elapid snake species and their main effects on the organism (modified from Birrell et al. 2007)

Toxin type	Effect
Neurotoxins	**Neurological signs (mydriasis, paralysis, etc.)**
Prothrombin activators	**Consumptive coagulopathy**
Phospholipases A2	**Neurological signs, muscle degeneration, necrosis, inflammation**
SVMP	Hemorrhage, oedema, hypotension, hypovolemia, inflammation, and necrosis
C-type lectins	Oedema, increased vascular permeability

Neurotoxins, prothrombin activators and phospholipases A2 (highlighted) represent the three major groups of components found in tiger snake venom.

1.1.3 CLINICAL PRESENTATION OF SNAKE ENVENOMATION IN DOGS

Clinical effects of snake envenomation include local, general, neurological, haematological and myotoxicity signs (Tabl.V).

Clinical effects at the bite site

At the bite site, the envenomation can cause locally painful and redness, swelling, and bruising. It is usually local minor effects although necrosis may occur. Fang puncture or scratch marks can be visible and helpful for the diagnosis (Geoffrey K. Isbister et al. 2013). A study on 53 dogs envenomed by *Vipera berus* showed a varying degree of swelling in the area of the bite. The bite site was located in the head/nose in 77% of dogs, in the hind limb in 13%, the front limb in 6%, the neck and prepuce in 2% each (Lervik, Lilliehöök, and Frendin 2010). Nevertheless, in Elapids envenomation, snakebite is not always visualized, as in other studies of tiger snake bites in which wounds were seen in only 9 on 125 dogs (Barr 1984) and 1 on 104 dogs (Indrawirawan et al. 2014) and were generally found on the face. It can be due to the

difficulty of identification of the wound (and the fact that the face has less hair than other parts of the body) as well as a lower sever local response.

General signs

Non-specific signs such as ptyalism (10%), vomiting (38% to 42% according to the authors) and generalised muscle weakness/collapse (around 30%) or reduced level of consciousness (14%) are common signs in tiger snake envenomation on dogs (Barr 1984, Indrawirawan et al. 2014). Collapse is usually described but often followed by rapid recovery (29%, Indrawirawan et al. 2014). Tachycardia, tachypnea or dyspnea are usually found in tiger snake envenomation, controversially elevated rectal temperature is not usual. Draining lymph nodes may also be tender (Barr 1984). These findings are also observed in humans envenomed by tiger snake (Geoffrey K. Isbister, Brown, and ASP Investigators 2012).

Neurological signs

Neurologic signs are also a common finding on dogs following a snake envenomation, including by tiger snake. Experiments on mice have shown the relationship between the dose and the severity of the disease, suggesting that these signs depend on the amount of venom injected at the bite site (Lewis 1994).

The earliest signs are usually disorientation and ptosis, which develops from one to several hours after the bite. Mydriasis and loss of pupillary light reflex, which is consistent with a paralysis of oculomotor nerve, are common findings and more particularly the most commonly observed signs in a study on tiger snake envenomation (76% and 66% of dogs respectively) (Barr 1984). In a more recent study on 21 dogs envenomed by tiger snake, mydriasis was observed on 29% of the dogs and reduced pupillary reflex on 24%. These numbers can be underestimate since they only include tiger snake envenomation confirmed by SVDK, professional snake handler or scale counting and do not include “suspected tiger snake envenomation” (Indrawirawan et al. 2014). In a study on 53 dogs, 73% of them had an affected mental status on arrival at the hospital, but this study was concerning only Viper envenomation (Lervik, Lilliehöök, and Frendin 2010). Then paralysis of facial, peripheral and glossopharyngeal muscles may occur and thus conduct to respiratory failure. Generalized paralysis is an important feature of tiger snakebites, but clinical evidences of flaccid paralysis take several hours to become apparent.

These neurological effects are usually reversed with antivenom if given prior to development of major paralysis. If many hours after the bite, the patient presents a major paralysis requiring intubation and ventilation, it may be necessary to continue this treatment for days, weeks, and very occasionally, even over a month (Fry 1999; Hodgson et al. 2003; Geoffrey K. Isbister et al. 2012).

Haematological signs

Coagulopathies are well known in snake envenomation and may result in clinical signs such as increased bleeding (linked to decreased blood coagulability or to endothelial damages), hypovolemic shock and secondary organ damage, macro- and microthrombosis, such as pulmonary thromboembolism (Goddard et al. 2011). Australian Elapids venom toxins act as procoagulants, causing in-vivo activation of the coagulation system, but in most cases, this does not result in massive thrombosis and consequent embolic disease, but rather causes consumption of coagulation factors, resulting in clinical anticoagulation. It may cause important abnormalities of clinical laboratory tests, but generally does not result in clinically significant bleeding (White 2005).

Bleeding from bite site or venupuncture site (hematoma, ecchymosis), oral and nasal cavity (epistaxis, petechial haemorrhages), gastrointestinal (melena), urinary and intracranial sites, may occur (study non specific to tiger snake) (Geoffrey K. Isbister et al. 2013). Discolouration of the urine is a common finding, reported in 25% of the dogs envenomed by tiger snake (Barr 1984) but can be the result of haemoglobin, myoglobin or erythrocyte's presence.

Myotoxicity signs

Severe myolysis can occur with tiger snakebites, particularly if treatment is delayed or inadequate. Myotoxicity was seen in 59% of the dogs envenomed by tiger snake (Indrawirawan et al. 2014) and is generally characterized by increased Creatine Kinase (CK). The usual signs may occur, e.g. muscle tenderness, muscle weakness, pain on contracting muscles, myoglobinuria and later development of muscle wasting (Geoffrey K. Isbister et al. 2012).

Renal failure

No renal toxins have so far been isolated from tiger snake venoms, but renal failure is a common effect of tiger snakebites in humans, for cases with significant envenoming. Nevertheless it is not usual in dogs, in which only a few isolated cases are reported (Warrell 2010).

Histopathology findings

A study has described the examination of post mortem heart, lungs, kidneys and gastrocnemius muscle tissue from one dog envenomed by a tiger snake. Pathological evidence of procoagulant venom activity with formed thrombi has been observed in the lungs, heart, muscles and kidneys. This supports the proposal that an initial thrombotic state occurs in envenomed dogs, leading to the depletion of vital clotting factors and thus to coagulopathy. Nevertheles, it is unclear whether renal failure is a direct result of the venom's nephrotoxicity or a secondary effect of myoglobinuria (Jacoby-Alner et al. 2011).

Table V. Clinical presentation of snake envenomation

Clinical signs	Presentation
Local signs	**Not usualy visualized**. Pain, red, swelling and bruising Scratch marks
General signs	**Ptyalism, vomiting** **Collapse / Reduced level of consciousness** **Weakness** **Tachycardia**, **tachypnea, dyspnea** **Tender lymph nodes**
Neurological signs	Disorientation Ptosis, **mydriasis, loss of pupillary light reflex** **Ataxia, generalized weakness, paralysis**
Haematological signs	Mostly laboratory findings (bleeding, hypovolomic shock not frequent) **Discoloration of urines**
Myotoxicity signs	**Muscle tenderness, weakness, pain**

Clinical signs associated with snake envenomation include local, general, neurological, haematological and myotoxicity signs. The most frequently observed clinical signs in tiger snake envenomation on dogs are highlighted.

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1.1.4 CLINICOPATHOLOGICAL VARIABLES ASSOCIATED WITH OPHIDIAN ENVENOMATION

1.1.4.1 Biochemistry

Many biochemical abnormalities have been described in snake envenomation (Tabl. VI) and may help the veterinarian to detect pathologic effects. Myotoxicity is the most affected biochemistry parameter described on tiger snake envenomation in the literature. Other biochemical abnormalities are described in various snake species.

Muscle activity: myotoxicity

In humans, CK (Creatine Kinase) activity is an indicator of many factors (like the amount of venom injected, the delay between envenomation to presentation and the quantification of myonecrosis) (Nakada et al. 1984) but its use is limited in dogs since it is often difficult to know the exact time of snakebite (Goddard et al. 2011). In an in-vivo study on mice, from six Australian Elapid venoms, only those from *Pseudechis colletti guttatus* and *Pseudechis australis* produced a rise of CK-activity. They also showed that a fraction of the Elapid snake *Pseudechis colletti guttatus* venom, the Phospholipase A2, caused a dose-dependent increase of CK-level and myoglobinuria (Mebs, Ehrenfeld, and Samejima 1983). Elevated CK were found in most cases of several studies: 15 out of the 28 sampled dogs (54%) 24h after admission on Viperidae envenomation (Lervik, Lilliehöök, and Frendin 2010), 75 out of 109 dogs (69%) in another study on Viperidae envenomation (Aroch and Harrus 1999) and 43 out of the 45 dogs (96%) on tiger snake envenomation (Barr 1984). In a more recent study on tiger snake envenomation, myotoxicity was seen in 59% of dogs with tiger envenomation, with a median CK activity of 1,878 U/L; the highest CK value was 62,586 U/L (Indrawirawan et al. 2014). In some of these studies, it was suggested that CK values were within the reference values, mainly for samples that were collected too early after snakebite (before the release of plasma CK from degenerating muscle) or too late in the envenomation process (after excretion by the kidneys). The increased CK activity may be related to rhabdomyolysis induced by snake venom myotoxicity and also to intravascular hemolysis.

Hepatic variables: hepatotoxicity

Hepatic variables changes associated with snake envenomation have mostly been described in Viperidae family. In a study on 34 dogs envenomed by *Vipera berus*, 65% had a

serum concentration above reference values for at least one liver enzyme (Alanine Amino Transferase ALT, Alkaline Phosphatase ALP, Lactate Deshydrogenase GLDH and bile acids) during one or more examinations (on arrival, at 24h, at follow-up on the period of days 4-10 and days 9-23). But in most cases, the increase was mild, temporary and only one or two parameters were affected (Lervik, Lilliehöök, and Frendin 2010). High increased of these variables have also been described in dogs envenomed by *Vipera palaestinae.* These findings have suggested hepatocellular damages and cholestasis, as a consequence of vascular ischemia as well as direct damages by cytotoxin effects of snake venom. An increase to some degree in ALAT and ASAT may also be explained by muscle damages and by glucocorticoid treatments (Aroch and Harrus 1999, Goddard et al. 2011).

Renal variables: nephrotoxicity

According to Lervik, Lilliehöök, and Frendin (2010) and Segev et al. (2008), the serum creatinine was within the reference values for all dogs at all examinations. Nevertheless some cases of acute renal failure have been described in dogs' envenomation. For example, a dog bitten by en Elapid snake, the Red-bellied Black snake (*Pseudechis porphyriacus*), with signs of rhabdomyolisis and hemolysis (increased CK, bilirubine and ASAT) presented moderate increased creatinine, azotaemia and marked hyperphosphataemia consistent with renal failure. This dog presented evidence of shock, dehydration, with discoloured and reduced urine production and became totally anuric before euthanasia (Heller et al. 2006). Renal failure may be due to renal ischemia caused by vasoconstriction and procoagulant microthrombotic effect, nephrotoxic effects of myoglobinuria, hemoglobinuria, DIC (disseminated intravascular coagulopathy), toxic nephropathy, and hypovolemic shock (Goddard et al. 2011, Heller et al. 2007).

Other biochemical abnormalities:

Other abnormalities have been especially shown in dogs envenomed by *Vipera palaestinae* e.g.: hyperalbuminemia, hyperglycemia, hypocholesterolaemia, hypoalbuminemia and hyperproteinemia (Aroch and Harrus 1999; Segev et al. 2004; Segev et al. 2008). Moderate hyperalbuminemia could be the consequence of hemolysis and/or ischemic hepatopathy (Goddard et al. 2011). Hyperglycemia can be linked to stress and pain (Segev et al. 2004). Hypocholesterolaemia and hypoalbuminemia may be explained by capillary damages and changes in lipoprotein transport and metabolism due to the phospholipase A2 (Segev et al. 2004). Total cholesterol level was also assumed to be inversely correlated to the severity of the

envenomation in human cases (Winkler et al. 1993). These abnormalities are poorly described on tiger snake envenomation.

1.1.4.2 Heamatology markers

1.1.4.2.1 RBC abnormalities

Hemoconcentration, characterized by an increased in hematocrit and hemoglobin concentration is a common finding on envenomation by puff-adders from Viperidae family and cobras from Elapidae family (Segev et al. 2004; Segev et al. 2008; Lobetti and Joubert 2004; Aroch et al. 2010). This abnormality can be explained by fluid losses, associated with third spaces due to heamorrhagins inducing capillary damages and the shift of proteins and fluids from circulation to the inflamed tissue at the bite site. Splenic contraction may also be involved associated with excitement, pain, and stress and subsequent release of stored erythrocytes into circulation. Dehydration was eliminated as a prominent cause of increased hematocrit based on lack of clinical evidence (Lobetti and Joubert 2004; Goddard et al. 2011).

Haemolysis is well described in snake envenomation, especially in the Elapid red-bellied Black Snake. First evidence is a discolouration of serum (Heller et al. 2006). Another evidence is red urine, although it could be the result of bleeding, hemoglobinuria or myoglobinuria and thus imply hemolysis, rhabdomyolysis, or renal damages (Heller et al. 2006). Nevertheless, in an in-vivo study on experimental administration of tiger snake venoms in dogs, although haemolysis appears quickly with plasma haemolized in 30 mintes and even at low doses of venom injected, it didn't seem to progress significantly. Haemolysis is thought to be the consequence of lytic factor or phospholipase activity which damages the erythrocyte cell membrane and results in an influx of water, leading to erythrocyte swelling, stretching and, ultimately, haemolysis (Goddard et al. 2011; Heller et al. 2006). The haemolysis is sometimes accompanied by echinocytosis and spherocytosis (Aroch and Harrus 1999).

Anemia has been observed less frequently in dogs with snake envenomation (Goddard et al. 2011).

1.1.4.2.2 Leukocytes abnormalities

Leukocytosis also characterizes envenomation in dogs, suggesting an acute inflammatory reaction. It has been described on envenomation by Viperidae such as the puff adders (*Bitis arietans*) and the palestine viper (*Vipera palestinae*) and by cobras from the Elapidae family

(Lobetti and Joubert 2004; Aroch and Harrus 1999; Goddard et al. 2011; Segev et al. 2004). Leukocytosis was identified as a risk factor for mortality in dogs with *Vipera palaestinae* envenomations (Aroch and Harrus 1999; Goddard et al. 2011).

1.1.4.3 Hemostatic changes

1.1.4.3.1 Platelets abnormalities

Various degrees of thrombocytopenia are commonly found: for example, on dogs envenomed by *Vipera palestinae*, 51.9% have shown thrombocytopenia at the venom admission and 52% 24h post admission (Aroch and Harrus 1999). A fall in the platelet count is usually evident within 24 hours of the bite (Geoffrey K. Isbister 2010). In a mouse model, platelet numbers were significantly decreased 30 minutes after *Bothrops asper* snake venom injection and returned to normal after 12 hours (Lomonte, Tarkowski, and Hanson 1993). Several mechanisms could explain thrombocytopenia in envenomation such as vasculitis, sequestration of platelets in inflamed tissues, and consumption of platelets with potential development of disseminated intravascular (Goddard et al. 2011). It is poorly described in tiger snake envenomation.

Interaction of snake venom with platelets aggregation are reported. Some PLA2s seem to induce platelet aggregation by cleaving platelet membrane phospholipids, releasing arachidonic acid and forming arachidonic acid metabolites such as thromboxane A2. Another group of PLA2s inhibit platelet aggregation via the cleavage products (Lu, Clemetson, and Clemetson 2005).

1.1.4.3.2 Vascular abnormalities: coagulopathy

As seen earlier (part 1.1.2.2.), a large number of venom proteins act as procoagulants, sometimes as anticoagulants, that then create thrombosis, consumption of coagulant factors and thus may result in incoagulable blood and bleeding (Fry 1999; White 2005). Coagulopathy is a common finding in snake envenomation and Australian Elapids are amongst the most potent in causing procoagulant coagulopathy (White 2005).

Coagulation testing in envenomed dogs is mainly characterized by coagulopathy evidence such as increased Prothrombine Time (PT) and activated Partial Thromboplastin Time

(aPTT), decreased fibrinogen, increased Fibrin/ fibrinogen degradation products (FDP) or D-Dimer levels, hypoantithrombinemia activity. An observation on blood coagulation in cats and dogs naturally envenomed by tiger snake showed that all dogs (n=6) had an increased PT and an increased aPTT, and half of them (n=3) had increased FDP levels (Holloway and Parry 1989). Later, other studies showed similar results in Viper envenomation with prolonged PT and aPTT at admission (68.4% and 21.1% of the dogs respectively (Segev et al. 2004), 29% and 39% of the dogs in (Aroch et al. 2010)) and further prolonged aPTT a day later (75.8% of the dogs). Prolonged PT and aPTT were observed in 54% and 44% of the dogs respectively, at least once during the experiment. Hypoantithrombinemia was observed at least once in 56% of the cases and D-dimer concentrations were positive (>250 ng/mL) in 90% (Aroch et al. 2010). In these studies, the authors recommended that dynamic multiple coagulation tests should be realized as the incidence of abnormalities was associated with higher mortality. Thromboelastography may also be an helpful diagnosis of coagulopathy (Goddard et al. 2011).

Presence of thrombocytopenia, increased PT and aPTT may lead to the diagnosis of DIC (Disseminated Intravascular Coagulopathy). Nevertheless, in Elapid envenomation, and especially with tiger snake species, this consumptive coagulopathy associated with snakebites has been referred to as a VICC, Venom Induced Consumption Coagulopathy (Goddard et al. 2011). VICC is characterized by prolonged clotting times, depletion of fibrinogen and cofactors V and VIII, and high concentrations of FDPs. However, it does not include evidence of thrombus formation and organ failure. In a study on tiger snake envenomation on dogs, laboratory evidence of a coagulopathy was detected in 12 out of 21 dogs (57%) envenomed by confirmed tiger snake and in 34 out of 41 dogs (83%) with suspected tiger snake envenomation (Indrawirawan et al. 2014). These numbers are lower than those observed in human literature where VICC was observed in 95% of tiger snake victims (Geoffrey K. Isbister et al. 2012). It was suggested that dogs are less sensitive to the procoagulant effects of tiger snake venom than humans (Indrawirawan et al. 2014).

Table VI. Laboratory evidences of snake envenomation

Laboratory evidence	Affected parameters
Myotoxicity	**High CK levels**
Hepatotoxicity	High ALP, ALT, GLDH, bile acids levels
Nephrotoxicity	High Creatinine levels
RBC abnormalities	Haemoconcentration (increased Ht) Haemolysis (haemolized plasma, red urines) Anemia
Leukocytes abnormalities	Leukocytosis
Platelet abnormalities	Thrombocytopenia
Coagulopathy	**Increased PT, aPTT** **Raised D-Dimer concentration** **Low to undetectable fibrinogen (VICC)**

Laboratory evidences highlighted represent those mostly altered in tiger snake envenomation.
CK = Creatine Kinase, ALT = Alanine Amino Transferase, ALPalkaline phosphatase ALP, lactate deshydrogenase GLDH, PT = Prothrombin Time, aPTT = Activated Partial Thromboplastin Time, VICC= Venom Induced Consumption Coagulopathy.

1.1.5 DIAGNOSIS OF SNAKE BITES

The diagnosis of snake envenomation is lead by the association of several factors (Diag.2). First of all, the presence of envenomation should be done on arrival with the history, clinical evidence and laboratory test. If none of the clinical or laboratory evidence can be observed, further exams should be run some hours later with a neurological examination in order to exclude snakebite. If envenomation has been identified, then the vet should determine which snake or group of snakes is most likely to be involved - with use of pictures, scale counting, geographic prevalence and SVDK (Snake Venom Detection Kit).

If the diagnosis is established, appropriate antivenom that covers the likely snake(s) is given. Nevertheless, in veterinary medicine, due to financial reason or to a long elapse time between the bite and the animal presentation, antivenom is sometimes used directly after the history and examination. Then, the diagnosis would be based on clinical improvements following the antivenom administration (Geoffrey K. Isbister et al. 2013).

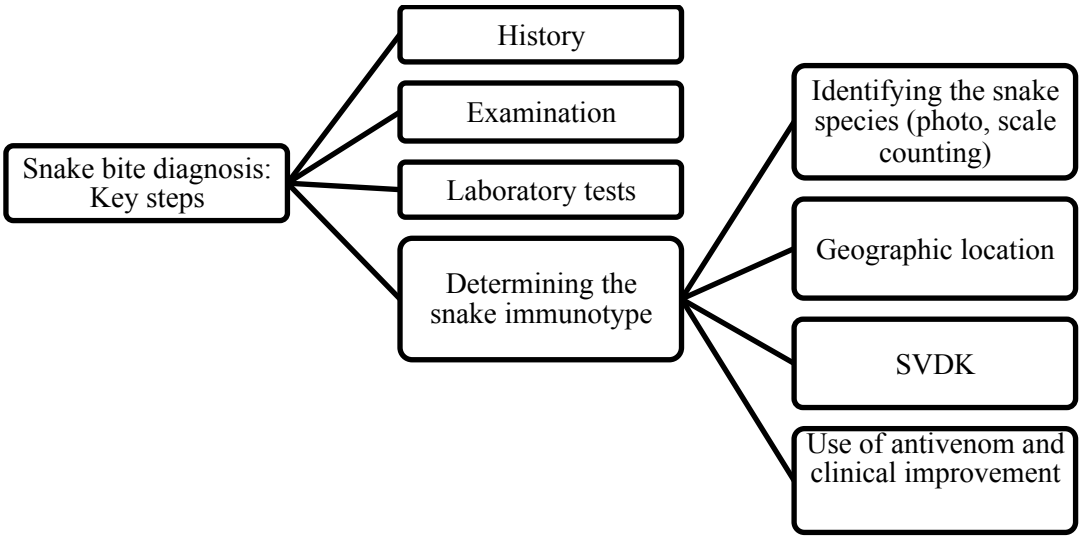


Diagram 2. Key steps for snakebite diagnosis

Two main steps are important to consider: diagnosis of snake envenomation and determination of the snake immunotype.

1.1.5.1 History

History is essential to make a diagnosis of snakebite. A detailed history of the circumstances of the bite (time, temperature, geographic location), of the witnessed snake (length, colouring, use of scale counting), of the first symptoms (vomiting, period of unconsciousness, collapse, etc.) and medical history as well should be taken into account, according to human's guide but applicable in domestic animals (White, 2013).

Nevertheless, as seen previously (see part 1.1.1), the identification of the snake by non-professional people, without any pictures taken or scale counting, is not reliable in the majority of cases.

1.1.5.2 Clinical evidence

Clinical evidence includes the clinical presentation (neurological, haematological and myotoxicity signs) presented previously (see part 1.1.3, Tabl. V). Fang marks at the bite site can be reliable clue of snakebite but is not always observed.

1.1.5.3 Laboratory tests

It is necessary to use clinical signs in conjunction with the history, the physical examination, the laboratory investigation and the venom detection kit. Iterative laboratory

testings and neurological assessments are recommended on admission, 6 hours and 12 hours after the bite (Ireland et al. 2010).

Like in human management, an initial full set of investigations should be made on dogs with a suspected snakebite. Nevertheless, some investigations traditionally done are not always available or cannot be conducted for financial reasons. In humans, it has been shown that the most appropriate combination of tests to reliably detect envenomed patients within 12 hours was INR (International Normalized Rates), aPTT and CK level associated with neurological examinations (Ireland et al. 2010).

1.1.5.4 Snake venom detection kit (SVDK)

Description

SVDK is a rapid sandwich enzyme immunoassay, used in order to detect the immunotype among the five major snakes in Australia (tiger, brown, black, death adder and taipan). The test gives a visual and qualitative result within 15 to 25 minutes and can detect as little as 0.01ng/mL of snake venom in a sample. Therefore, it is not an indication to give antivenom but its primary use is to assist veterinarians in choosing the most efficient antivenom therapy (Manufacturer data).

Principle

Antibodies against a particular immunotypes of the five snakes overrepresented in Australia are present in each well of the kit. First, the test sample is diluted in Yellow Sample Diluent, added to the test wells and incubated for 10 minutes at the room temperature. Any venom present in the test sample binds to the antibodies in the well and then, wells are washed to remove any unbound venom and conjugate. To finish, chromogen and peroxide solution are added to each well, resulting in the development of a blue color considered positive for a specific venom immunotype (Manufacturer data).

Sample selection

Test specimen options include bite site swab, affected clothing or bandage, urine, plasma or serum, heparinised whole blood (other anticoagulants may also be used), other tissue and biological fluids. Generally bite sites are harder to locate in veterinary cases and thus, urine or whole blood is commonly used. The best samples for venom detection are bite site swab (Manufacturer data; Heller et al. 2007).

Test Interpretation

If wells 1 to 5 show no colour change, no venom has been detected from the five most clinically important venom immunotypes. Tiger immunotype is detected if well 1 changes to blue first. In this case, the SVDK may have detected Tiger, Copperhead or Rough Scaled Snake venom (Fig.V). Clinical envenomation from these snakes should be treated with CSL tiger snake antivenom.

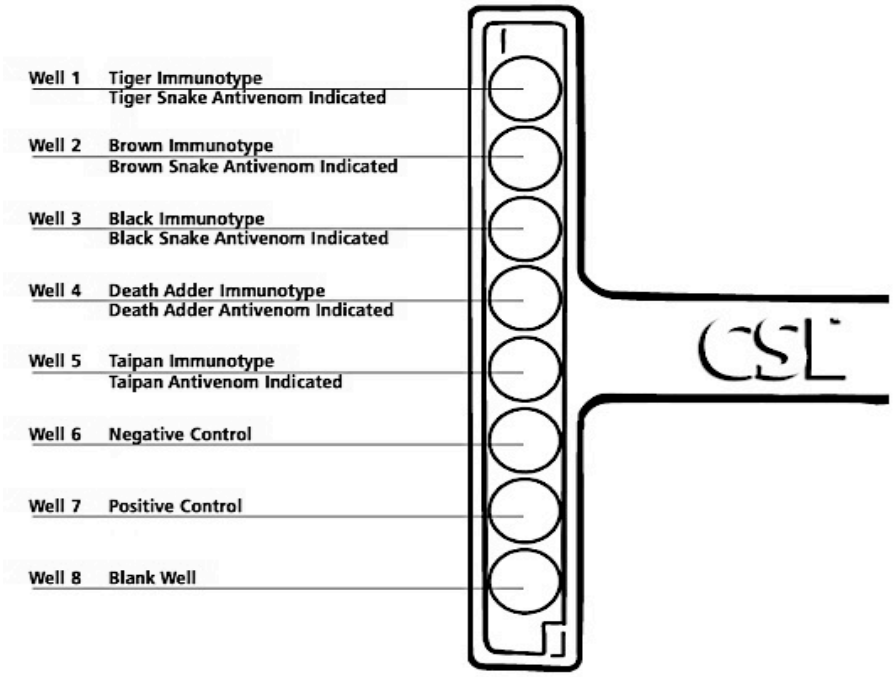


Figure V. Snake venom detection kit: correspondence of wells and snakes immunotypes and antivenom indicated (SVDK, manufacturer data)

Sensibility and specificity

The manufacturer has not published any information on the diagnostic sensitivity and specificity of this kit for snake envenomation.

In the veterinary population, the specificity of the test in urines samples is reported in only one study. There was no false positive in this study, suggesting a specificity of 100% (Ong, Swindells, and Mansfield 2010). Nevertheless, cross-reactivity to venoms from non venomous and mild venomous snakes have been shown in humans literature (Jelinek et al. 2004). These cross-reactions were also observed with other venomous Elapids, although they were found to be low and, remarkably, most apparent between greatly divergent venoms (Steuten et al. 2007). Moreover, in human literature, the SVDK has shown high rates of inaccuracy with a high level of false positives, false negatives and problems of cross-reactivities. This includes misdiagnosis of brown snake envenomation in one of the 10 cases of tiger snake envonomation in humans literature (Geoffrey K. Isbister et al. 2012), an incorrect

result in 14% of snakes where an expert identification was available and false negatives when no venom is detected in around 25% of envenomed patients (Geoffrey K. Isbister et al. 2012). Let's note that SVDK may not be of practical use if the severity of the case doesn't allow waiting for a result.

1.1.6 TREATMENT

1.1.6.1 First aid treatment

In human literature, first aid treatment includes immobilizing the patient in order to slow the systemic spread of the venom from the bitten area and thus delay the envenomation syndrome. Indeed, tiger snake venom is known to be distributed systemically within the lymphatic system and thus, the application of a firm crepe bandage with immobilization by a splint can effectively delay venom distribution through the body and can be also efficient on dogs. Pressure alone or immobilization alone did not delay venom movement (Sutherland, Coulter, and Harris 1979; Heller et al. 2007) .

1.1.6.2 Antivenom treatment

Production and composition

Antivenom is formulated from the serum of horses immunised with the venom of snakes (hyperimmune Immunoglobulins G, IgG, serum) (CSL, 2014). Antivenoms are obtained following a complex production process. After venoms collection and mixture from individual venomous snakes, the preparation is injected to the animals (generally horses). Once the immune response to the immunizing venom mixture has yielded satisfactory antibody levels, blood or plasma is collected from the immunized animals. Antivenoms are then prepared using diverse methods to obtain intact IgG molecules or secondary fragment antigen binding F(ab')2. Many fractionation processes exist leading to the degradation of many non-IgG proteins, and to the cleavage of IgG into bivalent F(ab')2 fragments by removal and digestion of the fragment cristallizable Fc fragment into small peptides (World Health Organization, 2010).

Formulation

Several formulations of antivenoms are available. Monovalent antivenoms are used against envenomation of one species of snake (Tabl. VII).

Bivalent antivenom allow to treat dogs envenomed by two species, and is especially used when no reliable identification was made and when two species are known to be easily confoundable or highly prevalent in a region. For example, the retrospective study conducted in Melbourne Hospital in Victoria noted that the manufacturers of tiger and brown snake antivenom recommend that both antivenoms (bilavent Tiger and Brown snake) should be administered in Victoria if the snake cannot be identified. Nevertheless, they think by their experience that monovalent tiger snake antivenom alone would cover nearly all envenomations from the Werribee area due to tiger snake prevalence in this area (Barr 1984).

Table VII. List of monovalent antivenoms available from CSL and corresponding snake venoms neutralised (modified from SVDK, manufacturer data)

Monovalent Antivenom	Snake Venoms neutralised
Tiger snake	Tiger snake (*Notehis scutatus)*, Copperhead (*Austrelaps superbus*) Red bellied black snake (*Pseudechis porphyriacus*) Rough Scaled snake (*Tropidechis carinatus*) Blue bellied snake (*Pseudechis guttatus*) Broad Headed snake (*Hoplocephalus bungaroides*) Pale Headed snake (*Hoplocephalus bitorquatus*) Stephen's Banded snake (*Hoplocephalus stephensi*) Sea snake
Brown snake	Common or Eastearn brown snake (*Pseudonaja textilis*) Dugite (*Pseudonaja affinis*) Western brown snake (*Pseudonaja nuchalis*)
Black snake	Mulga snake (*Pseudechis australis*) Papuan black snake (*Pseudechis papuanus*) Red bellied black snake (*Pseudechis porphyriacus*) Blue bellied snake (*Pseudechis guttatus*) Butler's mulga snake (*Pseudechis butleri)*
Death adder	Common death adder (*Acanthophis antarcticus*) Desert death adder (*Acanthophis Pyrrhus*) Northern deathnadder (*Acanthophis praelongus*) Pilbara death adder (*Acanthophis sp*)
Taipan	Taipan (*Oxyuranus scutellatus*) Inland taipan (*Oxyuranus microlepidotus*) Papuantaipan (*Oxyuranus scutellatus canni*)

A polyvalent antivenom neutralises the venom from all of the known medically important venomous snakes found in Australia. However, the bigger the required volume is, the higher the risk of adverse reactions is. Consequently, smaller volumes of monovalent antivenoms are more efficient and must be used if possible (SVDK manufactura).

Indication and posology

As seen previously, administration of antivenom is indicated wherever there is evidence of systemic envenomation: a positive result of SVDK in the absence of clinical evidence should not be a reason to give a treatment.

Two retrospective studies compared the benefits of antivenom on dogs envenomed by Crotalidae. Amongst the parameters evaluated, there were not any significant benefits associated with antivenom administration (Katzenbach and Foy 2015; McCown et al. 2009). Nevertheless, an important bias is highlighted by the fact that antivenoms were probably given when the severity of clinics was increased.

In human literature, Australian snake antivenom has been clearly proved to efficiently improve life-threatening systemic effects resulting from envenomation and especially to treat venom-induced coagulopathy (MacDonald, White, and Currie 2008). In Elapid envenomation, its use has been associated with considerable reduced or absent coagulopathy and myotoxicity after antivenom therapy (study on mulga snake) (Johnston et al. 2013; Heiner et al. 2013). The procoagulant effects have also been shown to be neutralized by the antivenom in a study on brown snake envenomation. In this study, the median venom concentration on blood sample of 27 envenomed patients was 20 ng/mL prior to antivenom and was not detected after antivenom administration (Geoffrey K. Isbister et al. 2007).

The posology is still a controversial issue in literature. In human medicine, the use of more than one vial or repeated doses of tiger snake antivenom is no longer recommended and the recovery of most clinical syndromes such as coagulopathy has not been found to improve following additional doses of antivenom (Geoffrey K. Isbister et al. 2012). Nevertheless, in the Australian Veterinary Journal, a review specifies that the amount of vials required depends on the amount of venom instilled and that tiger and brown snake species, may require substantially more than the recommended dosage (Heller et al. 2007).

Tiger snake antivenom therapy is more effective if it is given as soon as possible after the bite and delays in antivenom's administration may conduct to delayed response or slow recovery (Barr 1984). In a study on rattlesnakes (Viperidae family), antivenoms are recommended to be optimally given during the 4 hours following the snakebite, but they are still effective up to 24 hours or longer after envenomation (Juckett and Hancox 2002).

Adverse reaction

Acute adverse reactions occur on average in 5 to 7% of dogs envenomed by Viperidae or Crotalidae. Some clinical signs described are anaphylactic reactions, including fever, swelling and facial pruritus, vomiting, bradycardia, tachycardia, hypotension, and respiratory distress (Lund et al. 2013; McCown et al. 2009). Some other studies do not describe any adverse reactions (Aroch and Harrus 1999; Segev et al. 2004).

Delayed forms called serum sickness, have also been reported in humans from 4 days to 2 weeks after the antivenom's administration. There are no specific published reports of anaphylaxis in dogs due to Elapid snake antivenom's administration or serum sickness. However both effects are documented in humans (Heller et al. 2007).

1.1.6.3 Prophylactic treatment and symptomatic supportive treatment

Empirical therapy should include oxygen administration, wound management, fluid therapy and analgesic drugs.

Antimicrobial use

Local disinfection should always be done if local manifestations are visualized. Due to the potential presence of microbial agent, the prophylactic use of antibiotics has been questioned. Antibiotic prophylaxis has been shown to be inefficient in decreasing the incidence of local infection. Results from several studies (Jorge et al. 2004; Ericsson et al. 2006; Lervik, Lilliehöök, and Frendin 2010) are in accordance, as they found no clinical signs of infection in any envenomed dogs despite that only 19% of dogs were treated with antibiotics. Nevertheless, the use of antibiotics is still largely used in veterinary clinics: 85% to 90% of dogs received antibiotics in two studies (Lobetti and Joubert 2004; McCown et al. 2009).

Fluid therapy and blood transfusion

The type of fluid must be chosen after assessment of the clinical signs, the acid-base status, the degree of hypovolaemia, and the presence of renal failure and coagulopathy. Isotonic crystaloid solutions (70–90 mL/kg in fractioned doses) are more recommended than colloides use, associated with potential decrease in platelets (Armentano and Schaer 2011). Most of the time, blood transfusion is not required (around 7% in (Lobetti and Joubert 2004)) but it may be necessary in cases of uncontrolled bleeding due to coagulopathy (Barr 1984; Heller et al. 2007).

Analgesics

Analgesics should be given depending on clinical signs associated. Either lidocaine, buprenorphine, fentanyl and morphine may be given at the discretion of the clinician, although morphine should be avoided due to the risk of histamine release and hypotension (Armentano and Schaer 2011). Generally, non-inflammatory drugs and opiods are given to improve animal comfort (Lobetti and Joubert 2004).

Premedicants before antivenom administration

The use of premedicants to reduce the incidence of anaphylaxis secondary to snake antivenom administration is controversial within the literature. In human literature, adrenaline premedication appears to significantly reduce acute adverse reactions to antivenom administration without secondary effects (Williams et al. 2007; Premawardhena et al. 1999). The use of promethazine and hydrocortisone premedication observed in the same study was inefficient (Williams et al. 2007). Nevertheless, some side effects with the use of antihistaminics such as hypotension have been described and can be a non indication in coagulopathic patient (Heller et al. 2007; Armentano and Schaer 2011). Moreover, as seen previsouly, the incidence of acute reactions following antivenom administration in dogs is relatively low (see part 1.1.6.2).

Corticosteroids use

The use of corticosteroids is still very controversial. Indeed, some authors think that steroids may slow and diminish the response to antivenom and increase the risk of infection (Sutton, Bates, and Campbell 2011). In another study, no clinical or clinicopathological parameters have been shown to be modified with the use of a single dose of prednisolone 1mg/kg subcutaneously (Brandeker et al. 2015). Moreover, some studies have shown an increased mortality associated with the use of corticoids, suggesting that their use could even be harmful. Nevertheless, it has been highlighted that corticoids are more likely to be administrated if clinical signs were more pronounced which may influence on the mortality rate (Segev et al. 2004). Finally, inflammatory reaction induced by snake venom are commonly described (see part 1.2) and the use of corticosteroids may be helpful by inhibiting cyclooxygenases (COX) and thus preventing the release of arachidonic acid and other mediators of inflammation. No specific study has been conducted in tiger snake envenomation.

1.1.7 PROGNOSIS

Mortality rates in dogs are variable ranging from 1% to 30% (Lund et al. 2013; Armentano and Schaer 2011; Segev et al. 2004; Aroch and Harrus 1999). In the study on tiger snake envenomation, survival rates for dogs envenomed by this species and treated at the hospital were 67% (Barr 1984). In another study on tiger and brown snake envenomation, the survival rate for dogs that received antivenom was 95% (Indrawirawan YH, 2014).

According to Segev et al., dogs presented with the following variables were more likely to die: body weight below 15kg, limb envenomation, envenomation during the night, severe lethargy, hypothermia, systemic bleeding, shock, dyspnea, tachycardia, thrombocytopenia, and glucocorticosteroid therapy.

1.2 IMMUNE RESPONSE AFTER ENVENOMATION

1.2.1 IMMUNE SYSTEM: OVERVIEW

The immune system contents two related systems: the innate system and the adaptative system.

Epithelial barriers, resident neutrophils and macrophages, Natural Killer (NK) cells, polyreactive antibodies and the alternative pathway of the complement system provide innate immunity defences. Innate immunity response is relatively non-specific, provide instant defence but relatively weak and short one. Recognition of pathogen is mediated by transmembrane proteins known as toll-like receptors (TLRs). The innate immunity is provided by phagocytes (e.g. polymorphonuclear neutrophils PMN and resident macrophages), continuously present, rapidly activated and nonspecific in targeting pathogens. They detect and recognize non-specific pathogens, produce cytokines (such as IL-6, IL-1, TNF-α) that initiate the innate and the adaptative immune response and allow the recruitment of PMN to the site of infection, to finally phagocytize and kill the pathogen. Innate immunity is the immune response involved in envenomation. Dendritic cells, which are Antigen Presenting Cells (APC), make the link between innate and adaptative immunity and transport antigen from primary site of infection to secondary lymphoid tissue.

After the antigen recognition, allowing by the ACP, T lymphocytes multiply and differenciate in different types of cells, auxiliary lymphocytes able to produce cytokines and memory lymphocytes. B lymphocytes, after the action of cytokines secreted by auxiliary T lymphocytes, multiply and synthetize immunoglobulines. Once T and B lymphocytes of the adaptative immune system are activated within the lymphoid tissue, they migrate to the site of antigen and neutralize it (Fig. VI) (Day Michael J, veterinary immunology book, 2011).

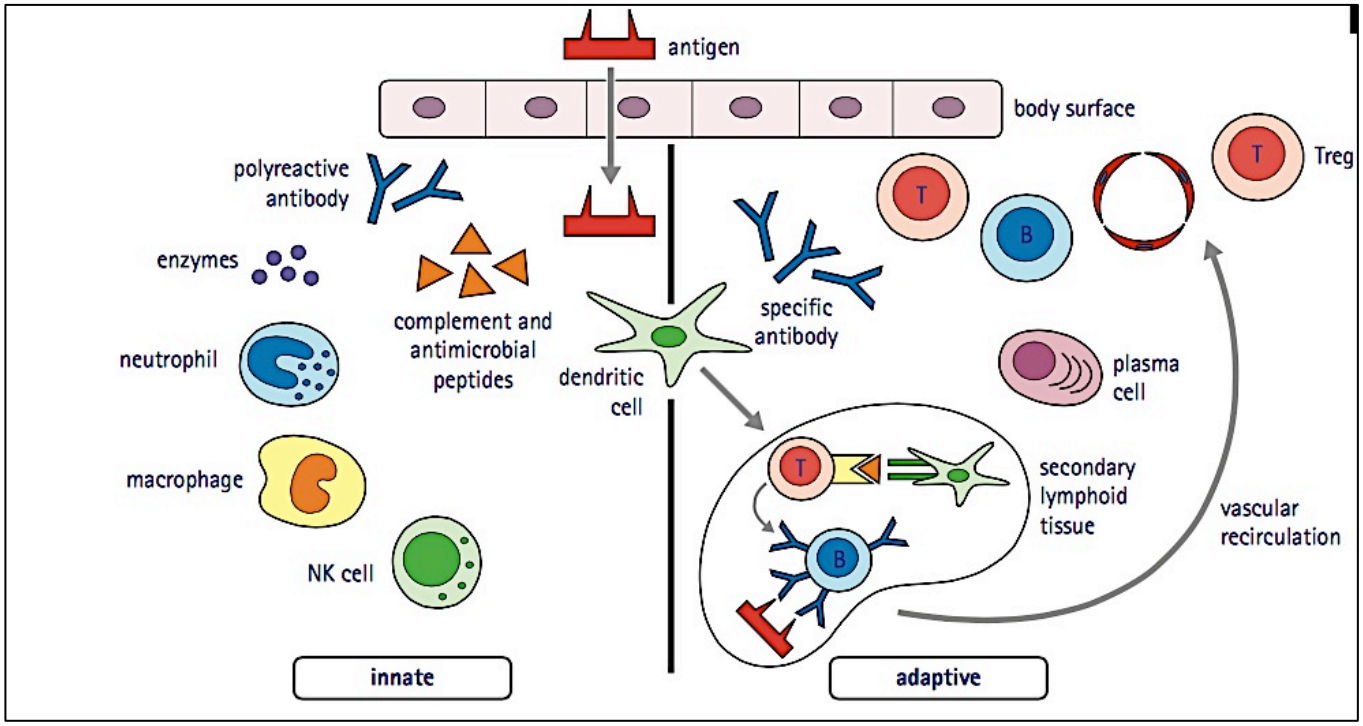


Figure VI. The innate and adaptive system (Day Michael J, Veterinary Immunology book, p11, 2011)

Once an antigen has been entering in the organism, the innate responses that include resident neutrophils and macrophages, NK cell, polyreactive antibodies and the alternative pathway of the complement system, provide innate immunity defences. Dendritic cells realize the transition to the adaptive system by transporting the antigen from primary site of infection to secondary lymphoid tissue. B and T lymphocytes are activated within the lymphoid tissue and then migrate to the site of antigen in order to neutralize it.
NK= Natural Killer, T= Lymphocyte T, B= Lymphocyte B

1.2.2 MAIN CARACTERISTICS OF THE INFLAMMATORY RESPONSE

The aim of the inflammatory response is to neutralize the pathogen, contain it, prevent its systemic spread and promote tissue repair once the pathogen is eliminated. At the site of inflammation, a complex network of soluble inflammatory mediators is released. These mediators include the initiating alarmins, histamines (derived from mast cells and a potent vasodilatator), chemokines (involved in the recruitment of leucocytes from the bloodstream) and cytokines (involved in the recruitment and activation of leucocytes), molecules of the alternative pathway of complement, coagulation factors and the vasoactive lipids (eicosanoids). The eicosanoids are derived from arachidonic acid released from phospholipids of damaged cell membranes by the action of phospholipases. Arachidonic acid may be converted to leukotrienes (leukotriene B4) by the action of lipooxygenases, or

to a range of prostaglandins (prostaglandin E2), thromboxanes (thromboxane A2) and prostacyclins (prostaglandins I2) by the cyclooxygenases (COX-1 and COX-2) (Day Michael J, Veterinary Immunology book, 2011).

In addition to local tissue inflammation, systemic signs of illness may accompany the inflammation. This effect largely relates to the release of a series of proinflammatory cytokines from activated macrophages. These include interleukin 1 (IL-1), interleukine 6 (IL-6) and tumor necrosis factor alpha (TNF-α). They interact directly with nerve cells in damaged tissue and are absorbed into the bloodstream, where they are able to access the brain to bind receptor molecules that mediate pyrexia, lethargy, and anorexia. These circulating cytokines also stimulate the liver's production of a collection of acute phase proteins such as C-reactive protein (CRP) promoting phagocytosis, serum amyloid A (SAA) acting as a leucocyte chemoattractant and having an immunosuppressive function.

The final stage of the inflammatory response is tissue repair. Macrophages phagocytize target by generating nitric oxide (NO) from arginine (M1 cells) or by generating ornithine (M2 cells). M2 macrophages produce fibroblasts and blood vessels including the cytokine transforming growth factors (TGF)-β, which promote the deposition of collagen and the growth of new vessels replacing areas of necrotic tissue (Day Michael J., Veterinary Immunology book, 2011).

1.2.2.1 Acute inflammation

Acute inflammation begins within a few minutes after tissue damage and is characterized by the five cardinal signs of inflammation: pain, swelling, heat, redness and loss of function. Neutrophils migrate in the first few hours following the acute inflammatory response. In parallel, the stretching of the vessel wall initiates coagulation involving the platelets and coagulation factors. The aim of this mechanism is to prevent spread of pathogens into the bloodstream. Finally, the organism is phagocytized and destroyed by the respiratory burst or by lyzsosomal enzymes (Day Michael J, Veterinary Immunology book, 2011).

1.2.2.2 Chronic inflammation

Chronic inflammation begins between 24h and 48h after the initiating insult and is characterized by the recruitment of blood monocytes into the tissue. Although macrophages are less quickly recruited than neutrophils involved in the innate immunity, they are more potent,

have a longer life span and a role in tissue repair by phagocytosing the debris left after apoptosis of neutrophils (Day Michael J., Veterinary Immunology book, 2011).

1.2.2.3 Complement system

The complement system is composed of a serie of approximately thirty plasma proteins. There are four complement pathways interacting sequentially in order to form an enzymatic cascade: classical, alternative, lectin, and terminal pathways. Classical and alternative pathways lead to the final production of complement factor C3b. The lectin pathway interacts with the classical system. The terminal pathway uses the factor C3b and leads to cell lysis, inflammation, opsonisation and interaction with other pathways (Fig. VII).

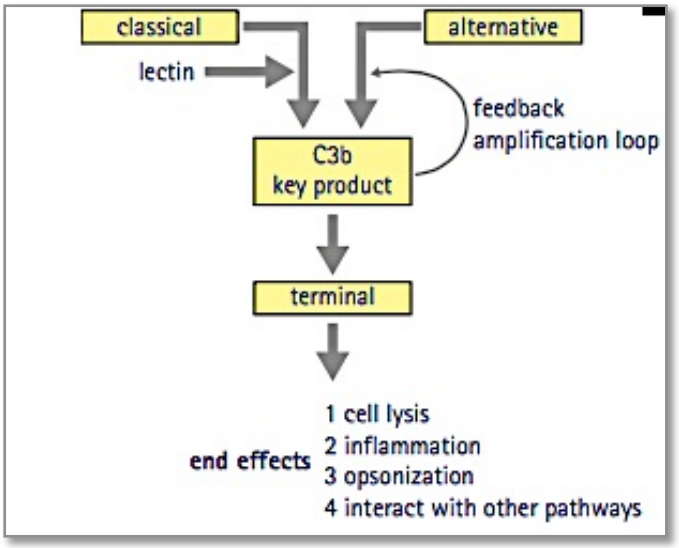


Figure VII. The four complement pathways and their interaction (Day Michael J., Veterinary Immunology book, p31, 2011)

Once the terminal complement is activated, thousands of MAC (Membrane Attack Complex) are generated within the membrane of the target cells and lead to cytolysis. The second major consequence of terminal complement activation is the generation of fragments, especially C3a, C3b and C5a, also named anaphylatoxins or chemoattractants. These anaphylatoxins have key roles in the tissue inflammatory response via several mechanisms. They are responsible for vessels vasodilation allowing vascular fluid and oedema, which may further dilute toxins produced locally. It also results in a local influx antibody and complement, participating in a local immune response in conjunction with mast cell degranulation. C3a and C5a activate neutrophils in order to enhance the phagocytic action and the release of

inflammatory mediators. A chemotactic gradient with the highest concentration of complement factors at the site where they were produced, allows an influx of these cells to the location of the antigen (Fig.VIII).

In some circumstances, the actions of C3a and C5a may cross the border between a useful role in local inflammation and a pathological inflammatory response. These molecules may cause massive mast cells degranulation and further expand the tissue inflammatory response (Day Michael J, Veterinary Immunology book, 2011).

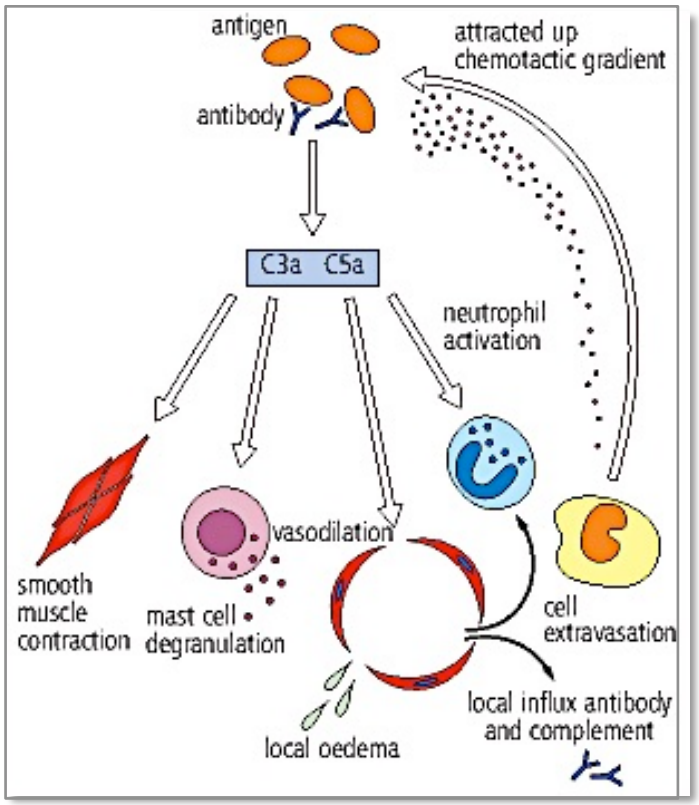


Figure VIII. Roles of complement factors C3a and C5a (Day Michael J, Veterinary Immunology book, p35, 2011)

The interaction between antigen and antibody activates the production of C3a and C5a that stimulates smooth muscle contraction, mast cell degranulation, neutrophil activation and vasodilatation of vessels responsible for local oedema and local influx antibody and complement (local immune response). The gradient of complement factors' concentration allows the influx of cells to the initial site of the antigen.

1.2.2.4 Involvement of cytokines in acute inflammatory responses

Numerous cytokines are involved in the inflammatory responses. Some of them will be discussed in this paragraph (Fig. XIII).

1.2.2.4.1 Cytokine overview

Cytokines are believed to be mediators that regulate all the phases of the inflammatory response and the outcome of the response is determined by their network (Arend and Gabay, 2004). Some cytokines may circulate systemically and have distant actions (endocrine). They are therefore one of the key means of communication between cells of the immune system (ACPs, T and B lymphocytes, NK cells) and between immune cells and other cells from the body.

Any single cell may produce a large number of cytokines and numerous cytokines may act on one target cell. Cytokines have multiple actions depending on the immunological circumstances and have different roles, from involvement in haemopoiesis and development of B and T lymphocytes, activation or suppression of immune cells to pro-inflammatory effects. It is possible to detect these cytokines by both cytokine gene transcriptions in tissue thanks to quantitative real-time reverse transcriptase-polymease chain reaction (RT-PCR) and the presence of cytokine protein in tissue or fluid samples using ELISA or complex bead-based multiplex assays (Khan 2008).

1.2.2.4.2 Multifunctional cytokines

IL-1, IL-6 and TNF-α are typical examples of multifunctional cytokines involved in the regulation of the immune response and inflammation. They show a wide variety of biological functions (Tabl. VIII) but each has also a particular characteristic function. IL-1 and TNF-α are considered as early or “alarm cytokines” with pleiotropic activities and the induction of a second wave of cytokines (IL-6, chemokines, IL-1 and TNF-α) and non cytokines mediators to amplify the inflammatory response.

IL-1, IL-6 and TNF-α are considered the most important pro-inflammatory cytokines, responsible for the initiation and propagation of the inflammatory response. IL-1 and IL-6 act on the synthesis of some Acute Phase Proteins (APPs), and are able to induce IL-6 release by fibroblasts. These interleukines induce fever, expression of adhesion molecules, hepatic

proteins, and T and B cells activation (Akira et al. 1990). TNF-α is known for its tumoricidal activities. All these cytokines are able to regulate the immune response, heamatopoiesis and inflammatory reactions (Akira et al. 1990).

IL-6 seems to play the most important role in the regulation of APPs. Indeed, it is the only cytokine able to stimulate the full spectrum of APP such as CRP synthesis in human hepatocytes (Castell et al. 1989). This cytokine is also recognized to be implicated in various inflammatory diseases such as rheumatoid arthritis, insulitis, thyroiditis and glomerulonephritis (Akira et al. 1990).

Interferon-α,β (IFN-α,β) are known for their antiviral, antibacterial, antifungal and antitumora effects. They also have a role in the immunomodulation of antibody production and can induce autoimmune and inflammatory reactions. In addition, IFN-β enhances macrophage production of several cytokines such as IL-1 and TNF (Paul William E., 1989).

IL-8 has a key role in attracting and activating neutrophils and has been described to be the main cause of this influx in many conditions (infection, ischemia, trauma, homeostasis damaging). Due to its long-lasting chemoattractant effects, it is thought to persist a long time in active form in tissue where it is released (Baggiolini and Clark-Lewis 1992).

Table VIII. Properties of IL-1, IL-6 and TNF-alpha (from Akira et al. 1990)

Interleukin	IL-1	IL-6	TNF- α
Producers	Macrophages, keratinocytes, endothelial cells, B cells, astrocytes, fibroblasts	Macrophages, T cells, B cells, keratinocytes, endothelial cells, fibroblasts, astrocytes, mesangial cells, bone marrow stroma cells	Macrophages, T cells, NK cells
Biological activities	Induction of: PGE2 synthesis Growth of fibroblasts Bone resorption ICAM-1 expression Fever Sleep Anorexia Synthesis of collagenase Growth and differentiation of T and B cells	Induction of: B cell differentiation Plasmocytoma growth Acute phase protein synthesis Macrophage differentiation Hematopoietic stem cell growth Maturation of megakaryocytes Neural cell differentiation Mesangial cell growth Myeloid leukemic cell differentiation	Tumoricidal activity Inhibition of lipoprotein lipase Induction of: Bone resorption Myeloid leukemic cell differentiation Procoagulant activity Growth and differentiation of B cells ICAM-1 expression

Producers and biological activities of one of the most pro-inflammatory cytokines

1.2.2.4.3 Cytokines and acute Phase Protein synthesis

The acute phase response is a non-specific inflammatory response that occurs shortly after any tissue injuries. It is characterized by changes in the concentrations of plasma proteins, called Acute Phase Proteins (APPs): negative APPs (e.g. albumin or transferrin) have decreased concentration levels while positive APPs (e.g. C-reactive protein [CRP], serum amyloid A [SAA], haptoglobin [Hp], alpha-1-acid glycoprotein [AGP], ceruloplasmin (Cp), and fibrinogen) have increased concentration levels. APPs are involved in the regulation of the immune response, the inflammation, and the protection against the infection, and there are also involved in the repair and recovery of damaged tissue. A single APP can have both a pro- and anti-inflammatory effect, with a specific blance between the two functions. Most of the positive APPs are glycoproteins synthesized mainly by hepatocytes. Pro-inflammatory cytokines act in an hormone-like manner, being generated at the inflammation site and transported to the liver via the bloodstream to induce APP synthesis (Cerón, Eckersall, and Martínez-Subiela 2005; Akira et al. 1990).

1.2.2.4.4 Cytokines and recruitment of leukocytes to inflammatory sites

The chemoattractant cytokines or chemokines are involved in the recruitment of leucocytes from blood into tissue (Fig IX.).

The main chemokine responsible for recruiting monocytes to inflammatory sites is the chemokine MCP-1. It has been demonstrated that this chemokine is involved in mediating early monocyte recruitment in murine peritonitis. Its rapid release by macrophages and also mesothelial cells allows immediate recruitment of monocytes from the circulation 1.5 to 2 hours after the stimulus (Henderson 2003). In addition to its chemoattractant activity, MCP-1 is also able to activate other cytokines (e.g. IL-l, IL-6) and enhance calcium flux and respiratory burst (Cavaillon 1994).

IL-1 and TNF-α induce the expression of adhesion molecules, such as the Intercellular Adhesion Molecule 1 (ICAM-1) and the Platelet Endothelial Cell Adhesion Molecule 1 (PECAM-1) on endothelial cells. Thus, the binding between leukocytes and endothelial cells stop the rolling of leukocytes and allow them to transmigrate into the affected tissue (Downey 1994). IL-1 and TNF-α also induce the secretion of chemokines, which further stimulate leukocytosis and the migration of neutrophils to the inflammation site. After penetration into the inflamed site, numerous cytokines are then produced by the activated leukocytes, which amplifies the inflammatory response (Akira et al. 1990). They have numerous other activities

such as osteoclast activation factor, cartilage degradation, fibroplasia and angiogenesis, adipocyte lipolysis, cachexia, etc. (Paul William E., 1989, p655).

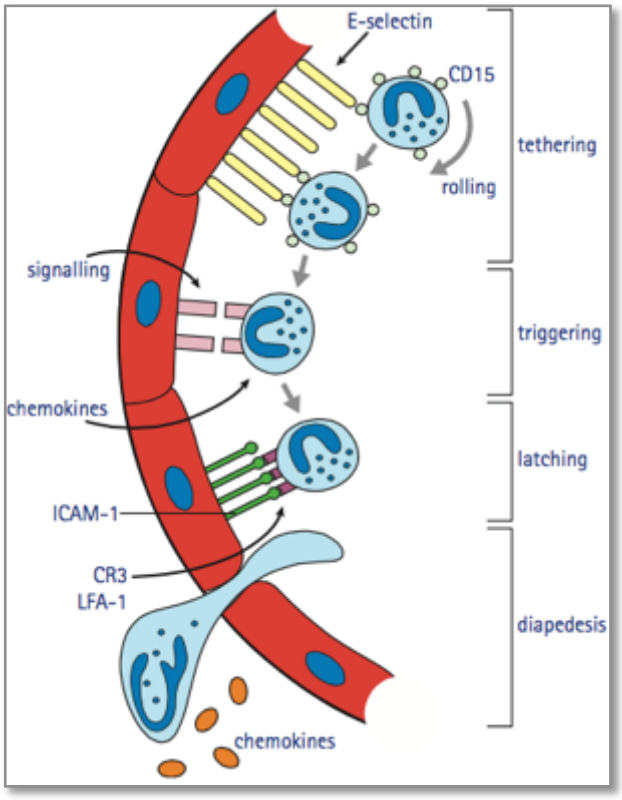


Figure IX. Leukocytes adhesion and involvement of chemokines (Day Michael J, Veterinary Immunology book, p63, 2011)

The first step to recruit leukocytes to the inflamed tissue is the tethering or rolling of polynuclear neutrophils on the endothelium of postcapillary venules. This step is made thanks to the binding between E-selectin (selectin family of adhesion molecule), expressed on the vascular endothelium and CD15, and expressed on the surface of neutrophiles. Then, chemokines and signaling molecules allow leukocytes to trigger to the vascular endothelium. A high affinity interaction between integrins on the neutrophil (CR3) and ICAMs on the endothelial cell allows the neutrophil to transmigrate into the affected tissue.

LFA-1= leucocyte function antigen-1, ICAM-1= intercellular adhesion molecule-1, CR3=complement receptor type 3,

1.2.2.4.5 Role of cytokines, neutrophil activity and neutrophil apoptosis: protective or deleterious inflammatory response

Once neutrophils have transmigrated into the affected tissue, they are able to combat the pathogene by several mechanisms such as the production of toxic oxygen radicals, the release of antimicrobial and lytic enzymes and the release of granule proteins (Bordon et al. 2013).

Moreover, they are also able to synthetize and release various cytokines (Marco Antonio Cassatella 1999; Marco A. Cassatella 2013).

Neutrophils have a very short lifetime. To maintain the cellular homeostasis, the death of neutrophils occurs in the absence of extracellular stimuli. Neutropenia can be caused by a rise in neutrophil death, which can increase bacterial and fungal infections. On the other hand, neutrophilia can be caused by delayed neutrophil death, which is associated with an increased inflammation after infection. Thus, maintaining appropriate neutrophil numbers or neutrophil homeostasis is critical during infection and/or inflammation. In the resolution of inflammation, the accumulated neutrophils need to be removed (Bordon et al. 2013).

The apoptosis plays a major role in eliminating neutrophils, controlling the duration and intensity of the inflammatory response and therefore the extent of neutrophil-mediated tissue damage (Fig.X). Many components may delay the apoptosis of neutrophils, leading to secondary necrosis and the release of pro-inflammatory mediators from the phagocyte which may further delay the inflammatory response (Simon 2003; Heasman et al. 2003). Glucocorticoids play a role in the resolution of inflammation since they facilitate clearance of apoptotic cells (Heasman et al. 2003).

Thus, there is a relative balance between the need for inflammation to stimulate repair and the need to limit inflammation because of the additional tissue damage (Butterfield, Best, and Merrick 2006).

In the example of the pneumonia in humans, when this balance is respected, the acute inflammation response is protective and self-limited. Once the infection is controlled, cytokines also act to restore homeostasis, including the modulation of neutrophil apoptosis (Haslett 1999). On the other hand, a cytokine storm results in a deleterious inflammation and poor clinical outcomes (fig. XI) (Bordon et al. 2013)

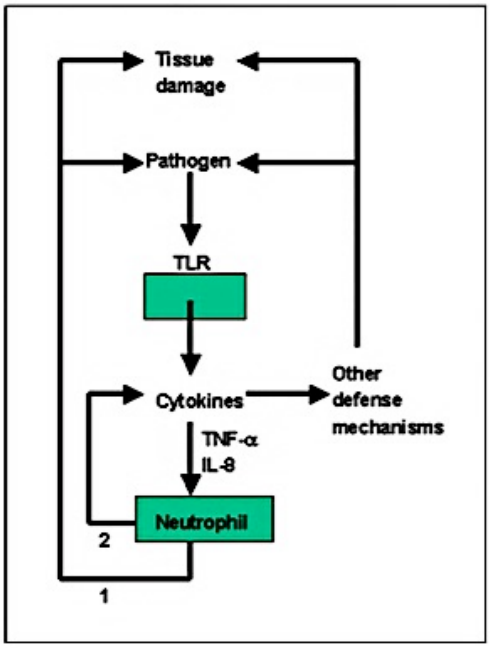


Figure X. Inflammatory response and role of neutrophils (Simon 2003).

Once the pathogen is recognized by TLR-expressing cells, pro-inflammatory cytokines are generated. Neutrophils are recruited to the site of inflammation and contribute to the elimination of pathogens (1) and also regulate the inflammatory response by the generation of cytokines (2). As neutrophils can cause tissu damage, their numbers need to be regulated.
TLR= Toll-like receptor

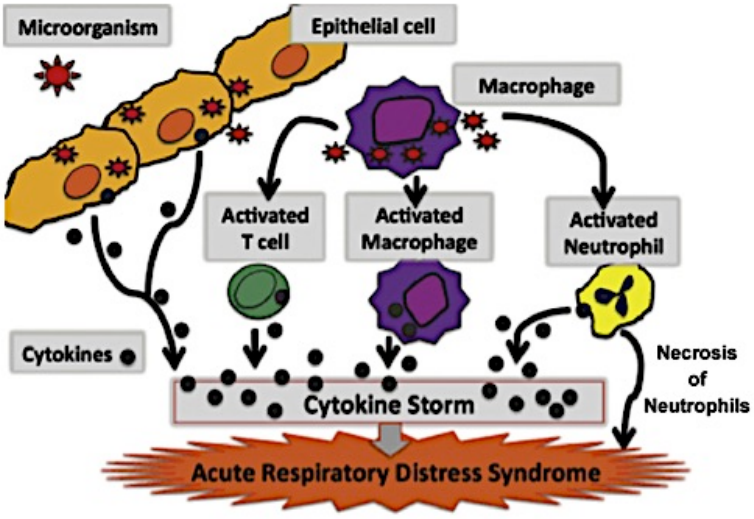


Figure XI. Example of cytokine storm and deleterious inflammation in a case of pneumonia (Bordon et al. 2013)

1.2.2.4.6 Fever induction

Fever is a part of the acute phase reaction representing an adaptive mechanism against infection: inhibition of bacterial growth, stimulation of APP synthesis, increased bactericidal activities of neutrophils and macrophages (Saper and Breder 1994). A multi-pathway mechanism was suggested for the induction of fever – including pyrogenic cytokines such as TNF, IL-1, IL-6, and IFN acting on CNS and inducing PGD (Netea, Kullberg, and Van der Meer 2000)

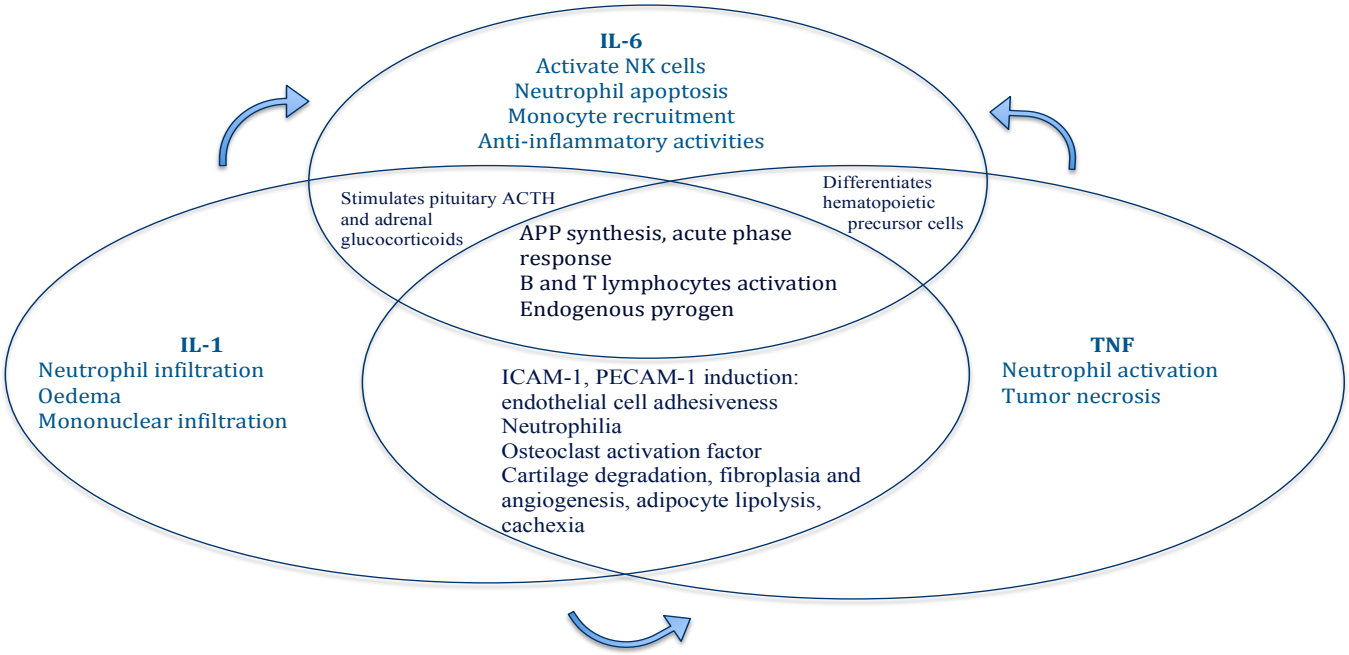


Figure XII. Summary of numerous activities of IL-1, IL-6 and TNF and interaction between cytokines (modified from Paul William E., p656, 1989).

IL= Interleukin, TNF= Tumor necrosis factor, MCP-1= Monocyte chemotactic protein, ICAM-1= intercellular adhesion molecule-1, PECAM= Platelet Endothelial Cell Adhesion Molecule 1
Dotted arrows represent the stimulation of synthesis.

1.2.2.5 Interleukin-6: principal characteristics and interests

IL-6 is produced by many cells including macrophages, T cells, B cells, fibroblasts, endothelial cells, astrocytes, mesangial cells, bone marrow stroma cells. It was originally known as a T cell derived lymphokine that induced antibody production in B cells. Thus, its primary role is to induce IgM, IgA and IgG production. IL-6 also acts on T-cells by the stimulation of another cytokine production (IL-2) (Akira et al. 1990). Since it is able to increase the secreation of adrenocorticotropic hormones and glucocorticoids, the rise of IL-6 could explain lymphopenia and leucocytosis (Paul William E., p656, 1989).

1.2.2.5.1 Pro and anti-inflammatory activities, hematopoiesis activity

As said previously (part 1.2.2.4.2), IL-6 acts as an important pro-inflammatory cytokine by activating the immune system (APPs stimulation). Acute inflammation is characterized by an initial infiltration of neutrophils. IL-6, in conjunction with chemokines produced by endothelial cells and vascular elements, leads to the attraction of neutrophils in the initial phase (Scheller et al. 2011).

Moreover, there are several evidences that IL-6 is implied in the shift from neutrophils to monocytes in inflammation. Neutrophils show a high interest in an organism's defense against injury thanks to its capacity to synthesize oxygen metabolites and various enzymes. However, they might be toxic for surrounding tissue and might induce inflammatory diseases. Therefore they have to be rapidly negatively regulated (part. 1.2.2.4.5). IL-6 induces neutrophil apoptosis and the phagocytosis of apoptotic neutrophils induced MCP-1 (monocyte chemotactic protein) production by macrophages. Then, both neutrophil elimination and chemokine shift lead to monocytes recruitment (Fig.XIII). Thus, IL-6 plays a protective role by decreasing neutrophil and favoring monocytes recruitment, allowing the initiation of an immune response (Kaplanski et al. 2003). In addition of its role in the acute neutrophil infiltration's resolution, IL-6 is also necessary for the recruitment and the anti-apoptosis of T lymphocytes as well as for B and T cells' differentiation by acting in the adaptive system – that we will not develop here (Scheller et al. 2011).

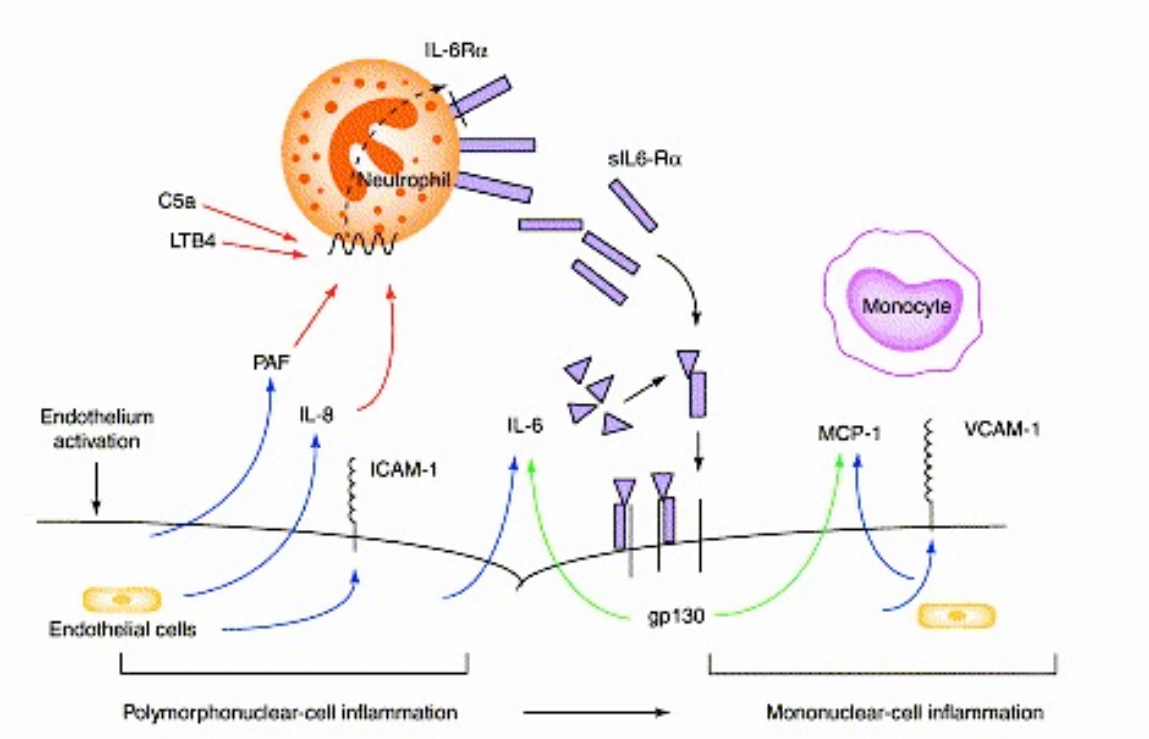


Figure XIII. Implication of IL-6 in the transition from neutrophil to monocyte recruitment (Kaplanski et al. 2003)

Endothelial activation by pro-inflammatory molecules leads to PAF, IL-8, leuko-endothelial adhesion molecule (ICAM-1) and IL-6 expression (blue arrow). Chemoattractants recruit neutrophils and induce IL-6-Rα shedding from their membranes (red arrow). The combination of IL-6 with its receptors IL-6-Rα enables binding to gp130 on endothelial cell membrane (black arrow) and increases both IL-6 and MCP-1 endothelial cell production (green arrow) but not IL-8, favoring a transition from neutrophil to monocyte recruitment. ICAM=intercellular adhesion molecule; IL=interleukin; MCP=monocyte chemo-tactic protein; PAF=platelet activating factor; sIL-6-R-soluble IL-6 receptor; VCAM=vascular-cell adhesion molecule.

Another interesting role of IL-6 is its function of thrombopoietin. Indeed, neutralized thrombopoietin in mice treated with IL-6 showed an enhanced hepatic thrombopoietin mRNA expression and an increased thrombopoietin level. This study suggests that IL-6 induces thrombocytosis through thrombopoietin (Kaser et al. 2001).

Reports on mice studies have also showed that IL-6 presents anti-inflammatory (or regenerative) properties by inducing the regeneration of epithelial cells after DSS (dextran, sodium, sulfate) induced damage (Scheller et al. 2011).

1.2.2.5.2 Interleukin-6: beneficial and deleterious effects

The severity of diseases has been associated with excesses of both pro- and anti-inflammatory cytokines, particularly IL-6 and IL-10. Indeed, the pro-inflammatory cytokine

response is normally regulated by the anti-inflammatory components of the immune system. However, if this balance in not respected, deleterious effects may result (Bordon et al. 2013).

In several studies, the presence of IL-6 was determined to be either positive or detrimental (Fig. XIV). Indeed, IL-6 seemed to be particularly important in controlling infectious diseases associated with intracellular parasites (*Trypanosoma cruzi*) and gram-negative bacteria *(Escherichia Coli)* - especially since it allows the recruitment of neutrophils *(Dalrymple et al. 1996; Gao and Pereira 2002).*

Nevertheless, it is recognized that a persistent elevation of cytokines is associated with a higher risk of death, and mortality seems to be correlated with the ratio of IL-6 to IL-10 (Bordon et al. 2013). Indeed, IL-6 was mostly considered as deleterious and was shown to be a marker of disease severity in sepsis and an association between IL-6 levels and poor outcome was found in patients with sepsis (Hack et al. 1989; Remick et al. 2005). Moreover, it was proved that the use of IL-6 antibodies contributes to improve survival in sepsis induced by cecal ligation/puncture in mice. Even if no potential hemodynamic effects (cardiovascular alteration) have been associated to IL-6 on dogs (Preiser et al. 1991), IL-6 was shown to increase C5a receptor, known to have deleterious effects during the early phases of sepsis. The blockage of IL-6 results in a significantly reduced expression of C5aR production in lungs, livers, kidneys, and heart, leading to greatly improved survival (Riedemann et al. 2003).

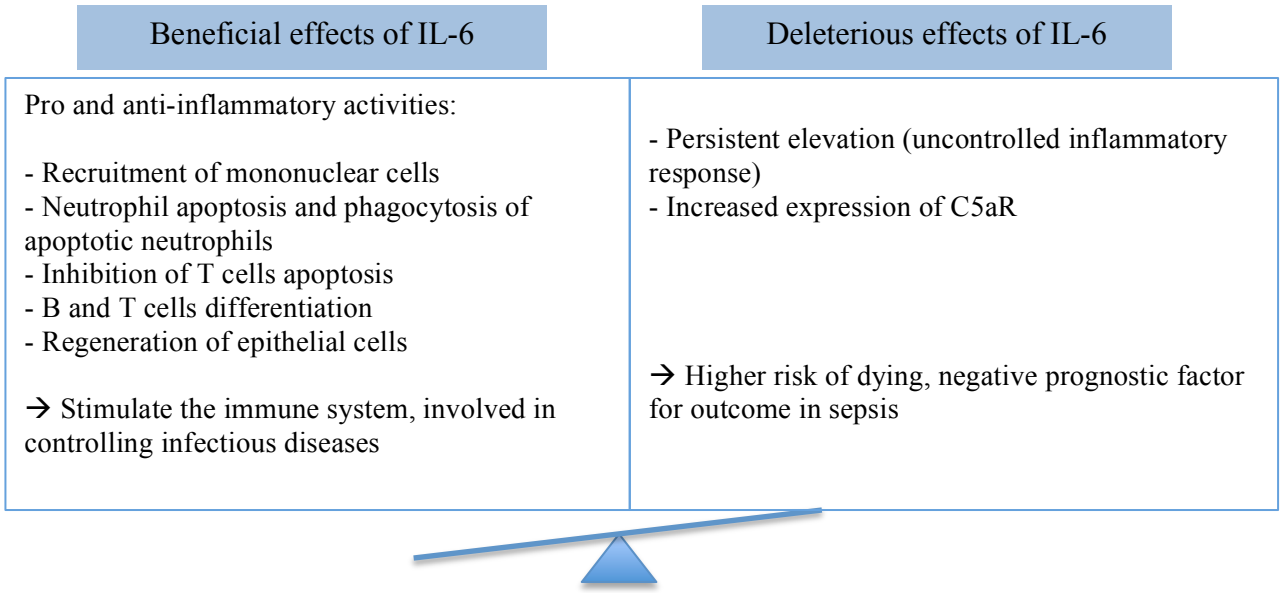


Figure XIV. Main activities of IL-6. Simplified figure of beneficial versus deleterious effects of IL-6.

IL-6 has pro- and anti-inflammatory activities involved in the activation of the immune system and in the control of infectious diseases. IL-6 is overall known for its deleterious effects. Deleterious effects are observed in case of persistent elevation of cytokines (unachieved or altered balance between pro- and anti-inflammatory components) and through the increase in C5aR, associated with higher mortality and poor prognosis in sepsis.

1.2.2.5.3 Interleukin-6: cytokine measurement in body fluids

Several factors can influence cytokine measurement in body fluids (Fig.XV).

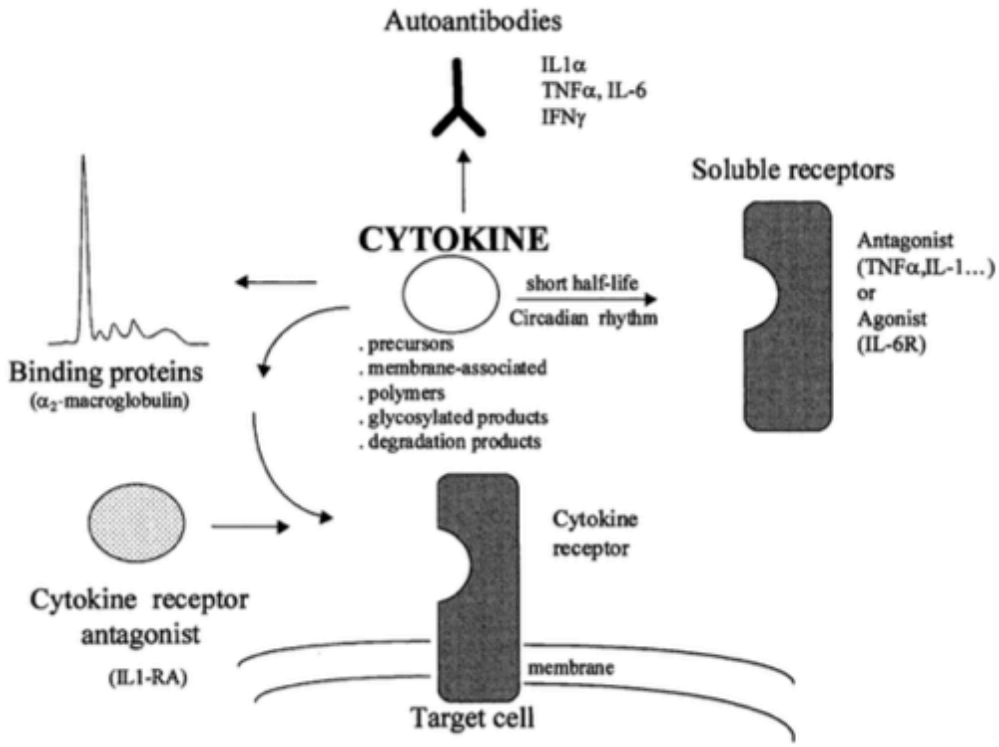


Figure XV. Factors affecting cytokine measurement in body fluids (Bienvenu et al. 2000).

Autoantibodies are very similar to the antibody developed by the immune assay and can bind to cytokine. All cytokine soluble receptors represent a potential source of interference in immunoassays. Circadian rhythms were reported for IL-6 (with a peak in the morning). Specific receptor antagonist (IL-1-RA) can interfere with IL-6. Non-specific inhibitors, represented by plasma proteins, with α2- macroglobulin, can bind to cytokine but with a low affinity.
IL-1-RA=Interleukin 1 rheumatoid arthritis, TNF-a=Tumor necrosis factor alpha, IL-1=Interleukin 1, IL-6R=Interleukin 6 receptor.

To measure cytokines, two methods are described: bioassays, based on various biological response, and immunoassay, based on the specific use of monoclonal antibodies. Due to their high specificity, immunoassays have rapidly become the reference. Radioimmunoassays (essentially immunoradiometric assay) or ELISA were developed using a first monoclonal antibody to bind the cytokine, and a second antibody (monoclonal or polyclonal) for the revelation. Many commercial kits are now available with ELISA microplate format. The

detection limit of the majority of cytokine kits is around 5pg/ml, i.e. greater than the circulating concentrations of many cytokines not only in physiological, but also, sometimes, in pathological conditions (Bienvenu et al. 2000).

1.2.2.5.4 Interleukin-6: interests of investigation

The half-life of cytokines is very short (usually less than ten minutes), leading therefore to brief peaks in blood (Bienvenu et al. 2000). Low concentrations of IL-1 and TNF are sufficient to activate inflammatory response. As a result, it can be difficult to detect systemic IL-1 and TNF in the serum, while IL-6 levels are quite abundant. Moreover, as IL-6 is induced by IL-1 and TNF rather than by direct effects, it is also an evidence of IL-1 and TNF productions (Voronov, Apte, and Sofer 1999).

The proinflammatory cytokine interleukin-6 (IL-6) has a longer plasma half-life than tumor necrosis factor alpha (TNF-α) or interleukin-1-beta have (Rau et al. 2007). The kinetic of IL-6 was measured by ELISA in a canine model of sepsis induced by endotoxin. IL-6 levels peaked at 3h and changes in serum IL-6 remained increased during 24h (Song et al. 2012).

Plasma interleukin-6 response is known to be predictive for severity and mortality in canine systemic inflammatory response syndrome and sepsis. It is considered to be a good marker of the severity of a systemic bacterial infection and thus can reasonably be considered as a valuable biomarker, providing information on the pathogenesis, diagnosis, and prognosis of systemic inflammatory response (Rau et al., 2007).

1.2.3 IMMUNE SYSTEMIC RESPONSE TOWARDS SNAKE VENOM

The immune response towards snake venom depends on several factors such as the structure and the concentration of the venom toxins or the dose of venom that is administered. Most studies on the immune systemic response towards snake venom are on Viperidae snakes. Thus, venom components will be further compared.

1.2.3.1 Evidence and mechanisms of inflammation induced in snake envenomation

Inflammation is a common finding in snake envenomation with several evidences: oedema, inflammatory cell infiltrate, and inflammatory mediators.

1.2.3.1.1 Oedema

An oedema, as a result of remodelling in vascular permeability, is the first local evidence of an inflammatory process implication in snake envenomation. In mice envenomed by Viperidae, an oedema develops over the first thirty minutes after venom injection, seems to reach a peak one or two hours post venom injection according to the authors, and then decreases (Picolo et al. 2002; Moreira et al. 2012). In a study of dogs envenomed by *Vipera palaestinae*, 99.6% of dogs showed local swelling and oedema (Segev et al. 2004). It was suggested that the oedema formation is the result of vasoactive components induced by the venom (Moreira et al. 2012).

1.2.3.1.2 Influx of leucocytes

A second common process of inflammatory response to envenomation is the influx of leucocytes, especially neutrophils, to the site of venom injection in order to contain and eliminate the pathogen (Part 1.2.2). In vivo on mice, this was described in the peritoneal cavity of mice after injection of *Bothrops asper* and *Bothrops atrox* venoms (Viperidae family). According to the authors, an increased of leukocytes was lasting from 1h to 48h after venom injection, reaching a peak between 4h and 6h (Moreira et al. 2012; Escocard et al. 2006; Stella R Zamuner et al. 2001).

At the beginning (3h to 24h), the predominant cells among these leucocytes observed were neutrophils (Stella R Zamuner et al. 2001), although another peak of neutrophils was also

reported 48h post venom injection (Escocard et al. 2006). Mononuclear leukocytes seem to appear later (48h).

In response to the presence of the pathogens in snake envenomation, neutrophils were shown to regulate the release of inflammatory mediators (cytokines and chemokines) and induce phagocytosis, associated with an enhanced expression of iNOS (inducible nitric oxide synthase) by macrophages (Escocard et al. 2006; Stella R Zamuner et al. 2001). Neutrophils do not seem to play a role in local tissue damage such as myonecrosis or haemorrhage but may contribute to delay the regenerative response in muscles (Teixeira et al. 2003).

1.2.3.1.3 Inflammatory mediators

Cytokines and complement play an important role in this inflammatory process which were specifically described in vipers (*Bothrops asper, Vipera russelli, Ammodytes, Meridionalis, Aspis*), rattlelsnakes (*Crotalus*) and Elapids (Farsky et al. 2000; Stella R. Zamuner et al. 2005; Langhorn et al. 2014; Santhosh et al. 2013; Szold et al. 2001; Tambourgi et al. 1994). Most studies concern snake venom of Viperidae family.

Acute phase protein: C-reactive protein (CRP)

According to Brandeker et al. (2015), in a study on dogs envenomation by *Vipera berus*, CRP concentration was increased in 80% of dogs. A correlation between degree of oedema swelling and CRP concentration was observed, bringing evidence of inflammation resulting from the envenomation. CRP concentration was often in the normal reference range at admission of the envenomed dogs despite the presence of swelling, but it was highly increased 24h later.

Eicosanoids and cytokines

Interleukin 6 (IL-6), interleukin 1B (IL-1B), tumor necrosis factor (TNF-α), interferon-γ (IFN-γ), chemokine MCP-1 and eicosanoids (prostaglandins PGD2, PGE2, thromboxane TXA2 and leucotrienes LTB4), were found to have increased concentrations at different time, in the serum sample and in the site of injection of *Bothrops* venom in mice (Rucavado et al. 2002; Petricevich et al. 2000; Stella R. Zamuner et al. 2005; Moreira et al. 2012).

The molecules which concentrations increased the earliest are some eicosanoids, thought to be involved in the influx of leucocytes, with higher PGD2 level at 30min after venom injection. Moreover, the observation of cyclooxygenases COX-2 level's increase between 1h

and 4h after *Bothrops asper* venom (BaV). It is thought that COX-1 initiates the production of eicosanoids, followed by the induction of COX-2 to stimulate PGDs production in the late periods of inflammatory process induced by the envenomation (Moreira et al. 2007).

The concentration of IL-6 also increased in the early stages (1h to 3-6h post venom administration according to the authors) (Stella R. Zamuner et al. 2005; Moreira et al. 2012; Lomonte, Tarkowski, and Hanson 1993).

TNF-α levels were found higher 1h after injection of BaV, and no detectable production was observed at the later time intervals studied. MCP-1 levels increased between 1h and 8h with a peak at 4h (Moreira et al. 2012). IFN-γ levels also increased after *Bothrops* venom injection (Stella R Zamuner et al. 2001). Numerous other cytokines and eicosanoids are thought to be involved in the infiltration of leukocytes to the injection site, associated with neutrophil activation and the activation of adhesion molecules on endothelial cells (Stella R Zamuner et al., 2005; C. Teixeira et al. 2009).

Nitric Oxide production

The mechanism by which snake venom induces the production of Nitric Oxide (NO) was not well described (Petricevich et al. 2000) until Zammuner et al. strongly suggested a role for IFN-γ as a mediator of macrophage iNOS synthesis induction by the venom. NO was assumed to be involved in tissue damage through its ability to form peroxynitrite after reaction with locally generated superoxide anions. NO is also involved in hypotension observed on snake envenomation (Stella R Zamuner et al. 2001).

Activation of the complement system

In-vitro studies, where *Bothrops* snake venom was administrated to human plasma, have shown activation of the complement cascade (C3a, C4a, C5a fragments) (Pidde-Queiroz et al. 2010; Farsky et al. 2000). By pre-incubating neutrophils with anti-rat C5a receptor, a dose-dependent inhibition of the migration of neutrophils was observed, suggesting that C5a is involved in the in-vitro neutrophils recruitment. Moreover, in C5a-deficient mice, no migration of neutrophils was observed which lead the authors to the conclusion that C5a is also involved in in-vivo neutrophils recruitment and is an important component of the inflammatory response to envenomation (Farsky et al. 2000). In accordance with this study, Rodrigues (2004) indicated that the complement system is involved in neutrophiles accumulation, but not in the hemorrhage, at the site of venom components injection. It was shown in some Elapids (genera

Micrurus and *Naja*) that their venoms contain components able to activate the complement system in a dose related way (Tambourgi et al. 1994).

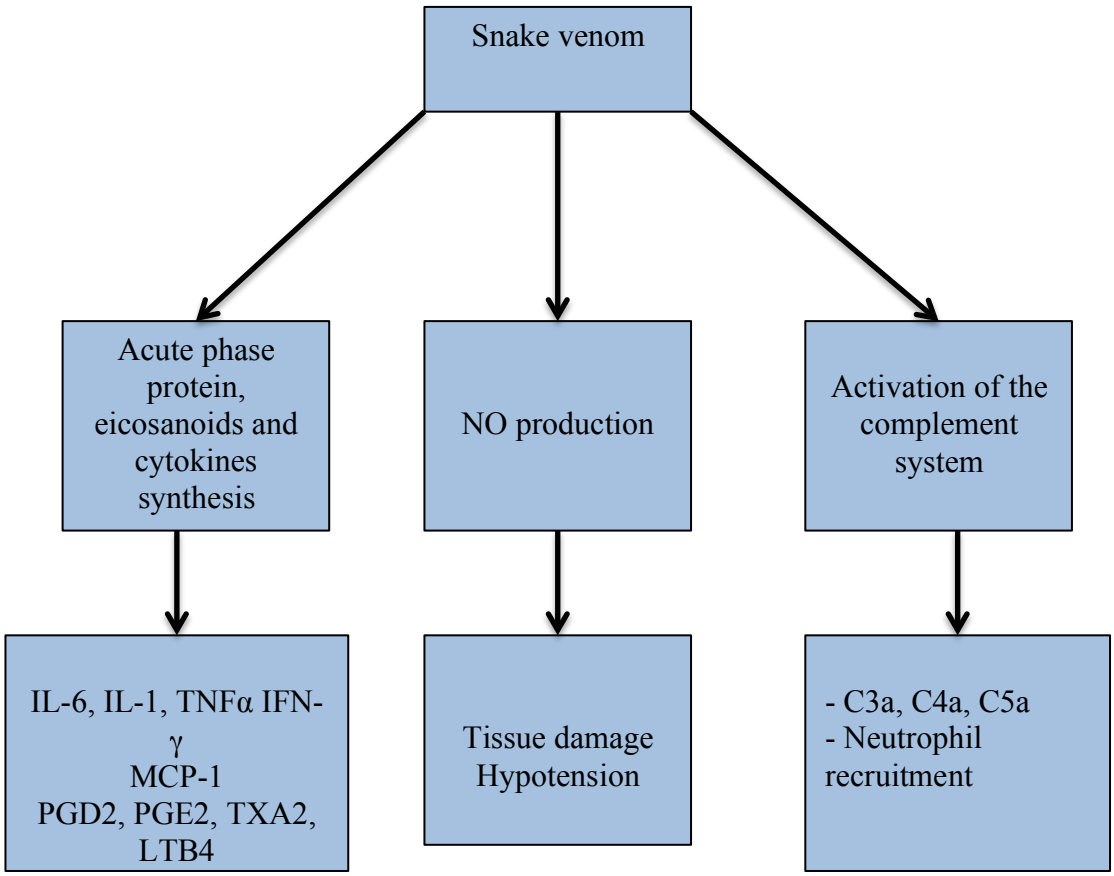


Figure XVI. Effects of snake venom on the production and the activation of mediators (modified from Luna, da Silva, and Pereira 2011).

IL-6=Interleukin 6, IL-1=Interleukin 1B, TNFα=Tumor necrosis factor α, IFN-γ=interferon-γ, MCP-1=chemokine, PGD2=Prostaglandin D2, PGE2=Prostaglandin E2, TXA2= Thromboxane A2, LTB4= Leucotrienes B4

1.2.3.2 Venom components acting in the inflammatory response

As seen previously, snake venom are composed in majority with toxic proteins, lipids and other molecules which act as immunogenic components and induce toxic effects (Leon et al. 2011). It has been shown that various venom components such as phospholipase A2 (PLA2) and snake venom metalloproteinases (SVMP) can cause the release of inflammatory mediators (Cher et al. 2003; Birrell et al. 2007). The role of these components in the inflammatory process has been particularly studied in the literature (fig. XVIII).

Role of metalloproteinases

First of all, SVMP play many roles in the local tissue damage and pro-inflammatory process (Fig. XVIII). Besides their action on the haemostatic system (procoagulant effects, inhibition of platelets aggregation, haemorrhage, blister formation) (see part 1.1.2.2) and besides their role on myonecrosis and dermatonecrosis, metalloproteinases have both direct and indirect actions on the inflammatory response. Indeed, metalloproteinases of *Bothrops asper* venom, isolated and injected in mice are reported to induce oedema (Gutiérrez et al. 1995). They are able to activate the complement system, even though other mechanisms might be actors too (Farsky et al. 2000). SVMP are also involved in the release of inflammatory mediators (cytokines, TNF-α), which can further exacerbate the oedema formation by increasing the endothelial permeability (Rucavado et al. 2002). The extent of local necrosis after snakebite is highly affected by the release of cytokines induced by metalloproteinases. Chemotactic factors, important in the neutrophils recruitment during inflammation, were shown to be induced by metalloproteinases and complement activation (C5a), but did not imply other components such as PLA2 or myotoxins (Farsky et al. 2000). Part of metalloproteinases also activate endogenous non-haemorrhagic metalloproteines, called Matrix Metalloproteinases (MMPs), which are able to degrade the extracellular matrix (Rucavado et al. 2002).

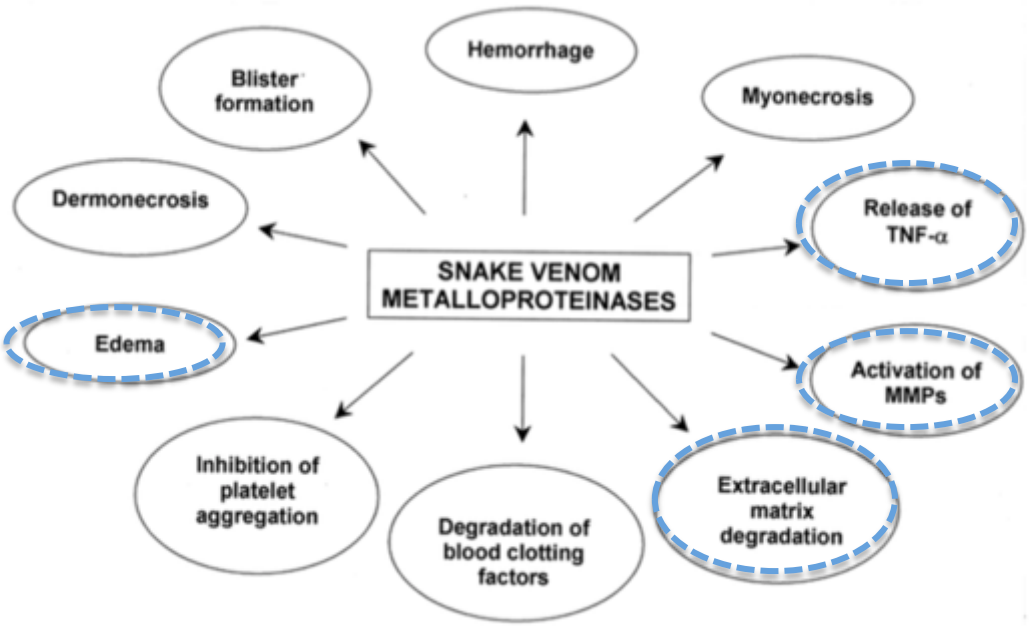


Figure XVII. Metalloproteinases and their roles in the pathogenesis of local tissue damage (Rucavado et al. 2002)

: *Roles of metalloproteinases in the immune response and tissue damage*
MMPs= Matrix Metalloproteines; TNF- α= Tumor Necrosis Factor α

Role of PLA2

PLA2s, important myotoxins of snake venom, have been isolated from *Bothrops asper* venom and their roles have been characterized in several studies (Fig.XVIII).

First, PLA2s are thought to be other actors in the oedema formation (Chaves, Barboza, and Gutiérrez 1995; Milton M Kanashiro 2002; Zuliani et al. 2005). The rapid plasma extravasation (in the first 15 minutes after PLA2s administration), suggested the involvement of vasoactive mediators derived from mast cell granules (Zuliani et al. 2005). It was also reported that their action in oedema formation is in part related to damage in smooth muscle of lymphatic vessels (Mora et al., 2008) .

Secondly, they are involved in myonecrosis events associated with higher CK level serum in mice, 1 hour after PLA2s injection, and damage in striated muscle cells too (Milton M Kanashiro 2002).

Besides, an important influx of leukocytes with mainly neutrophils was observed following the administration of a certain dose of PLA2 of *Bothrops asper* venom. This influx of neutrophils was maximal 6 hours after PLA2 injection (Kanashiro et al., 2002) (Zuliani et al. 2005). Since the treatment with anti adhesion molecules monoclonal antibodies decreased the neutrophil influx induced, the authors conclude that PLA2s also up-regulate the expression of cell adhesion molecules, involved in neutrophils recruitment (Zuliani et al. 2005). As seen previously, numerous mediators are involved in neutrophils recruitment. It was demonstrated that PLA2s up-regulate the eicosanoids levels (LTB_4 and TXA_2) and both PLA2s are able to induce an augmentation of IL-1 and IL-6 levels in the peritoneal fluid of mice after injection (Zuliani et al. 2005). Last, a study on potential actions of Crotalus venom on immune and neuroendocrine function showed that PLA2s were able to enhance plasma glucose, ACTH, corticosterone, and TNFα plasma levels and thus stimulate both hypothalamo pituitary adrenal axis and immune functions during the acute phase response of the inflammatory process (Chisari et al. 1998).

Other venom components

Other serine proteases (BpirSP27 and BpirSP41, from *Bothrops asper* venom) were described to play a role in the inflammatory response to snake envenomation. They may activate the complement pathway but play a minor role on the pro-inflammatory response by inducing only a small oedema formation and weak leucocytes recruitment (Menaldo et al. 2013).

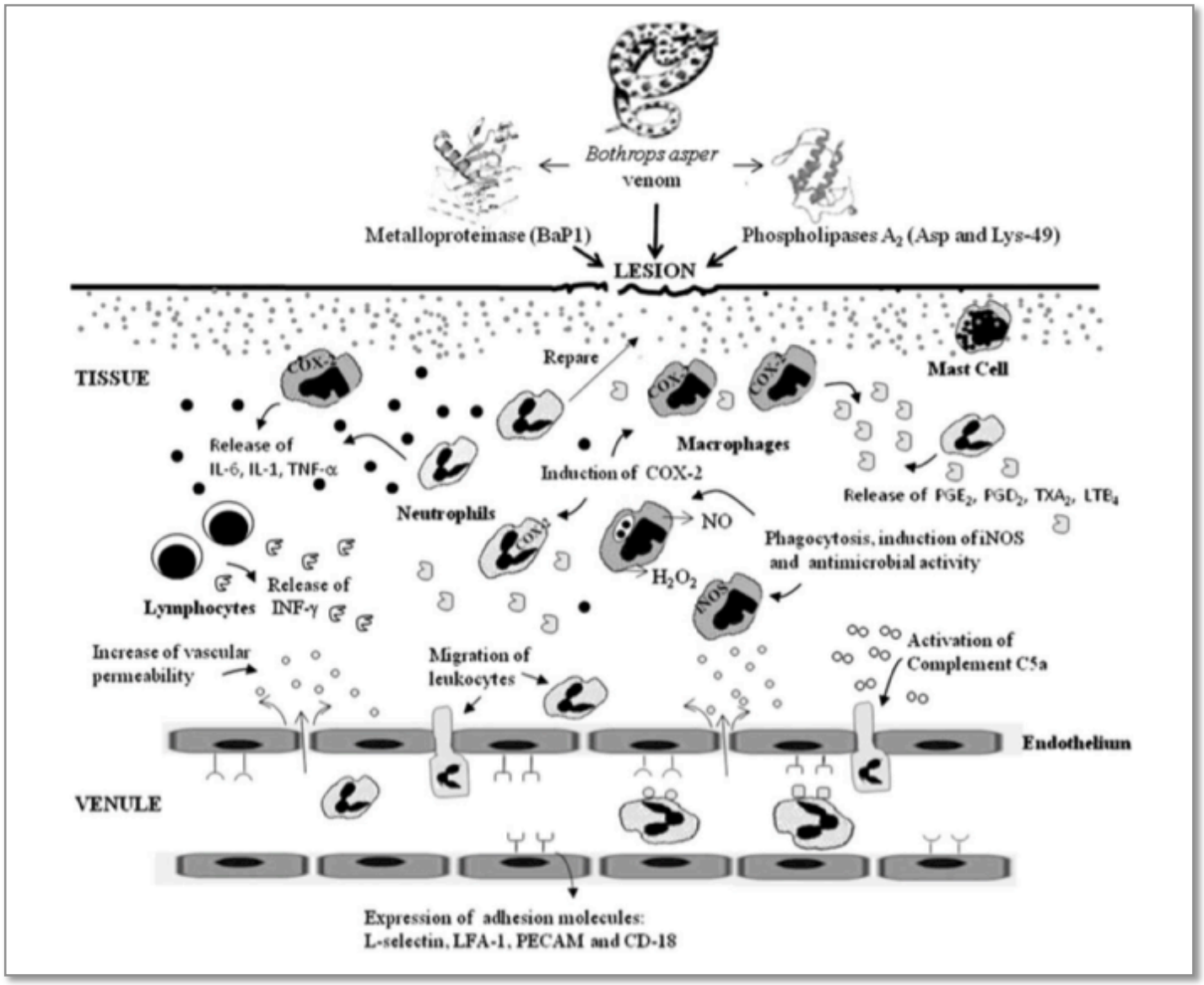


Figure XVIII. Schematic summary of the inflammatory action of a metalloproteinases and phospholipases A2 of *Bothrops asper* venom (C. Teixeira et al. 2009)

Metalloproteinases and phospholipases A2 are thought to be the major components responsible for the inflammation response at the snakebite site. The venom is able to activate the complement system, responsible for leukocyte recruitment (activation, up-regulation of adhesion molecules, transmigration and chemotaxis). The venom also induces the expression of the iNOS and the consequent production of NO by leukocytes. The release of TNF-α seems to be related to expression nitric oxide whereas the other cytokines and eicosanoids are associated with induction of leukocytes recruitment. The venom up-regulates the expression of COX-2 by both macrophages and neutrophils, further source of eicosanoids in envenomed sites.

BaP1=Bothrops asper Protein 1, COX=Cyclooxygenase, PGE=Prostaglandins E, TXA2=Thromboxane A2, LTE=Leukotriene E, IL-6=Interleukine 6, IL-1=Interleukine 1, TNF-α=Tumor necrosis factor α, INF-γ=Interferon γ, NO=Nitric oxide, iNOS=Inducible Nitric Oxide Synthetase, LFA-1=Lymphocyte Function-Associated antigen-1, PECAM=Platelet Endothelial Cell Adhesion Molecule

1.2.3.3 Venom components and inflammation in Elapids

The immune systemic response towards snake venom was described mainly in Viperidae. As said previously, Elapid venom contains mainly neurotoxins, prothrombin activators, and PLA_2s. Neurotoxins and prothrombin activators are more implicated in neurological effects and coagulopathy and do not seem to be implied in the inflammatory response. PLA2s seem to be the main venom component responsible for inflammation in Elapids. SVMP was also identified in some Elapid species but remains predominantly described in Viperidae. Nevertheless, it seems that some clinical effects attributed previously to PLA2s in Elapids, such as necrosis and inflammation could be (also) caused by the SVMPs.

1.2.3.4 Neutralization of the systemic inflammatory response by current treatment

Antivenom was proved to be efficient against the main threatening systemic effects resulting from envenomation (e.g. coagulopathy) (see part 1.1.6.2). Nevertheless, the use of antivenom seems to be partly inefficient against local manifestation induced by the inflammatory response to snake envenomation.

Numerous studies were conducted on *Bothrops jaraca* (Viperidae family). In a first study, the neutralization of lethal and hemorrhagic effects of *Bothrops jaraca* venom was more effective than the neutralization of myonecrosis and edema. Antivenom was not effective on the oedema formation, whatever time or route of antivenom administration (J. M. Gutiérrez 1981). In two another studies, antivenom was shown to reduce oedema and pain only if antivenom was injected in the very early stages: 15 minutes after venom administration (Picolo et al. 2002) or only if given in the first 30 minutes after venom injection (Araújo et al. 2007).. The myonecrotic effects were observed to be partially neutralized by antivenom; this only in the case where antivenom was administrated rapidly after venom injection (Chaves, Barboza, Gutiérrez 1995). The authors suggested that the lack of antivenom effectiveness on local manifestations of inflammation is based on the extremely fast manifestation of oedema and hyperalgesia after venom injection and their association with the release of endogenous mediators. Moreover, it is also thought that locally, the amount of antibodies is insufficient and delayed. Therefore, the time interval between snakebite and antivenom administration as well as the route of serum administration should be very important. However, the time between the snakebite and the animal's presentation is unpredictable and is often over an hour in

veterinarian medicine (J. M. Gutiérrez 1981; Picolo et al. 2002). No specific study was conducted on the neutralization of the inflammatory response thanks to tiger snake antivenom.

Looking at the lack of antivenom effectiveness against the management of clinical signs due to inflammatory response to envenomation, some studies suggest complementing this antivenom treatment. According to their observation on mice, indomethacin (non-steroidal anti-inflammatory, acting as a cyclooxygenase inhibitor) and prazosin (α-adrenergic blocker) and dexamethasone (inhibitor of phospholipase A) were able to reduce the formation of oedema induced by B. asper venom (Chaves, Barboza, and Gutiérrez 1995). More recently, another study demonstrates that neither antivenom nor anti-inflammatory drugs (dexamethasone or, indomethacin) become effective if they are administered alone 45 minutes after *Bothrops asper* venom administration. Nevertheless, the association of dexamethasone and antivenom or indomethacin and antivenom appears to reduce significantly paw oedema when injected 45 minutes after venom induction (Araújo et al. 2007). The inflammatory response seems to be characterized by the release of endogenous mediators, induced by some venom components, and also by the participation of metabolites and arachidonic acid. Therefore according to these findings, the association of anti-inflammatory drugs, such as dexamethasone or indomethacin, with antivenom could be a rational alternative to treat the local reaction induced by venoms. So far, no study in this area was conducted on tiger snake envenomation.

3. PART 2. EXPERIMENTAL PROTOCOL, RESULTS AND DISCUSSION

3.1. OBJECTIVES AND HYPOTHESIS

Despite the severity of the condition, the mechanism and characteristics of the post-envenomation syndrome that follows after the initial obvious crisis have only been insufficiently studied in dogs. Based on the known effects of components of Australian snake venom, it is likely that a marked systemic inflammatory response evolves in snake bitten dogs. If this is indeed the case, new therapeutic avenues open up to optimize post-envenomation care in dogs.

We proposed a pilot study in order to determine the systemic inflammatory response in tiger snake envenomation and antivenom administration in dogs, through the evaluation of plasma IL-6 levels. The study measured whether there is evidence of inflammation occurring due to the snake venom and the antivenom administered. It was thought to improve treatment options for snake envenomed dogs in the future by providing novel information on the potential utility of anti-inflammatory treatment in dogs with snake bite in the future.

We have hypothesized that a plasma marker of inflammation, the IL-6, was altered at presentation compared to a control group of healthy animals, and further increased after antivenom administration.

The aim of this project is to determine to which extent tiger snake envenomation in dogs needs to be considered as a condition of systemic inflammation and whether clinical trials including non-steroidal or steroidal anti-inflammatory drugs are warranted.

3.2. MATERIAL AND METHODS

3.2.1. POPULATION: INCLUSION CRITERIA

Naturally snake envenomed dogs (n=10) enrolled in this prospective observational study were followed from the initial presentation to the emergency service of the University of Melbourne Veterinary Hospital (UMVH) until hospital discharge or death. Healthy dogs (n= 4) have been included in this study as control group**.** The study protocol was approved by the University of Melbourne Animal Ethics Committee (AEC) prior to any research being

conducted and informed consent was obtained from the owners of all dogs enrolled in the study.

Dogs were enrolled if they weight more than 5 kg, had a haematocrit > 30% at admission, had not receive any antivenom prior to admission or experience a prior snake envenomation within the preceding four weeks. Enrolment occurred based on the association of several factors: history of the dog, clinical evidence of snake envenomation, including combination of general symptom, haematological, myotoxicity and neurological signs. Diagnosis of tiger snake envenomation was considered to be reliable if tiger snake was witnessed and identified by scale counting or if the snake venom detection kit (SVDK) revealed tiger snake immunotype.

3.2.2. GENERAL DATA

General data were recorded following a questionnaire obtained via client interview and physical exam findings collected at the time of admission.

Confounding variables

Confounding data were collected if known or suspected to influence the clinical presentation and the inflammatory response. They include historical information with concomitant disease and current medical treatment, date and location of snakebite, environmental temperature of snakebite, time from snakebite to presentation, the number of antivenom vials administrated, and drugs administrated.

Clinical variables

In order to assess severity of illness of the dogs, clinical variables present prior to admission (e.g. vomiting, weakness or collapse) and at hospital admission (e.g. clinical signs of vomiting, weakness or collapse, muscle tremors, ataxia, dilated pupils, absent pupillary light reflex, respiratory arrest) were obtained. Heart rate, respiratory rate, Sp02, rectal temperature and systolic blood pressure assessed by oscillatory or Doppler method were also measured.

Clinical outcome

Clinical outcome was determined as survival to hospital discharge, duration of hospitalization, mode of death and in the case of euthanasia, as to whether this was primarily due to financial constraints or due to futility.

3.2.3. CLINICOPATHOLOGICAL DATA

At the time of presentation, blood was collected from a cephalic, saphenous or jugular vein, placed in an EDTA and Heparin tube. Clinicopathological variables including biochemistry, electrolyte analysis, venous blood gas and coagulation time were assessed according to the choice of the clinician responsible for the animal.

3.2.4. INFLAMMATORY MARKERS MEASUREMENTS: IL-6 LEVELS

Whole blood samples (2 mL) were collected in sodium citrate tubes at presentation of the animal to the emergency service (i.e., prior to antivenom administration) (T0), within the two first hours after antivenom administration (T1), 18 hours (T18), and 24 hours (T24) after admission. Only one sample was taken from the control cases. Samples were centrifuged with a speed of 3800Rpm (3200G)[1], under 5°C for 5 minutes. Then, the supernatant plasma was immediately placed in polypropylene microtainer tubes and stored at -80°C until analysis for a maximum period of 6 months, without any freeze-thaw cycle. After collection, samples were frozen in a maximum delay of 3 hours.

In the literature, EDTA tubes are recommended since chelating agents avoid any potential cytokine production after sampling. Heparin should be avoided as it is often contaminated by endotoxins, which strongly induce cytokine synthesis. Plasma or serum has to be decanted very rapidly to prevent any cytokine degradation or adsorption. Samples are recommended to be stored frozen (–80°C). The thawing-freezing cycles should be avoided, even though three cycles seem to have no influence on IL-6 determination (Bienvenu et al. 2000).

The prototypical inflammatory mediator IL-6 was determined by using a quantikine ELISA kit standard[2], validated to determine relative mass values for naturally occurring canine IL-6. Samples were brought to the temperature room before use. 50 µL of assay diluent were added to each well. 100 µL of Standard dilution (=10ng of recombinant canine IL-6), control or sample were then added. Plate was covered and incubated at room temperature for 2 hours on an orbital microplate shaker. Each well was aspirated and plate washed 5 times. Then, 200 µL of Conjugate solution were added to each well. Plate was covered with a new plate sealer and incubated at room temperature for 2h on the shaker. Each well was aspirated again and plate washed 5 times. 120 µL of substrate solution were added to each well and plate was incubated

[1] *Allegra 6R Refrigerated Benchtop Centrifuge, Beckman Coulter, USA*
[2] *Quantikine ® ELISA, Canine IL-6, R&D system, USA &Canada*

at room temperature on the benchtop for 30 minutes (avoiding light). Finally, 120 µL of Stop solution were added to each well. The absorbance of each well was read at 450nm within 30 min (by a microplate reader).

The detection level of IL-6 (sensitivity of the assay) was considered to be 11.8pg/mL, according to R&Dsystem (canine IL-6 Quantikine ELISA kit).

3.2.5. STATISTICS

A mann-Whitney test was realized to compare IL-6 levels to control cases for each time. A wilcoxon matched-pairs test was realized in order to compare IL-6 levels differences between each time. The level of significance was considered to be $p<0,05$.

3.3. RESULTS

3.3.1. DOGS AND SNAKEBITE INFORMATION

The study included 10 dogs (tabl.IX). There was no significant difference between females and males. Their average age was 4.4 years-old. Only pure breeds were presented. Their average weight was 19.6Kg. One dog (case n°6) had a concomitant disease (skin allergy), currently treated with Atopica (cyclosporine). Another dog (case n°2) had incoagulant rat bait ingestion 12 months prior. And a last dog (case n°9) had a previous history of snake envenomation 4 months before.

Snakebite was seen in 7 cases out of 10 (70%) without any scale counting determined.

All snake envenomation occurred in West Melbourne. The time of admission ranged from 12:00 pm to 10:15 pm. The average local temperature was 26.7°C (with a minimum of 21.1°C and a maximum of 31.7°C).

The time spent between snakebite to admission was determined in 7 cases out of 10 and varied from 30 minutes to 80 minutes. First samples (T0) were collected at the admission (after clinical examination). Samples, collected after antivenom administration (T1), were taken on average 86 minutes after administration, ranging from 15 to 165 minutes. Samples collected at T18 and T24 were collected 18h and 24h after admission. The delay between snakebite and sample collection at T1 was on average 167 minutes (ranging from 110 to 206 minutes).

Table IX. Signalment and history

Cas n°	1	2	3	4	5	6	7	8	9	10
Age (year, month)	5y6m	4y	2y4m	1y6m	3y	10y10m	2y	7y	5y3m	5y3m
Gender	MN	MI	F	F	M	FS	FS	F	FS	MN
Breed	CKC	GSP	Am. Staff.	Dachs.	JRT	CKC	Schn. m.	Akita	Am. Staff.	Border Collie
Time from snake bite to admission (h)	01:20	U	U	U	00:30	00:40	01:00	00:34	01:00	0 :45

Age, gender, breed and time from snakebite to admission for each dog included in the study.

MN=Male Neutered, MI=Male Intact, F=Female Intact, FS=Female Spayed, U=Unknown, CKC=Cavalier King Charles, GSP=German shorthaired pointer, Am. Staff.=American Staffordshire Bullterrier, Dachs.=Dachshund, JRT=Jack Russel Terrier, Schn. m.= Schnauzer miniature

3.3.2. CLINICAL FINDINGS

All dogs were reported to present clinical signs before admission. The most common clinical signs prior admission were collapse (on 5 out of 10 dogs) and vomiting (on 3 out of 10 dogs), followed by weakness and hypersalivation (2 dogs) and diarrhea (1 dog).

At the initial examination, abnormal findings included vomiting and collapse (3 dogs), hypersalivation (2 dogs), nausea (1 dog), muscle tremors (1 dog). Mydriasis and loss of pupillary light reflexes were observed in three dogs. Mentation was altered in one dog, which was hyperexcited. One dog presented ataxia for a few hours.

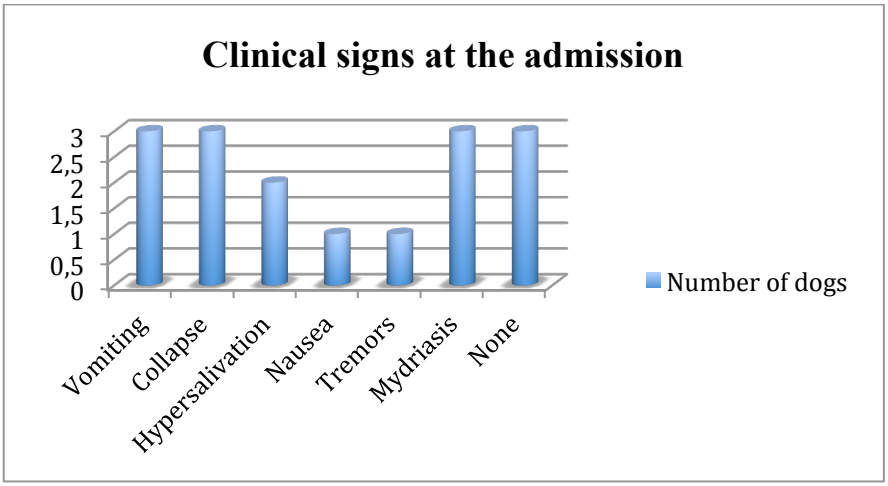


Diagram 3. Clinical signs present at the admission (on 10 dogs)

Only one dog (case n°1) presented tachycardia (with a HR=160bpm). Tachypnea was reported in four dogs (BR>40mpm). Two dogs presented hyperthermia (39.2°C and 39.4°C). Systolic blood pressure was determined by either Oscillometry or Doppler on five dogs, and was considered as normal (between 110 and 131 mmHg).

3.3.3. BICHEMISTRY, HAEMATOLOGY AND CELL BLOOD COUNT, COAGULATION TIMES

The time spent between the bite to the admission was known in 7 cases out of 10 and was on average 55 minutes (between 30 and 80 minutes). First blood samples were taken immediately after admission (T0), depending on clinician's decision.

Serum biochemistry[3] was performed on nine dogs (Tabl.X). CK was determined for 8 out of 10 dogs and was increased for 7 out of these 8 dogs (from 379 to 25,571mmol/L). Total proteins were increased in 2 ou of 10 dogs. ALKP were measured for three dogs and severely increased in one dog (case n°6). Bilirubinemia was slightly increased in one dog. Electrolyte analysis was performed on six dogs (Tabl.XI) and showed moderate to slight hypokaliema in 3 out of 6 dogs.

Table X. Laboratory diagnosis: biochemistry

	Ref	Case n°1	Case n°2	Case n°3	Case n°4	Case n°5	Case n°6	Case n°7	Case n°9	Case n°10
Total protein [g/dL]	*52-82*	60	60	70	71	**84**	60	66	50	**92**
Albumin [g/dL]	*23-40*		**45**				40		**67**	33
Glucose [mmol/L]	*4.11-7.95*	6.00	4.53	7.90		**9.90**	6.2	5.8		5.75
Creatinine [mcmol/L]	*44-159*		105				74			89
Urea [mmol/L]	*2.5-9.6*		5,8				3			7.5
ALKP [U/L]	*23-212*		25				**1993**			163
ALT [U/L]	*10-125*		**226**				78			**138**
Bilirubin [mcmol/L]	*0-15*			2			2		**18**	
CK	*<100*	**13,319**	**1,583**		**25,571**	**1,362**	**379**	**1,021**	<100	**19,940**

Biochemistry results at the admission for each dog included in the study. Main abnormalities are increased CK (7 out of 8 dogs), increased hepatic parameters (3 out of 6). ALT=aminotransferase, ALKP=alkaline phosphatase. No data for case n°8.

[3] *IDEXX catalyst DX Chemistry Analyzer, Australia*

Table XI. Laboratory diagnosis: electrolytes

	Ref	Case n°1	Case n°2	Case n°3	Case n°6	Case n°7	Case n°9
Na [mmol/L]	*145-158*	146	145	146	146	**142**	144
K [mmol/L]	*3.6-5.8*	3.6	**2.9**	**3.2**	**3.5**	3.9	4.4
Cl [mmol/L]	*105-122*	113	119	120	121	117	115

Electrolyte analysis results for six dogs at the admission Moderate to slight kypokaliema in 3 dogs. No data for cases n°4, 5, 8 and 10.

Blood cell counts[4] included only haematocrit and platelets (PLT) were determined respectively on nine and eight dogs respectively (Tabl.XII). Haematocrit was increased on 7 out of 9 dogs. 1 out of 8 dogs had thrombocytosis and 4 out of 8 dogs had thrombocytopenia. Serum was haemolysed on 9 out of the 10 dogs.

Table XII. Laboratory diagnosis: cell counts

	Ref	Case n°1	Case n°2	Case n°3	Case n°4	Case n°5	Case n°6	Case n°7	Case n°9	Case n°10
Haematocrit [%]	33.6-47.4	44	**52**	**52**	47	**62**	**49**	**58**	**56**	**92**
Platelets [x10^3/mcL]	200-500	203	244	**183**	**556**	**180**	**158**	**156**		254

Haematocrit and platelets results at the admission for each dog included in the study. Increased haematocrit (7 out of 10 dogs) and both thrombocytopenia (4 out of 8 cases), thrombocytosis (1 out of 8 cases) are noticed. No data for case n°8.

Acid base status and lactate[5] were assessed on six dogs (Tabl. XIII): hyperlactatemia was present on 5 out of these 6 dogs. Systolic blood pressure was in the reference range for all dogs tested.

Table XIII. Laboratory diagnosis: acid base status

	Ref	Case n°1	Case n°2	Case n°3	Case n°5	Case n°6	Case n°7	Case n°9
pH	7.350-7.440	**7.319**	**7.257**	7.401		7.393	**7.195**	7.408
HCO3 [mmol/L]	22-22	21,5		19,3		21,7	16,2	
PvCO2 [mm Hg]	33.0 - 52	43.1	41.8	31.7	35	36.4	32	34.3
Lactate [mmol/L]	0.6 - 2.5	**3**	**4.4**	**2.8**	**3.4**	1.4	**9.1**	

Acid base status results at the admission for each dog included in the study. A slight acidosis is noted. No data for cases n°4, 8 and 10.

[4] *IDEXX Procyte Dx Hematology Analyzer, Australia*
[5] *Radiometer ABLFlex800 Analyser, country, year*

Prothrombin (PT) and activated partial thromboplastin times (aPTT)[6] were collected in EDTA and were at least increased for 9 of the 10 dogs (Tabl. XIV).

3.3.4. DIAGNOSIS OF SNAKE ENVENOMATION

Snakes were witnessed for all dogs. A picture was taken for one of the snakes and clinicians confirmed diagnosis. Snake venom detection kits (SVDK)[7] were performed on 2 out of 9 dogs using urine and serum. Results revealed in both cases tiger snake immunotype (Tabl. XIV).

Table XIV. Major criteria for laboratory diagnosis of envenomation

	Case n°1	Case n°2	Case n°3	Case n°4	Case n°5	Case n°6	Case n°7	Case n°8	Case n°9	Case n°10
PT (14-19s)	**NA**	**NA**	10	**44**	10	**NA**	**NA**	**NA**	**NA**	**NA**
aPTT (100-120s)	**>300**	**>300**	103	**>300**	**>300**	**163**	**>300**	**>300**	**>300**	**>300**
Colour of serum	Haem.	Haem.	Haem.	Haem.	Haem.	Normal	Haem.	Haem.	Haem.	Haem
CK	<100	**13,319**	**1,583**		**25,571**	**1,362**	**379**	**1,021**	<100	**19,940**
SVDK	**Yes U**	No	**Yes S**	No	No	No	No	No	No	No
SVDK results	Tiger		Tiger							
2nd SVDK	Yes W	Yes U	No	No	No	Yes W	No	No	Yes W	Yes U
SVDK results	Neg	Neg				Neg			Neg	Neg

Coagulation times, color of serum, CK, SVDK test at admission and sample used for it and potential second SVDK test were recorded in order to make a diagnosis of envenomation. Increased PT (8/10), aPTT (9/10), haemolized serum (9/10), increased CK (7/9), positive SVDK (2/2) were considered in favor of snake envenomation.
PT=Prothrombin time, aPTT=activated Partial Thromboplastin Time, CK=Creatin Kinase, SVDK=Snake Venom Detection Kit, U=Urine sample, S=Serum sample, W=Whole blood sample, 2nd SVDK=SVDK repeated after antivenom administration, NA=Non Applicable, Haem. =Haemolized

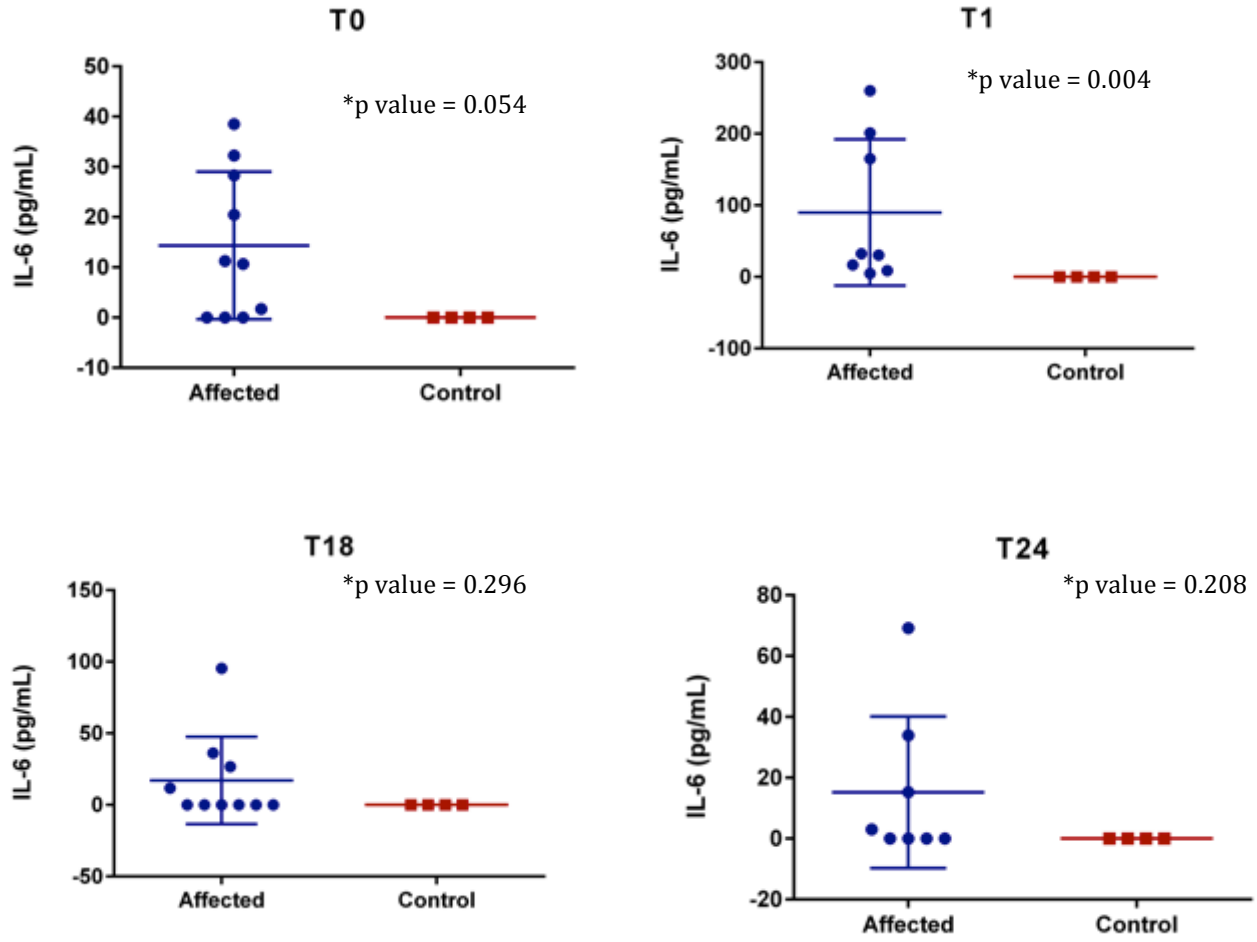
[6] *IDEXX Coag Dx Analyser*
[7] *Snake Venom Detection Kit, CSL Limited, VIC, Australia*

3.3.5. KINETIC FOLLOW-UP OF IL-6

IL-6 was quantified at each time following envenomation (T0, T1, T18, T24) except for two dogs (case n°3 and case n°5) in which IL-6 was measured at only T0 and T18 (due to some reasons such as the lack of serum for analysis, owner's decision to take the dog home or material availability).

The serum concentration ranges from 0pg/mL to 260pg/mL with an average of 33pg/mL (Graph. 1). IL-6 was indictable for all control cases. The average IL-6 concentration was above the detection level at each time and IL-6 was significantly more increased at T1 during the first two hours after antivenom injection compared to the control case (mean=89.9pg/mL, ranging from 4.7 to 260.0pg/mL, p-value=0.004 for difference between control and affected dogs by Mann-Whitney test) (Graph. 2). There were no significant differences between IL-6 levels at T0, T18 and T24 compared to control cases ($p>0.05$, by Mann-Whitney test). Moreover, IL-6 levels were significantly higher at T1 compared to IL-6 levels at T0 and at the other times (p-values < 0.005 for difference between levels at T1 and each other times T0, T18 an T24, Wilcocxon matched-paired signed rank test). There were no significant differences comparing IL-6 levels at T0, T18, T24 (p-values>0.9, Wilcocxon matched-paired signed rank test).

Graphic 1. Mean, median and quartile of IL-6 levels for each time in affected and healthy dogs

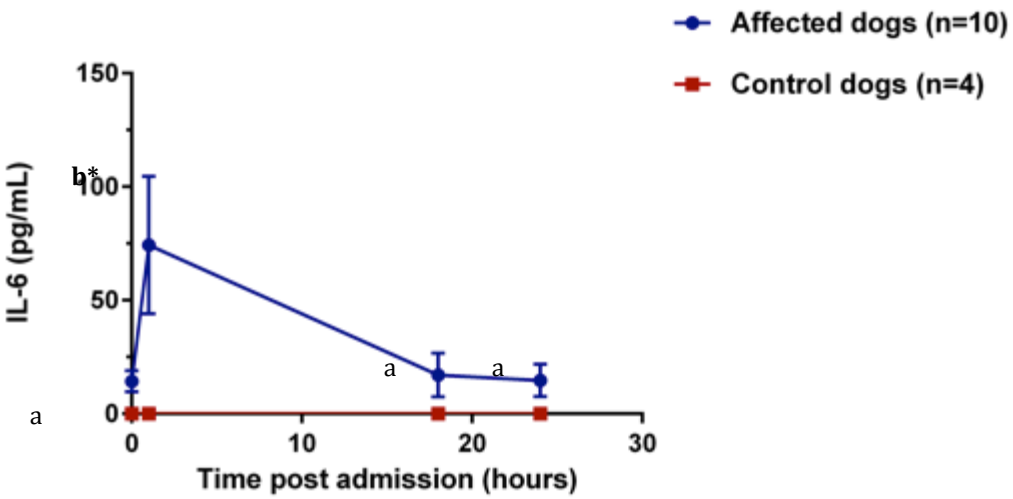


IL-6 serum concentration was determined at the admission (T0), in the first two hours after the admission (T1), 18 hours after the admission (T18) and 24 hours after the admission (T24). IL-6 levels ranged from 0pg/mL to 260pg/mL. IL-6 was indictable for all control cases. No data for two dogs at T1 and T24.

Tall bar=mean, small bar=standard error of mean (SEM)

**p-value is Mann-Whitney Test comparing healthy controls and affected dogs*

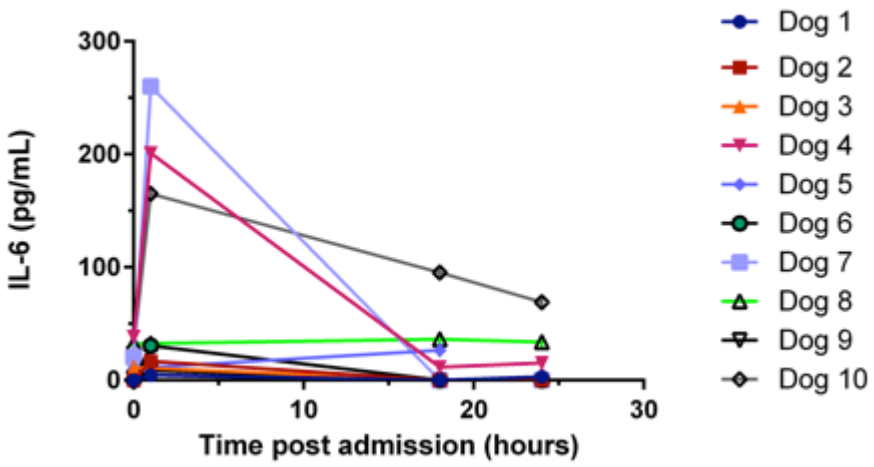
Graphic 2. Evolution of mean IL-6 levels depending on time post admisison



Evolution of mean IL-6 levels after admission at the hospital. The mean IL-6 concentration is above the detection level at each time and IL-6 is significantly increased at T1 (b), after antivenom injection, compared to T0 and to the other times (Wilcoxon matched paired test). Bar=standard error of mean (SEM).*

Three dogs showed significantly increased IL-6, at at least two times, especially at T1 (cases n°4, 7 and 10). Three dogs (cases n°1, 3 and 9) showed IL-6 levels below detection level for all times (including the dog n°3 for which data was only obtained at T0 and T18). Two dogs (cases n°2 and n°6) had IL-6 levels above detection level only at T1 and one dog (case n°5) had IL-6 levels above detection level only at T18 (data unknown at T1 and T24). A last dog (case n°8) had IL-6 levels slightly increased (33.8pg/mL on average) and stable at all times (Graph. 3).

Graphic 3. **Evolution of IL-6 levels for each dog**



Evolution of IL-6 levels measured at the admission, after antivenom administration, 18 hours and 24 hours after admission for each dog.

3.3.6. TREATMENT AND OUTCOME

Six dogs out of ten received 1 vial (3,000-4,000 units) and four dogs received 2 vials (6,000-8,000units) of the antivenom. A bivalent tiger snake / brown snake antivenom was used in the majority of dogs (8 out of 10 dogs) and a monovalent antivenom was administrated in two dogs in which SVDK revealed tiger snake immunotype. All dogs received fluid therapy (Lactated Ringer's solution at 2.5mg/Kg/h) and analgesia (Buprenorphine at 0.02mg/Kg IV or Methadone at 0.1mg/Kg depending on the dog's comfort).

Results of SVDK were used to guide repeated administration of antivenom for 5 dogs out of 9 and all results were negative. Therefore, no further antivenom administration was made.

Hypersensitivity was noted for one dog (case n°1) and was characterized by facial puritis and erythema affecting skin around mouth and chin. An antihistaminic (Chlorpheniramin) was given and the antivenom administration was stopped. No further signs were noticed. One day after admission, one dog (case n°10) presented a progressive and rapid decrease in haematocrit (until 13%) and was suspected of mediated immune hemolytic anaemia. He received a red blood cell transfusion on day 2 of its hospitalization.

All dogs were discharged alive. No mechanical ventilation was required. The median duration of hospitalization was 1.7 days (ranging from 1 to 5 days). The cost on discharge was on average 980 AUS dollars.

3.4. Discussion

In this study, a marker for systemic inflammation, Interleukin-6 and also clinical and clinicopathological parameters were investigated.

3.4.1. THE INFLAMMATORY RESPONSE

Snake envenomation was described as a complex inflammatory reaction in which cytokines and acute phase proteins play a major role. In a study on *Bothrop asper* envenomation, IL-6 was thought to be involved in leucocyte influx neutrophil activation and maturation as well as in the stimulation of molecules' adhesion on endothelial cells to form a cellular infiltrate (Stella R. Zamuner et al. 2005).

Evidence of inflammatory systemic response

IL-6 levels were above the detection level for 4 out of 10 dogs at T0, for 6 out of 8 dogs at T1, for 3 out of 10 dogs and 3 out of 8 dogs at T18 and T24. Unlike our hypothesis, plasma markers of inflammation were not significantly altered at dogs' presentation (T0) but only at T1 – during the two first hours following antivenom administration. In clinically healthy dogs, the serum concentration was never above 0pg/mL. There were some big variations between animals for IL-6 values, indicating different effects following inflammation of affected animals. Unfortunately, there is no study in which a reference interval for IL-6 in dogs has been calculated. However, similar values and disparity of IL-6 levels in dogs studies with healthy controls were reported. For example, IL-6 concentration in dogs with hepatitis and hepatic tumours were between 1 to 20 pg/mL (Neumann, Kaup, and Scheulen 2010). Dogs with local inflammation induced by intramuscular injection of turpentine oil had increased IL-6 levels ranging from 0 to 204.4 pg/mL (Yamashita et al. 1994). In our study, the IL-6's mean concentration for dogs envenomed by snakes is comparable to the one of dogs with primary hepatic tumour (Neumann, Kaup, and Scheulen 2010). Given the very low reference value, any increase of IL-6 can be easily recognized. Thus, in the present study, even if IL-6 was significantly increased at T1 only, there is still evidence of systemic inflammatory response for numerous dogs at all collection times.

IL-6 levels, clininical and clinicopathological correlation

Clinically, among the dogs with very high IL-6 levels, one dog presented only vomiting at the admission, another dog had weakness and hypersalivation and the last one had collapsed

before admission. Nevertheless, these signs were also observed on dogs with the lowest IL-6 levels. On the three dogs with the highest IL-6 levels, one had increased CK (19.940 mmol/L), one had CK within the reference (379 mmol/L) and CK was not measured for the third one due to financial reasons. High CK levels were also observed for dogs without any increase in IL-6 levels. Moreover, the lack of haematological and biochemical tests for some dogs make any biological correlation difficult. Haemolized plasma was observed on both, dogs with high IL-6 levels and dogs with low IL-6 levels. Since the collected samples for biochemical and haematological analysis were taken generally at the admission, it could have been interesting to analyze these parameters at each time in order to follow their evolutions. The longest hospitalization concerned a dog with a high IL-6 level but the duration remained quite similar than the one for other dogs, no further correlation could be made. Thus, no clinical or biological correlation could be made with the animals that had the highest levels of IL-6.

IL-6 levels and antivenom administration

In our study, the inflammatory response is more important at T1, after antivenom administration. Indeed, at T1, a higher number of dogs have their IL-6 level more severely affected by an increase compared to T0. Nevertheless, the delay between antivenom administration and snakebite was (if known) quite similar between dogs with or without high IL-6 levels. Thus, no correlation could be made with delayed antivenom administration. The number of vials administrated varied from one to two, and more antivenom was given to dogs with either high and low IL-6 levels. No correlation could be made between delayed antivenom administration or antivenom posology and IL-6 levels.

Antivenom administration, current standard treatment, was found to further exacerbate the venom induced inflammatory up-regulation and constitute a second pro-inflammatory hit (León et al., 2011, Stone et al., 2013). Indeed, two kinds of reactions induced by antivenom administration have been described: early reactions and late reactions. Late reactions correspond to a syndrome called “serum sickness” - it can appear 5 to 20 days after treatment and do not concern our study. Early reactions occur during the first hours following antivenom administration and the pathogenic mechanisms involved include pyrogenic and anaphylactic reactions with Ig-E and non Ig-E mediated reactions. Pyrogenic reactions are mainly characterized by hyperthermia, myalgia, nausea, sweating, tachycardia, and vasodilation with a secondary fall in blood pressure. They mainly imply bacterial lipopolysaccharides (LPS) in biologically-derived pharmaceuticals, stimulating monocytes and macrophages to produce cytokines such as IL-1, IL-6, INF-γ and TNF- α (León et al., 2011, Stone et al., 2013) and thus

could explain any rise in IL-6 levels. In the study of Stone et al., they noticed that patients presented astonishing levels of immune activation after antivenom administration. This immune activation was observed whether a reaction occured or not. Nevertheless, increases in inflammatory mediator levels (IL-6, IL-10, TNF-α) were highest in patients who also had pyrogenic reactions. Nevertheless, in our study, the dogs with the most altered IL-6 at T1 (dogs n°4, n°7 and n°10), showed none or slight signs of pyrogenic reaction (no hyperthermia and no hypotension for all dogs / only tachycardia at 152bpm and 150bpm for two dogs). Thus, in our study, pyrogenic reactions did not seem to be related to the inflammatory response noticed. As antivenom is formulated from hyperimmune IgG serum (CSL antivenom, 2014), it is possible that antibodies to antivenom participate to the induction of the inflammatory response. Stone et al. have suggested that antivenoms contain aggregates of immunoglobulins that have the ability to crosslink IgE receptors on the surface of mast cells resulting in non-allergen specific mast cell degranulation. In that study, they also noted an increase in IL-6 levels even when no hypersensitivity reaction to antivenom occurred. One dog in our study showed facial pruritus consistent with a hypersensitivity reaction, but IL-6 level was below the detection limit of the assay for each time (4,7pg/mL at T1). Considering the massive increase in IL-6 levels after antivenom administration, even if no pyrogenic or anaphylactic reaction have been noted, an immune response to antivenom, in addition with the inflammatory response induced by the venom itself, could not be excluded.

IL-6 levels and time effect

IL-6 levels were elevated at the early stages of envenomation, starting at the admission - T0 which was estimated in average to be 55 minutes after snakebite (4 out of 10 dogs). On the other hand, less dogs had increased IL-6 levels at T18 (3 out of 10 dogs) and T24 (3 out of 8 dogs) and increases were not significant. This early induction in IL-6 release was also found between 1 and 3 to 6 hours, peaking 3 hours after intraperitoneal injection of *Asper bothrop* venom in mice, returning to normal after 12 hours (Moreira et al. 2012). Nevertheless, in the present study, IL-6 was still above the reference value 18 hours and 24 hours after admission for 3 out of 10 dogs.

These results are also consistent with previous works in humans envenomed by snakes who showed elevated concentrations of IL-6, IL-8 and TNF-α. The inflammatory response in children following a *Bothrops* snake envenomation was studied by Ávila-Agüero et al. (2001) and showed an increase of IL-6 and IL-8 during the first 12-24 hours. Cytokine concentrations were detectable prior to antivenom administration and returned to normal 72 hours later. In another study of humans envenomed by Sri Lankan snakes (Stone et al. 2013), plasma

concentrations of IL-6, IL-10, C4a and C5a were significantly higher in envenomed patients before they received antivenom and IL-6 concentration increased rapidly and significantly after antivenom administration. These higher values after antivenom administration, also detected in our study can lead to question if there is an influence of antivenom administration on the inflammatory response as seen previously, or whether this is just a time effect between antivenom administration and the time required to elaborate IL-6 and to release it in the circulation. In a study on the kinetic of IL-6 changes, in which dogs received an intraveinous bolus of high dose lipopolysaccharide solution to induce sepsis, IL-6 levels increased at 1 hour, peaked at 3 hours and remained high until 24 hours (Song et al. 2012). In our study, the delay between snakebite and sample collection at T1 was on average 167 minutes (ranging from 110 to 206 minutes). The delay between antivenom administration and sample collection at T1 was on average 86 minutes (ranging from 15 to 165 minutes). Thus, the release of IL-6 in the circulation could match with the time required for the elaboration of the inflammatory response to the envenomation. Moreover, a bias exists in our study due to the lack of data (i.e. snakebites unknown for three dogs, including one of the dogs with highest IL-6 levels) and the fact that there is a wide disparity of delays between antivenom administration and sample collection at T1 due to some management reasons at the ICU (e.g. personnel avaibility, animals priority, samples collected at the same time for both the experiment and blood analysis as required by the clinician in order to minimize the number of venipunctures).

Among the three dogs for which IL-6 reached the highest levels at T1, one dog (dog n°4) had no determined time of snakebite. For another dog (dog n°7), blood was collected at T1 15 minutes after antivenom administration, corresponding to 130 minutes after snakebite; thus, in this case, elevation of IL-6 seemed pretty quick to be explained by the antivenom and thus would probably be due to the envenomation itself. For the last dog (dog n°10), the delay was 120 minutes between antivenom administration and blood collection at T1 and 165 minutes between snakebite and blood collection at T1. Thus, no conclusion could be made (delay was considered to be too similar). In this way, no correlation could be clearly made between the time required by the organism to elaborate IL-6 and the antivenom administration time or the envenomation time itself.

Adjuvant treatment

The previous considerations could lead to the perspective of new therapeutic objectives in conjunction with antivenom therapy regarding tiger snake envenomation. Since, on one hand, only one dog showed a reaction to antivenom administration of poor severity (pruritus) in our study, and, on the other hand, the literature (Armentano and Schaer 2011) showed low

prevalence of reactions for dogs, antihistamine drugs do not appear to be systematically necessary in tiger snake envenomation.

The use of corticosteroids and nonsteroidal anti-inflammatory drugs (NAIDs) is very controversial. Secondary effects (nephropathy and gastrointestinal ulceration) associated with NAIDs can be a fatal complication in envenomed patients and no in-vivo prospective canine studies have been performed to justify its use at this time (Armentano and Schaer 2011). Most of the current literature does not recommend the use of glucocorticoids. Moreover, a lot of these studies are retrospective studies and study design limitations have been raised. For example, a retrospective study on 327 dogs envenomed by *Vipera palaestinae* showed higher mortality with glucocorticoid therapy. But the authors also added that clinicians tend to give glucocorticoids therapy to more severely affected dogs first (Segev et al. 2004). The same limitation was found in a retrospective study on 53 Swedish dogs envenomed by *Vipera berus*, in which the group of dogs treated with glucocorticoids showed a trend towards a greater degree of swelling. But once more, the severity of clinical signs at presentation may have influenced the clinician to add glucocorticoids (Lervik, Lilliehöök, and Frendin 2010). A more recent prospective study, using randomly placebo in a clinical trial on dogs envenomed by *Vipera berus*, concludes that frequent use of glucocorticoids in dogs is not necessary since it did not significantly affect any clinical or clinicopathological parameters studied (Brandeker et al. 2015). Nevertheless, these studies looked at several snake species and none was conducted on tiger snake envenomation. No dog received any anti-inflammatory treatment in our study.

However, there are also some literatures to support glucocorticoids use. Indeed, the participation of arachidonic acid and metabolites as well as the release of endogenous mediators induced by venom components can justify its use. The stimulation of the inflammatory response induced by PLA2 and SVMP, important component of snake venom, have been particularly studied in *Bothrops asper* (Farsky et al. 2000; Kanashiro et al., 2002; Zuliani et al. 2005). For mice, dexamethasone administration in conjunction with serum therapy were shown to be beneficial to treat inflammatory oedema caused by *Bothrops jararaca* (Araújo et al. 2007). Moreover, a wide variety of glucocorticoid drugs can be administered, from anti-inflammatory to immunosuppressive doses, and in multiple species, rendering a clinical interpretation of the limited research data very difficult (Armentano and Schaer 2011).

3.4.2. CLINICAL AND CLINICOPATHOLOGICAL FEATURES

Clinical signs

Dogs with mild envenomation were presented with non-specific clinical signs such as collapse and vomiting, which are common findings in dogs with envenomation. None of the dogs involved in the study had local signs of snakebite e.g. oedema, erythematous area, etc. According to an experimental study of 1994 in which tiger snake venom was injected in dogs with various dose rates, envenomation results primarily in neurological, myolitic anc coagulopathic clinical syndromes. Three categories of signs were defined as pre-paralytic, paralytic and lethal or delayed signs. Dogs envenomed by tiger snake presented collapse due to systemic hypotension, vomiting, salivation, defaecation and tachypnea in the pre-paralytic stage, skeletal muscle paralysis, coagulopathic signs, oliguria with or without haemoglobinuria or myoglobinuria in the paralytic stage and mydriasis, slow or absent pupillary reflex, stiff gait, ataxia, signs or renal failure in the sublethal stage (Lewis 1994; Heller et al. 2007). In our study, neurological signs included mainly mydriasis, which was also previously described to be the most common clinical sign - occuring in 76% of dogs with tiger snake envenomation (Barr 1984). Other neurological signs such as hindleg ataxia and flaccid paralysis were also observed in respectively 35% and 41% of dogs, we did not observe those signs in our study. In the same study, weakness and vomiting were frequent clinical signs following tiger snake envenomation (for respectively 37% and 53% of cases). In a study looking at clinical syndromes associated to humans envenomed by tiger snake, all envenomed patients developed Venom Induced Consumption Coagulopathy (VICC), neurotoxicity (53%) or myotoxicity (11%) alone or in combination. Systemic symptoms were the most common findings (80%) with nausea, vomiting and headache (44%, 35%, 34% respectively) (Geoffrey K. Isbister et al. 2012).

Clinical signs observed after snake envenomation are variable depending on snake species involved, probably given the different toxins in their venoms. For example, altered mental status was not often observed in our study, unlike in the study of (Lervik, Lilliehöök, and Frendin 2010) in which 73% of dogs envenomed by *Vipera berus* had an affected mental status on arrival at the hospital. Diarrhea was also seen in one third of the dogs envenomed by this species (Brandeker et al. 2015). It is also the most common clinical sign in humans. However, gastrointestinal signs (e.g. diarrhea) are not frequently reported in dogs with tiger snake envenomation.

Alteration in biochemical and haematological parameters

The most affected biochemical parameters were CK since almost all dogs presented moderate to severe CK levels increase. This is probably caused by muscle damages at the snakebite. Myotoxicity is defined as an elevated creatine kinase level associated with myalgia and/or muscle tenderness and is thought to be mainly due to notexin, one of the most important toxins of tiger snake venom. Notexin is a potent myotoxic and presynaptic PLA2 (Fry 1999), that induces a rapid local and generalised skeletal muscle myodegeneration, even after low doses of venom according to a study on histological examination of experimentally envenomed dogs tissues (Lewis 1994). Hyperlactataemia was present in several dogs and may be the result of an increased oxygen consommation by muscles. A slight lactic acidosis was noted in some dogs, without any abnormality of systolic blood pressure. The dog with increased ALKP received also cyclosporin for skin allergy, and thus no link with the envenomation was established. Except for significant CK level increases, the biochemical changes in our study were mild, as it was in other studies in which no significative alteration other than for CK were reported (Barr 1984; Geoffrey K. Isbister et al. 2012).

Haemoconcentration, characterized by an increased in haematocrit, was observed in 7 out of 10 dogs in our study. It was also found in another study on an Elapid snake *(Naja annulifera*) (Lobetti and Joubert 2004). However, it was only rarely described in Elapids and more frequently reported in Viperidae envenomation (puff-adders and viper) (Segev et al. 2004; Aroch et al. 2010; Lobetti and Joubert 2004). In these last studies, abnormality was explained by fluid losses and translocation of proteins and fluids from the circulation to the inflamated tissue at the bite site. Nevertheless, if there is evidence of local response to envenomation by these species, there is typically no local response observed in dogs with tiger snakebite. Splenic contraction may also be involved associated with excitement, pain, stress and subsequent release of stored erythrocytes into circulation (Goddard et al. 2011).

We noticed that plasma serum was almost always hemolysed, which could suggest hemolysis. It is thought to be the consequence of lytic factor or phospholipase activity contained in tiger snake venom (Goddard et al. 2011). Moreover, almost all dogs presented signs of coagulopathy, as reflected by strong increase of PT and aPTT, a common finding in Elapid snake envenomation (Holloway and Parry 1989; Geoffrey K. Isbister et al. 2012; Geoffrey K. Isbister 2010). A slight thrombocytopenia was observed in 4 out of 10 dogs, which is usually more common in Viperidae envenomation (Goddard et al. 2011). Based on the current diagnostic criteria for DIC, including prolonged PT and aPTT, thrombocytopenia, increased concentration of D-dimers or fibrin degradation products (FDPs), and fibrinogen depletion, we observed some of the criteria compatible with potential disseminated

intravascular coagulation. Prolonged PT and aPTT may be the result of decreased prothrombin and fibrinogen concentrations and increased FDPs, which are probably the direct results of the venom procoagulant action, rather than the beginning of disseminated (or localised) intravascular coagulation. If venom is slowly absorbed from the envenomation site, its blood concentration will be low, and, fibrinogen will be consumed without intravascular fibrin deposition (or thrombus formation) (Holloway and Parry 1989). This consumptive coagulopathy associated with snakebites has been referred as a VICC (Venom Induced Consumption Coagulopathy) described in several different snake species envenomation including Elapids (Goddard et al. 2011). VICC is characterized by prolonged clotting times, depletion of fibrinogen and cofactors V and VIII, and high concentrations of FDPs, but does not include evidence of thrombus formation and organ failure. The VICC results from the activation of the coagulation pathway due to components of Elapid venoms, including procoagulants (such as prothrombin activators, FV and FX activators). The VICC also differs from rapid onset and resolution, which was observed in all our cases. The time from the bite to the complete defibrination can vary from 15 minutes to many hours and sometimes days for some species of Elapid. Spontaneous bleeding is rare following bites by these snakes but any vascular damages can cause major bleeding (White 2005). In our study, there was no correlation between haemostatic abnormality and severity of envenomation. All dogs were discharged alive.

Concerning the treatment and the outcome, antivenom administrated was always the bilavent tiger snake and brown snake immunotype. On the retrospective study piloted at the Melbourne University Hospital from March 1973 to March 1983, both antivenoms (tiger and brown snake immunotypes) were administrated in Victoria if the snake could not be identified but they also precised that from their experience, tiger snake antivenom alone would cover nearly all envenomations from the Werribee area (Barr 1984).

The number of vials administrated was correlated to clinical signs observed after the first antivenom administration and did not seem to be associated with the severity of clinical signs at the arrival at the hospital. A longer hospitalization was associated with CK level increase. Indeed, hospitalization was recommended as long as CK level did not decrease sufficiently from its highest concentration. No dog died during hospitalization and the disease evolution was benign with a mental status returning to normal in all dogs within the first day. Nevertheless, the outcome was biased by the fact that dogs envenomed more than 24 hours before admission were not enrolled. Indeed, when the time of the snakebite was known, the average delay between snakebite and antivenom administration was 83 minutes (ranging from

45 to 130 minutes), which is a quite short delay and we know that the shorter the delay between snakebite and antivenom administration the better the prognosis. Antivenom therapy is more effective if given as soon after the bite as possible. Previous works have shown that a delay in administration of antivenom is followed by delayed response or slow recovery (Barr 1984). Outcomes of our study are thought to be relatively good because admission occurred within the same day of envenomation and antivenom was administrated rapidly.

3.4.3. LIMITATIONS

This study has some limitations. The first limitation is the small number of dogs considered, which maked comparison and correlation very difficult.

The SVDK was determined in only two cases and there were the only cases confirmed positive for tiger snake immunotype. This immunotype is shared by the tiger snake (*Notechis scutatus)*, the common copperhead (*Australeps superbus*) and the rough scaled snake (*Tropidechis carinatus*) (White J, 2013). However, the tiger snake is the only species known to exist in the western suburbs of Melbourne (Mirtschin et al. 1998) where the University of Melbourne Veterinary Hospital is located. In one case, owners brought a picture of the snake, which was recognized to be a tiger snake by clinicians. Thus, diagnosis can be reasonably considered as reliable for these three cases. The SVDK could not be used in all cases due to financial request from the owner. Nevertheless, a combination of an appropriate history, clinical signs, laboratory tests, marked prolongation of coagulation times, an elevated CK and knowledge of experienced clinicians were considered to be sufficient for the diagnosis of snake envenomation.

For this pilot study, biochemistry and haematology were only assessed depending on the clinician's decision and owners' one, therefore the lack of data for several dogs made an accurate interpretation difficult. Systematic and iterative biochemical and haematological tests could have been helpful in diagnosis as well as to better characterize the severity of the envenomation and to support evidence of an inflammatory response (e.g. anemia, leukocytosis, etc.).

In the same perspective, there was a lack of data concerning the exact time of snakebite and thus the exact delay between snakebite, IL-6 measurement and antivenom administration. Unfortunately, as snake envenomations on dogs are not always observed by their owner, it is a

very difficult parameter to control. Moreover, time collection after antivenom administration was very difficult to control because depending on the avaibility of personnel in the ICU and on the clinician decision to collect blood for the animal's health.

Systemic and sometimes local effects induced by snake venom are characterized by an acute inflammatory response with accumulation of leukocytes and release of numerous mediators. Many authors, working on the inflammatory response induced by venom of Viperidae snakes, investigated the release of numerous cytokines, chemokines and/or other mediators such as IL-1, IL-6, IL-8, IL-10, TNF- α, CRP, Regulated on Activation Normal T cell Expressed and Secreted (RANTES) (Lomonte, Tarkowski, and Hanson 1993; Stone et al. 2013; ÁVILA-AGÜERO et al. 2001; Zamuner et al. 2005). Nevertheless, due to financial and practical reasons, IL-6 was the only cytokine measured in this study. Thus, as these mediators have different biological activities and peak at different times into the blood circulation after stimulation, it could have been interesting to have an idea of the levels and the kinectics of these different mediators to better characterize the inflammatory response following tiger snake envenomation.

Blood has been collected in citrated tubes prior to plasma production, which could increase IL-6 levels. Nevertheless, citrated tubes were used for all IL-6 blood samples taken. A storage at -80°C was made for a maximum of 6 months in order to fulfill storage conditions that should not influence our results (Kenis et al. 2002).

3.5. Conclusion

This study showed evidence of a systemic inflammatory response occurring on dogs after tiger snake envenomation, with the increase of a systemic inflammation marker, the interleukin-6.

There was a wide disparity between animals for IL-6 values, which indicates the existence of different effects following inflammation of affected animals. Unfortunately, there is no study in which a reference range was calculated for IL-6 in dogs. Thus, any increase in IL-6 levels was easily recognized and showed evidence of systemic inflammatory response for numerous dogs at all various times of blood sample collection.

No clinical or biological correlation could be made with the studied animals that had the highest levels of IL-6. No correlation could be made either between delayed antivenom administration or antivenom posology and the increase of IL-6 levels. Pyrogenic reactions did not seem to be related to the inflammatory response noticed.

This systemic inflammatory response was highly influenced by delay after envenomation and may be even more important after antivenom administration. As antivenom is generated from hyperimmune IgG serum, it was previously supposed that antibodies to antivenom might participate to the induction of the inflammatory response.

The increased IL-6 levels after antivenom administration could be consistent with the time required to elaborate IL-6 in response to the venom. However, an exacerbation of the venom induced inflammatory response by the antivenom itself could not be excluded. An attenuation of this inflammatory response may be exploited as a new therapeutic avenue.

This study has some limitations such as a limited number of cases, limited hematological and biochemical number of analysis, and the fact that only one marker of the systemic inflammatory response (IL-6) have been studied.

Moreover, there is a serious lack of prospective studies on the use of anti-inflammatory medicine on dogs, especially regarding tiger snake envenomation. Considering the inflammatory response following tiger snake envenomation, it is suggested that the use of anti-inflammatory therapy in tiger snake envenomation should further be investigated.

AGREMENT SCIENTIFIQUE

En vue de l'obtention du permis d'imprimer de la thèse de doctorat vétérinaire

Je soussignée, Séverine BOULLIER, Enseignant-chercheur, de l'Ecole Nationale Vétérinaire de Toulouse, directeur de thèse, certifie avoir examiné la thèse de **COSTET** Juliette intitulée **«Inflammatory response to naturaliy occurring tiger snake envenomation in dogs, with a special emphasis on IL-6.»** et que cette dernière peut être imprimée en vue de sa soutenance.

Fait à Toulouse, le 4 octobre 2016
Docteur Séverine BOULLIER
Enseignant chercheur
de l'Ecole Nationale Vétérinaire de Toulouse

Vu :
La Directrice de l'Ecole Nationale Vétérinaire de Toulouse
Isabelle CHMITELIN

Vu :
Le Président du jury :
Professeure Bettina COUDERC

Vu et autorisation de l'impression :
Président de l'Université
Paul Sabatier
Monsieur Jean-Pierre VINEL
Le Président de l'Université Paul Sabatier
par délégation,
La Vice-Présidente de la CFVU

Régine ANDRE-OBRECHT

Conformément à l'Arrêté du 20 avril 2007, article 6, la soutenance de la thèse ne peut être autorisée qu'après validation de l'année d'approfondissement.

Bibliography

Akira, S., T. Hirano, T. Taga, and T. Kishimoto. 1990. "Biology of Multifunctional Cytokines: IL 6 and Related Molecules (IL 1 and TNF)." The FASEB Journal 4 (11): 2860–67.

Alvarez, J., and J. García-Sancho. 1989. "Inhibition of Red Cell Ca2+-Dependent K+ Channels by Snake Venoms." Biochimica Et Biophysica Acta 980 (2): 134–38.

Araújo, S. Dias, A. de Souza, F. P. B. Nunes, and L. R. C. Gonçalves. 2007. "Effect of Dexamethasone Associated with Serum Therapy on Treatment of Bothrops Jararaca Venom-Induced Paw Edema in Mice." Inflammation Research 56 (10): 409–13.

Arend, William P., and Cem Gabay. 2004. "Cytokines in the Rheumatic Diseases." Rheumatic Diseases Clinics of North America 30 (1): 41–67, v – vi.

Armentano, Robert A., and Michael Schaer. 2011. "Overview and Controversies in the Medical Management of Pit Viper Envenomation in the Dog: Management of Pit Viper Envenomation in the Dog." Journal of Veterinary Emergency and Critical Care 21 (5): 461–70.

Aroch, I., and S. Harrus. 1999. "Retrospective Study of the Epidemiological, Clinical, Haematological and Biochemical Findings in 109 Dogs Poisoned by Vipera Xanthina Palestinae." The Veterinary Record 144 (19): 532–35.

Avila-agüero, Maria L., Maria M. Paris, Shuxian Hu, Phillip K. Peterson, José Maria Gutierrez, Bruno Lomonte, Idis Faingezicht, Snakebite Study Group, and others. 2001. "Systemic Cytokine Response in Children Bitten by Snakes in Costa Rica." Pediatric Emergency Care 17 (6): 425–29.

Baggiolini, Marco, and Ian Clark-Lewis. 1992. "Interleukin-8, a Chemotactic and Inflammatory Cytokine." FEBS Letters 307 (1): 97–101.

Barr, S. C. 1984. "Clinical Features Therapy and Epidemiology of Tiger Snake Bite in Dogs and Cats." Australian Veterinary Journal 61 (7): 208–12.

Bienvenu, J., G. Monneret, N. Fabien, and J. P. Revillard. 2000. "The Clinical Usefulness of the Measurement of Cytokines." Clinical Chemistry and Laboratory Medicine 38 (4): 267–85.

Birrell, Geoff W., Stephen T. H. Earl, Tristan P. Wallis, Paul P. Masci, John de Jersey, Jeffrey J. Gorman, and Martin F. Lavin. 2007. "The Diversity of Bioactive Proteins in Australian Snake Venoms." Molecular & Cellular Proteomics 6 (6): 973–86.

Bordon, Jose, Stefano Aliberti, Rafael Fernandez-Botran, Silvia M. Uriarte, Madhavi J. Rane, Padmaraj Duvvuri, Paula Peyrani, Letizia Corinna Morlacchi, Francesco Blasi, and Julio A. Ramirez. 2013. "Understanding the Roles of Cytokines and Neutrophil Activity and Neutrophil Apoptosis in the Protective versus Deleterious Inflammatory Response in Pneumonia." International Journal of Infectious Diseases 17 (2): e76–83.

Brandeker, Erika, Anna Hillström, Sofia Hanås, Ragnvi Hagman, and Bodil Ström Holst. 2015. "The Effect of a Single Dose of Prednisolone in Dogs Envenomated by Vipera Berus – a Randomized, Double-Blind, Placebo-Controlled Clinical Trial." BMC Veterinary Research 11. p1-8

Broad, A. J., S. K. Sutherland, and A. R. Coulter. 1979. “The Lethality in Mice of Dangerous Australian and Other Snake Venom.” Toxicon 17 (6): 661–64.

Butterfield, Timothy A., Thomas M. Best, and Mark A. Merrick. 2006. “The Dual Roles of Neutrophils and Macrophages in Inflammation: A Critical Balance between Tissue Damage and Repair.” Journal of Athletic Training 41 (4): 457.

Cassatella, Marco A. 2013. “L33. Neutrophil in Immunity: A Key Modulator.” La Presse Médicale, 16th International Vasculitis & ANCA Workshop, 42 (4, Part 2): 594–95.

Cassatella, Marco Antonio. 1999. “Neutrophil-Derived Proteins: Selling Cytokines by the Pound.” In Advances in Immunology, edited by Frank J. Dixon, 73:369–509. Academic Press. http://www.sciencedirect.com/science/article/pii/S0065277608607919.

Castell, José V., Maria J. Gómez-Lechón, Martina David, Tilo Andus, Thomas Geiger, Ramón Trullenque, Ricardo Fabra, and Peter C. Heinrich. 1989. “Interleukin-6 Is the Major Regulator of Acute Phase Protein Synthesis in Adult Human Hepatocytes.” FEBS Letters 242 (2): 237–39.

Cavaillon, J. M. 1994. “Cytokines and Macrophages.” Biomedicine & Pharmacotherapy = Biomédecine & Pharmacothérapie 48 (10): 445–53.

Cerón, Eckersall, and Silvia Martínez-Subiela. 2005. “Acute Phase Proteins in Dogs and Cats: Current Knowledge and Future Perspectives.” Veterinary Clinical Pathology 34 (2): 85–99.

Chaves, Fernando, Milagro Barboza, and JoséMaría Gutiérrez. 1995. “Pharmacological Study of Edema Induced by Venom of the Snake Bothrops Asper (terciopelo) in Mice.” Toxicon 33 (1): 31–39.

Cher, Charmian D. N., Arunmozhiarasi Armugam, Ramkumar Lachumanan, Marelyn-Wintour Coghlan, and Kandiah Jeyaseelan. 2003. “Pulmonary Inflammation and Edema Induced by Phospholipase A2: Global Gene Analysis and Effects on Aquaporins and Na+/K+-ATPase.” The Journal of Biological Chemistry 278 (33): 31352–60.

Chippaux, J. -P., V. Williams, and J. White. 1991. “Snake Venom Variability: Methods of Study, Results and Interpretation.” Toxicon 29 (11): 1279–1303.

Chisari, A., E. Spinedi, M. J. Voirol, A. Giovambattista, and R. C. Gaillard. 1998. “A Phospholipase A2-Related Snake Venom (from Crotalus Durissus Terrificus) Stimulates Neuroendocrine and Immune Functions: Determination of Different Sites of Action.” Endocrinology 139 (2): 617–25.

Cull-Candy, S. G., J. Fohlman, D. Gustavsson, R. Lüllmann-Rauch, and S. Thesleff. 1976. “The Effects of Taipoxin and Notexin on the Function and Fine Structure of the Murine Neuromuscular Junction.” Neuroscience 1 (3): 175–80.

Dalrymple, Stacie A., Robyn Slattery, Dee M. Aud, Mala Krishna, Linda A. Lucian, and Richard Murray. 1996. “Interleukin-6 Is Required for a Protective Immune Response to Systemic Escherichia Coli Infection.” Infection and Immunity 64 (8): 3231–35.

Datyner, M. E., and P. W. Gage. 1973. “Presynaptic and Postsynaptic Effects of the Venom of the Australian Tiger Snake at the Neuromuscular Junction.” British Journal of Pharmacology 49 (2): 340–54.

Day Michael J. 2011.Veterinary immunology: Principles and practice. First Edition. p11-70. ISBN-13: 978-1840761436

Downey, G. P. 1994. “Mechanisms of Leukocyte Motility and Chemotaxis.” Current Opinion in Immunology 6 (1): 113–24.

Ericsson, Charles D., Christoph Hatz, Thomas Junghanss, and Mauro Bodio. 2006. “Medically Important Venomous Animals: Biology, Prevention, First Aid, and Clinical Management.” Clinical Infectious Diseases 43 (10): 1309–17.

Escocard, Rita de Cássia Mothé, Milton Masahiko Kanashiro, Jorge Hudson Petretski, Juliana Azevedo-Silva, Eulógio Carlos Queiroz de Carvalho, Wilmar Dias da Silva, and Thereza Liberman Kipnis. 2006. “Neutrophils Regulate the Expression of Cytokines, Chemokines and Nitric Oxide Synthase/nitric Oxide in Mice Injected with Bothrops Atrox Venom.” Immunobiology 211 (1–2): 37–46.

Farsky, Sandra H. P., Luís Roberto C. Gonçalves, José M. Gutiérrez, Adriana P. Correa, Alexandra Rucavado, Philippe Gasque, and Denise V. Tambourgi. 2000. “Bothrops Aspe-Snake Venom and Its Metalloproteinase BaP–1 Activate the Complement System. Role in Leucocyte Recruitment.” Mediators of Inflammation 9 (5): 213–21.

Fry, Bryan Grieg. 1999. “Structure–function Properties of Venom Components from Australian Elapids.” Toxicon 37 (1): 11–32.

Gao, Wenda, and Miercio A Pereira. 2002. “Interleukin-6 Is Required for Parasite Specific Response and Host Resistance to Trypanosoma Cruzi.” International Journal for Parasitology 32 (2): 167–70.

Goddard, Amelia, Johan P. Schoeman, Andrew L. Leisewitz, Salome S. Nagel, and Itamar Aroch. 2011. “Clinicopathologic Abnormalities Associated with Snake Envenomation in Domestic Animals.” Veterinary Clinical Pathology / American Society for Veterinary Clinical Pathology 40 (3): 282–92.

Gutiérrez, JoséMaría, Marjorie Romero, Cecilia Díaz, Gadi Borkow, and Michael Ovadia. 1995. “Isolation and Characterization of a Metalloproteinase with Weak Hemorrhagic Activity from the Venom of the Snake Bothrops Asper (terciopelo).” Toxicon 33 (1): 19–29.

Gutiérrez, José María, Alexandra Rucavado, Fernando Chaves, Cecilia Díaz, and Teresa Escalante. 2009. “Experimental Pathology of Local Tissue Damage Induced by Bothrops Asper Snake Venom.” Toxicon 54 (7): 958–75.

Hack, C. E., E. R. De Groot, R. J. Felt-Bersma, J. H. Nuijens, R. J. Strack Van Schijndel, A. J. Eerenberg-Belmer, L. G. Thijs, and L. A. Aarden. 1989. “Increased Plasma Levels of Interleukin-6 in Sepsis.” Blood 74 (5): 1704–10.

Hardy, Margaret C., Jonathon Cochrane, and Rachel E. Allavena. 2014. “Venomous and Poisonous Australian Animals of Veterinary Importance: A Rich Source of Novel Therapeutics.” BioMed Research International 2014.

Harris, J. B. 1985. “Phospholipases in Snake Venoms and Their Effects on Nerve and Muscle.” Pharmacology & Therapeutics 31 (1): 79–102.

Harris, J. B., R. Vater, M. Wilson, and M. J. Cullen. 2003. “Muscle Fibre Breakdown in Venom-Induced Muscle Degeneration.” Journal of Anatomy 202 (4): 363–72.

Haslett, C. 1999. “Granulocyte Apoptosis and Its Role in the Resolution and Control of Lung Inflammation.” American Journal of Respiratory and Critical Care Medicine 160 (5 Pt 2): S5–11.

Heasman, S. J., K. M. Giles, C. Ward, A. G. Rossi, C. Haslett, and I. Dransfield. 2003. “Glucocorticoid-Mediated Regulation of Granulocyte Apoptosis and Macrophage Phagocytosis of Apoptotic Cells: Implications for the Resolution of Inflammation.” Journal of Endocrinology 178 (1): 29–36.

Heiner, Jason D., Vikhyat S. Bebarta, Shawn M. Varney, Jason D. Bothwell, and Aaron J. Cronin. 2013. “Clinical Effects and Antivenom Use for Snake Bite Victims Treated at Three US Hospitals in Afghanistan.” Wilderness & Environmental Medicine 24 (4): 412–16.

Heller, J., Kl Bosward, Dr Hodgson, and R. Pottie. 2006. “Anuric Renal Failure in a Dog after Red-Bellied Black Snake (Pseudechis Porphyriacus) Envenomation.” Australian Veterinary Journal 84 (5): 158–62.

Heller, J, Dj Mellor, Jl Hodgson, Swj Reid, Dr Hodgson, and Kl Bosward. 2007. “Elapid Snake Envenomation in Dogs in New South Wales: A Review.” Australian Veterinary Journal 85 (11): 469–79.

Henderson, R. B. 2003. “Rapid Recruitment of Inflammatory Monocytes Is Independent of Neutrophil Migration.” Blood 102 (1): 328–35.

Hill, F. W. G. 1979. “Snake Bite in Dogs.” Australian Veterinary Journal 55 (2): 82–85.
Hite, L. A., L. G. Jia, J. B. Bjarnason, and J. W. Fox. 1994. “cDNA Sequences for Four Snake Venom Metalloproteinases: Structure, Classification, and Their Relationship to Mammalian Reproductive Proteins.” Archives of Biochemistry and Biophysics 308 (1): 182–91.

Hodgson, Wayne C., Christer O. Eriksson, Paul F. Alewood, and Bryan G. Fry. 2003. “Comparison of the in Vitro Neuromuscular Activity of Venom from Three Australian Snakes (Hoplocephalus Stephensi, Austrelaps Superbus and Notechis Scutatus): Efficacy of Tiger Snake Antivenom.” Clinical and Experimental Pharmacology & Physiology 30 (3): 127–32.

Hodgson, Wayne C., and Janith C. Wickramaratna. 2006. “Snake Venoms and Their Toxins: An Australian Perspective.” Toxicon 48 (7): 931–40.

Holloway, Sa., and Bw. Parry. 1989. “Observations on Blood Coagulation after Snakebite in Dogs and Cats.” Australian Veterinary Journal 66 (11): 364–66.

Hutton, R. A., and D. A. Warrell. 1993. “Action of Snake Venom Components on the Haemostatic System.” Blood Reviews 7 (3): 176–89.

Ireland, Graham, Simon GA Brown, Nicholas A. Buckley, Jeff Stormer, Bart J. Currie, Julian White, David Spain, and Geoffrey K. Isbister. 2010. “Changes in Serial Laboratory Test Results in Snakebite Patients: When Can We Safely Exclude Envenoming?” Medical Journal of Australia 193 (5): 285.

Indrawirawan YH, Sheridan GI, McAlees TJ, 2014. Clinical features of Mainland tiger and Eastern brown snake envenomation in dogs and cats in Melbourne. Australian Veterinary Practitioner 44 (4) p704-712

Isbister, Geoffrey K. 2010. "Snakebite Doesn't Cause Disseminated Intravascular Coagulation: Coagulopathy and Thrombotic Microangiopathy in Snake Envenoming." Seminars in Thrombosis and Hemostasis 36 (4): 444–51.

Isbister, Geoffrey K., S. G. A. Brown, and ASP Investigators. 2012. "Bites in Australian Snake Handlers--Australian Snakebite Project (ASP-15)." QJM: Monthly Journal of the Association of Physicians 105 (11): 1089–95.

Isbister, Geoffrey K., Simon G. A. Brown, Colin B. Page, David L. McCoubrie, Shaun L. Greene, and Nicholas A. Buckley. 2013. "Snakebite in Australia: A Practical Approach to Diagnosis and Treatment." Medical Journal of Australia 199 (11). https://www.mja.com.au/journal/2013/199/11/snakebite-australia-practical-approach-diagnosis-and-treatment.

Isbister, Geoffrey K., Margaret A. O'Leary, Matthew Elliott, and Simon G. A. Brown. 2012. "Tiger Snake (*Notechis* Spp) Envenoming: Australian Snakebite Project (ASP-13)." Medical Journal of Australia 197 (3). https://www.mja.com.au/journal/2012/197/3/tiger-snake-notechis-spp-envenoming-australian-snakebite-project-asp-13.

IUCN. 2009. "*Notechis Scutatus*: Cogger, H.: The IUCN Red List of Threatened Species 2010: e.T169687A6666605. Available on http://www.iucnredlist.org/details/169687/0.

Jacoby-Alner, T.E., N. Stephens, K.M. Davern, L. Balmer, S.G.A. Brown, and K. Swindells. 2011. "Histopathological Analysis and in Situ Localisation of Australian Tiger Snake Venom in Two Clinically Envenomed Domestic Animals." Toxicon 58 (4): 304–14.

Jelinek, George A., Charles Tweed, Dania Lynch, Tony Celenza, Brian Bush, and Nick Michalopoulos. 2004. "Cross Reactivity between Venomous, Mildly Venomous, and Non-Venomous Snake Venoms with the Commonwealth Serum Laboratories Venom Detection Kit." Emergency Medicine Australasia: EMA 16 (5-6): 459–64.

J. M. Gutiérrez, F. Chaves. 1981. "Neutralizacion de Los Efectos Locales Del Veneno de Bothrops Asper Por Un Antiveneno Polivalente." Toxicon 19 (4): 493–500.

Johnston, C. I., S. G. A. Brown, M. A. O'Leary, B. J. Currie, R. Greenberg, M. Taylor, C. Barnes, J. White, G. K. Isbister, and ASP investigators. 2013. "Mulga Snake (Pseudechis Australis) Envenoming: A Spectrum of Myotoxicity, Anticoagulant Coagulopathy, Haemolysis and the Role of Early Antivenom Therapy - Australian Snakebite Project (ASP-19)." Clinical Toxicology (Philadelphia, Pa.) 51 (5): 417–24.

Jorge, M. T., C. Malaque, L. A. Ribeiro, H. W. Fan, J. L. C. Cardoso, S. A. Nishioka, I. S. Sano-Martins, et al. 2004. "Failure of Chloramphenicol Prophylaxis to Reduce the Frequency of Abscess Formation as a Complication of Envenoming by Bothrops Snakes in Brazil: A Double-Blind Randomized Controlled Trial." Transactions of The Royal Society of Tropical Medicine and Hygiene 98 (9): 529–34.

Juckett, Gregory, and John G. Hancox. 2002. "Venomous Snakebites in the United States: Management Review and Update." American Family Physician 65 (7): 1367–74.

Kaplanski, Gilles, Valérie Marin, Félix Montero-Julian, Alberto Mantovani, and Catherine Farnarier. 2003. “IL-6: A Regulator of the Transition from Neutrophil to Monocyte Recruitment during Inflammation.” Trends in Immunology 24 (1): 25–29.

Kaser, A., G. Brandacher, W. Steurer, S. Kaser, F. A. Offner, H. Zoller, I. Theurl, et al. 2001. “Interleukin-6 Stimulates Thrombopoiesis through Thrombopoietin: Role in Inflammatory Thrombocytosis.” Blood 98 (9): 2720–25.

Katzenbach, Julia E., and Daniel S. Foy. 2015. “Retrospective Evaluation of the Effect of Antivenom Administration on Hospitalization Duration and Treatment Cost for Dogs Envenomated by \iCrotalus Viridis : 113 Dogs (2004-2012): Antivenom in Dogs Envenomated by \iC. Viridis.” Journal of Veterinary Emergency and Critical Care 25 (5): 655–59.

Kenis, Gunter, Charlotte Teunissen, Raf De Jongh, Eugène Bosmans, Harry Steinbusch, and Michael Maes. 2002. “Stability of Interleukin 6, Soluble Interleukin 6 Receptor, Interleukin 10 and CC16 in Human Serum.” Cytokine 19 (5): 228–35.

Khan, Manzoor M. 2008. Immunopharmacology. Boston, MA: Springer US. p33-59. Available on http://link.springer.com/10.1007/978-0-387-77976-8.

Langhorn, Rebecca, Frida Persson, Björn Ablad, Amelia Goddard, Johan P. Schoeman, Jakob L. Willesen, Inge Tarnow, and Mads Kjelgaard-Hansen. 2014. “Myocardial Injury in Dogs with Snake Envenomation and Its Relation to Systemic Inflammation.” Journal of Veterinary Emergency and Critical Care (San Antonio, Tex.: 2001) 24 (2): 174–81.

Leon, Guillermo, Laura Sanchez, Andres Hernandez, Mauren Villalta, Maria Herrera, Alvaro Segura, Ricardo Estrada, and Jose Maria Gutierrez. 2011. “Immune Response Towards Snake Venoms.” Inflammation & Allergy - Drug Targets 10 (5): 381–98.

Lervik, Jessica B., Inger Lilliehöök, and Jan HM Frendin. 2010. “Clinical and Biochemical Changes in 53 Swedish Dogs Bitten by the European Adder - Vipera Berus.” Acta Veterinaria Scandinavica 52 (1): 26.

Lewis, P. F. 1994. “Common Tiger Snake Envenomation in Dogs and Mice—relationship between the Amount of Venom Injected and the Onset of Clinical Signs.” Australian Veterinary Journal 71 (5): 130–32.

Lobetti, R. G., and K. Joubert. 2004. “Retrospective Study of Snake Envenomation in 155 Dogs from the Onderstepoort Area of South Africa.” Journal of the South African Veterinary Association 75 (4): 169–72.

Lomonte, Bruno, Andrej Tarkowski, and Lars \AA Hanson. 1993. “Host Response toBothrops Asper Snake Venom.” Inflammation 17 (2): 93–105.

Luna, K. P. O., MB da Silva, and V. R. A. Pereira. 2011. “Clinical and Immunological Aspects of Envenomations by Bothrops Snakes.” Journal of Venomous Animals and Toxins Including Tropical Diseases 17.

Lund, Heidi S., Veronica Kristiansen, Anna V. Eggertsdóttir, Ellen Skancke, and Birgit Ranheim. 2013. “Adverse Reactions to Equine-Derived F(ab’) 2 -Antivenin in 54 Dogs Envenomated by \iVipera Berus Berus: Adverse Reactions to Equine-Derived Antivenin in Dogs.” \iJournal of Veterinary Emergency and Critical Care. p532-37

Lu, Q., J. M. Clemetson, and K. J. Clemetson. 2005. “Snake Venoms and Hemostasis.” Journal of Thrombosis and Haemostasis 3 (8): 1791–99.

MacDonald, Ellen, Julian White, and Bart J. Currie. 2008. “Current Use of Australian Snake Antivenoms and Frequency of Immediate-Type Hypersensitivity Reactions and Anaphylaxis.” Medical Journal of Australia 188 (8): 473–76.

Manufacturer data. 2007. CSL Snake Venom Detection Kit (SVDK). Available on http://www.csl.com.au/docs/92/398/SVDK_Product_Leaflet,0.pdf

Maria Fernandes, Cristina, Stella Regina Zamuner, Juliana Pavan Zuliani, Alexandra Rucavado, José Maria Gutiérrez, and Catarina de Fátima Pereira Teixeira. 2006. “Inflammatory Effects of BaP1 a Metalloproteinase Isolated from Bothrops Asper Snake Venom: Leukocyte Recruitment and Release of Cytokines.” Toxicon 47 (5): 549–59.

Markland, Francis S. 1998. “Snake Venoms and the Hemostatic System.” Toxicon 36 (12): 1749–1800.

McCown, Jennifer L., Kirsten L. Cooke, Rita M. Hanel, Galin L. Jones, and Richard C. Hill. 2009. “Effect of Antivenin Dose on Outcome from Crotalid Envenomation: 218 Dogs (1988-2006).” Journal of Veterinary Emergency and Critical Care (San Antonio, Tex.: 2001) 19 (6): 603–10.

Mebs, D., M. Ehrenfeld, and Y. Samejima. 1983. “Local Necrotizing Effect of Snake Venoms on Skin and Muscle: Relationship to Serum Creatine Kinase.” Toxicon: Official Journal of the International Society on Toxinology 21 (3): 393–404.

Meier J, Stocker KF. 1995. Biology and distribution of venomous snakes of medical importance and the composition of snake venoms. In: Meier J, White J, editors. Handbook of Clinical Toxicology of Animal Venoms and Poisons. CRC Press, Florida:367–412

Menaldo, Danilo L., Carolina P. Bernardes, Juliana C. Pereira, Denise S.C. Silveira, Carla C.N. Mamede, Leonilda Stanziola, Fábio de Oliveira, Luciana S. Pereira-Crott, Lúcia H. Faccioli, and Suely V. Sampaio. 2013. “Effects of Two Serine Proteases from Bothrops Pirajai Snake Venom on the Complement System and the Inflammatory Response.” International Immunopharmacology 15 (4): 764–71.

Milton M Kanashiro, Rita de Cássia M. Escocard. 2002. “Biochemical and Biological Properties of Phospholipases A2 from Bothrops Atrox Snake Venom.” Biochemical Pharmacology 64 (7): 1179–86.

Mirtschin, P. J., P. Masci, D. C. Paton, and T. Kuchel. 1998. “Snake Bites Recorded by Veterinary Practices in Australia.” Australian Veterinary Journal 76 (3): 195–98.

Mora, Javier, Rodrigo Mora, Bruno Lomonte, and José María Gutiérrez. 2008. “Effects of Bothrops Asper Snake Venom on Lymphatic Vessels: Insights into a Hidden Aspect of Envenomation.” PLoS Neglected Tropical Diseases 2 (10).

Moreira, Vanessa, Maria Cristina Dos-Santos, Neide Galvão Nascimento, Henrique Borges da Silva, Cristina Maria Fernandes, Maria Regina D’Império Lima, and Catarina Teixeira. 2012.

"Local Inflammatory Events Induced by Bothrops Atrox Snake Venom and the Release of Distinct Classes of Inflammatory Mediators." Toxicon 60 (1): 12–20.

Moreira, Vanessa, Stella Regina Zamuner, John L. Wallace, and Catarina de Fátima Pereira Teixeira. 2007. "Bothrops Jararaca and Crotalus Durissus Terrificus Venoms Elicit Distinct Responses Regarding to Production of Prostaglandins E2 and D2, and Expression of Cyclooxygenases." Toxicon 49 (5): 615–24.

Morita, Takashi. 2004. "Use of Snake Venom Inhibitors in Studies of the Function and Tertiary Structure of Coagulation Factors." International Journal of Hematology 79 (2): 123–29.

Morrison, J. J., J. H. Pearn, J. Covacevich, and J. Nixon. 1983. "Can Australians Identify Snakes?" The Medical Journal of Australia 2 (2): 66–70.

Murata, H., N. Shimada, and M. Yoshioka. 2004. "Current Research on Acute Phase Proteins in Veterinary Diagnosis: An Overview." The Veterinary Journal 168 (1): 28–40.

Nakada, K., F. Nakada, E. Ito, and F. Inoue. 1984. "Quantification of Myonecrosis and Comparison of Necrotic Activity of Snake Venoms by Determination of Creatine Phosphokinase Activity in Mice Sera." Toxicon: Official Journal of the International Society on Toxinology 22 (6): 921–30.

Netea, M. G., B. J. Kullberg, and J. W. Van der Meer. 2000. "Circulating Cytokines as Mediators of Fever." Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America 31 Suppl 5 (October): S178–84.

Neumann, Stephan, Franz-Josef Kaup, and Sonja Scheulen. 2010. "Interleukin-6 (IL-6) Serum Concentrations in Dogs with Hepatitis and Hepatic Tumours Compared with Those with Extra-Hepatic Inflammation and Tumours." Comparative Clinical Pathology 21 (5): 539–44.

Ong, R. K. C., K. Swindells, and C. S. Mansfield. 2010. "Prospective Determination of the Specificity of a Commercial Snake Venom Detection Kit in Urine Samples from Dogs and Cats." Australian Veterinary Journal 88 (6): 222–24.

Paul William E. Fundamental Immunology. 1989 Second Edition. United Stated: Raven Press. P647-651. ISBN: 0-88167-491-5

Petricevich, V. L., C. F. Teixeira, D. V. Tambourgi, and J. M. Gutiérrez. 2000. "Increments in Serum Cytokine and Nitric Oxide Levels in Mice Injected with Bothrops Asper and Bothrops Jararaca Snake Venoms." Toxicon: Official Journal of the International Society on Toxinology 38 (9): 1253–66.

Picolo, G., M. Chacur, J. M. Gutiérrez, C. F. P. Teixeira, and Y. Cury. 2002. "Evaluation of Antivenoms in the Neutralization of Hyperalgesia and Edema Induced by Bothrops Jararaca and Bothrops Asper Snake Venoms." Brazilian Journal of Medical and Biological Research 35 (10): 1221–28.

Pidde-Queiroz, Giselle, Maria de Fátima Furtado, Carlos F. Filgueiras, Lucas A. Pessoa, Mônica Spadafora-Ferreira, Carmen W. van den Berg, and Denise V. Tambourgi. 2010. "Human Complement Activation and Anaphylatoxins Generation Induced by Snake Venom Toxins from Bothrops Genus." Molecular Immunology 47 (16): 2537–44.

Preiser, J. C., D. Schmartz, P. Van der Linden, J. Content, P. Vanden Bussche, W. Buurman, W. Sebald, E. Dupont, M. R. Pinsky, and J. L. Vincent. 1991. “Interleukin-6 Administration Has No Acute Hemodynamic or Hematologic Effect in the Dog.” Cytokine 3 (1): 1–4.

Premawardhena, A. P., C. E. de Silva, M. M. Fonseka, S. B. Gunatilake, and H. J. de Silva. 1999. “Low Dose Subcutaneous Adrenaline to Prevent Acute Adverse Reactions to Antivenom Serum in People Bitten by Snakes: Randomised, Placebo Controlled Trial.” BMJ (Clinical Research Ed.) 318 (7190): 1041–43.

Queensland museum website. Available on: http://www.sciencentre.qm.qld.gov.au/Find+out+about/Animals+of+Queensland/Reptiles/Snakes/Common+and+dangerous+species/Western+Brown+Snake#.WAJI54RGyWc (consulted in October 2016)

Rau, Stefanie, Barbara Kohn, Constance Richter, Nora Fenske, Helmut Küchenhoff, Katrin Hartmann, Stefan Härtle, Bernd Kaspers, and Johannes Hirschberger. 2007. “Plasma Interleukin-6 Response Is Predictive for Severity and Mortality in Canine Systemic Inflammatory Response Syndrome and Sepsis.” Veterinary Clinical Pathology 36 (3): 253–60.

Remick, D. G., G. Bolgos, S. Copeland, and J. Siddiqui. 2005. “Role of Interleukin-6 in Mortality from and Physiologic Response to Sepsis.” Infection and Immunity 73 (5): 2751–57.

Riedemann, Niels C., Thomas A. Neff, Ren-Feng Guo, Kurt D. Bernacki, Ines J. Laudes, J. Vidya Sarma, John D. Lambris, and Peter A. Ward. 2003. “Protective Effects of IL-6 Blockade in Sepsis Are Linked to Reduced C5a Receptor Expression.” The Journal of Immunology 170 (1): 503–7.

Rodrigues, F. 2004. “The Complement System Is Involved in Acute Inflammation but Not in the Hemorrhage Produced by a Bothrops Atrox Snake Venom Low Molecular Mass Proteinase.” Molecular Immunology 40 (16): 1149–56.

Rosing, J., J. W. Govers-Riemslag, L. Yukelson, and G. Tans. 2001. “Factor V Activation and Inactivation by Venom Proteases.” Haemostasis 31 (3-6): 241–46. doi:48069.

Rosing, J., and G. Tans. 1992. “Structural and Functional Properties of Snake Venom Prothrombin Activators.” Toxicon: Official Journal of the International Society on Toxinology 30 (12): 1515–27.

Rucavado, Alexandra, Teresa Escalante, Catarina F. P. Teixeira, Cristina María Fernándes, Cecilia Díaz, and José María Gutiérrez. 2002. “Increments in Cytokines and Matrix Metalloproteinases in Skeletal Muscle after Injection of Tissue-Damaging Toxins from the Venom of the Snake Bothrops Asper.” Mediators of Inflammation 11 (2): 121–28.

Santhosh, M. Sebastin, M. Shanmuga Sundaram, K. Sunitha, K. Kemparaju, and K. S. Girish. 2013. “Viper Venom-Induced Oxidative Stress and Activation of Inflammatory Cytokines: A Therapeutic Approach for Overlooked Issues of Snakebite Management.” Inflammation Research 62 (7): 721–31.

Saper, Clifford B., and Christopher D. Breder. 1994. “The Neurologic Basis of Fever.” New England Journal of Medicine 330 (26): 1880–86.

Scheller, Jürgen, Athena Chalaris, Dirk Schmidt-Arras, and Stefan Rose-John. 2011. “The pro- and Anti-Inflammatory Properties of the Cytokine Interleukin-6.” \iBiochimica et Biophysica Acta (BBA) - Molecular Cell Research 1813 (5): 878–88.

Segev, G., D.g. Ohad, A. Shipov, P. H. Kass, and I. Aroch. 2008. “Cardiac Arrhythmias and Serum Cardiac Troponins in Vipera Palaestinae Envenomation in Dogs.” Journal of Veterinary Internal Medicine 22 (1): 106–13.

Segev, G., A. Shipov, E. Klement, S. Harrus, P. Kass, and I. Aroch. 2004. “Vipera Palaestinae Envenomation in 327 Dogs: A Retrospective Cohort Study and Analysis of Risk Factors for Mortality.” Toxicon: Official Journal of the International Society on Toxinology 43 (6): 691–99.

Shea, G. M. 2000. “The Distribution and Identification of Dangerously Venomous Australian Terrestrial Snakes.” Australian Veterinary Journal 77 (12): 791–98.

Simon, Hans-Uwe. 2003. “Neutrophil Apoptosis Pathways and Their Modifications in Inflammation.” Immunological Reviews 193 (1): 101–10.

Song, Ruhui, Junhwan Kim, Dohyeon Yu, Chul Park, and Jinho Park. 2012. “Kinetics of IL-6 and TNF-α Changes in a Canine Model of Sepsis Induced by Endotoxin.” Veterinary Immunology and Immunopathology 146 (2): 143–49.

Stone, Shelley F., Geoffrey K. Isbister, Seyed Shahmy, Fahim Mohamed, Chandana Abeysinghe, Harendra Karunathilake, Ariaranee Ariaratnam, Tamara E. Jacoby-Alner, Claire L. Cotterell, and Simon G. A. Brown. 2013. “Immune Response to Snake Envenoming and Treatment with Antivenom; Complement Activation, Cytokine Production and Mast Cell Degranulation.” Edited by Robert A. Harrison. PLoS Neglected Tropical Diseases 7 (7): e2326.

Sutherland, S. K., A. R. Coulter, and R. D. Harris. 1979. “Rationalisation of First-Aid Measures for Elapid Snakebite.” Lancet (London, England) 1 (8109): 183–85.

Sutton, N. M., N. Bates, and A. Campbell. 2011. “Canine Adder Bites in the UK: A Retrospective Study of Cases Reported to the Veterinary Poisons Information Service.” Veterinary Record 169 (23): 607–607.

Szold, O., R. Ben-Abraham, A. A. Weinbroum, T. E. Englender, D. Ovadia, M. Sorkine, C. Bon, R. Flaison, and P. Sorkine. 2001. “Antagonization of TNF Attenuates Systemic Hemodynamic Manifestations of Envenomation in a Rat Model of Vipera Aspis Snakebite.” Intensive Care Medicine 27 (5): 884–88.

Tambourgi, D. V., M. C. dos Santos, M. de F. Furtado, M. C. de Freitas, W. D. da Silva, and T. L. Kipnis. 1994. “Pro-Inflammatory Activities in Elapid Snake Venoms.” British Journal of Pharmacology 112 (3): 723–27.

Tans, G., and J. Rosing. 2001. “Snake Venom Activators of Factor X: An Overview.” Haemostasis 31 (3-6): 225–33.

Teixeira, Catarina, Yara Cury, Vanessa Moreira, Gisele Picolo, and Fernando Chaves. 2009. “Inflammation Induced by Bothrops Asper Venom.” Toxicon 54 (1): 67–76.

Teixeira, C. F. P., S. R. Zamunér, J. P. Zuliani, C. M. Fernandes, M. A. Cruz-Hofling, I. Fernandes, F. Chaves, and J. M. Gutiérrez. 2003. “Neutrophils Do Not Contribute to Local

Tissue Damage, but Play a Key Role in Skeletal Muscle Regeneration, in Mice Injected with Bothrops Asper Snake Venom." Muscle & Nerve 28 (4): 449–59.

Van den Enden MD, Erwin. 2015. Illustrated Lecture Notes on Tropical Medicine-Snakes-Clinic. Health and Medical Publishing Group. Available on: http://itg.author-e.eu/Generated/pubx/173/snakes/clinic.htm

Voronov, E., R. N. Apte, and S. Sofer. 1999. "The systemic inflammatory response syndrome related to the release of cytokines following severe envenomation". Journal of Venomous Animals and Toxins 5 (1): 5–33.

Warrell, David A. 2010. "Snake Bite." The Lancet 375 (9708): 77–88.

White, J. 1998. "Envenoming and Antivenom Use in Australia." Toxicon: Official Journal of the International Society on Toxinology 36 (11): 1483–92.

White, Julian. 2005. "Snake Venoms and Coagulopathy." Toxicon: Official Journal of the International Society on Toxinology 45 (8): 951–67.

White Julian, 2013. A clinician's guide to Australian Venomous bites and Stings. Australia: BioCSL. p51-168. ISBN: 9780646579986

White Julian. 2014. CSL Antivenom Handbook. Available on http://www.toxinology.com/generic_static_files/cslavh_antivenom_tiger.html

Williams, David J., Simon D. Jensen, Bill Nimorakiotakis, Reinhold Müller, and Kenneth D. Winkel. 2007. "Antivenom Use, Premedication and Early Adverse Reactions in the Management of Snake Bites in Rural Papua New Guinea." Toxicon 49 (6): 780–92.

Winkler, E., M. Chovers, S. Almog, S. Pri-Chen, M. Rotenberg, M. Tirosh, D. Ezra, and H. Halkin. 1993. "Decreased Serum Cholesterol Level after Snake Bite (Vipera Palaestinae) as a Marker of Severity of Envenomation." The Journal of Laboratory and Clinical Medicine 121 (6): 774–78.

World Health Organization. 2010. WHO guidelines for the production Control and Regulation of Snake Antivenom Immunoglobulins. Available on: http://www.who.int/bloodproducts/snake_antivenoms/SnakeAntivenomGuideline.pdf

Yamashita, Kazuto, Toru Fujinaga, Toru Miyamoto, Mitsuyoshi Hagio, Yasuharu Izumisawa, and Tadao Kotani. 1994. "Canine Acute Phase Response : Relationship between Serum Cytokine Activity and Acute Phase Protein in Dogs." Journal of Veterinary Medical Science 56 (3): 487–92.

Zamuner, Stella Regina, Juliana Pavan Zuliani, Cristina Maria Fernandes, José Maria Gutiérrez, and Catarina de Fátima Pereira Teixeira. 2005. "Inflammation Induced by Bothrops Asper Venom: Release of Proinflammatory Cytokines and Eicosanoids, and Role of Adhesion Molecules in Leukocyte Infiltration." Toxicon 46 (7): 806–13.

Zamuner, Stella R, José Maria Gutiérrez, Marcelo N Muscará, Simone A Teixeira, and Catarina F. P Teixeira. 2001. "Bothrops Asper and Bothrops Jararaca Snake Venoms Trigger Microbicidal Functions of Peritoneal Leukocytes in Vivo." Toxicon 39 (10): 1505–13.

Zamuner, Stella R., and Catarina F. P. Teixeira. 2002. “Cell Adhesion Molecules Involved in the Leukocyte Recruitment Induced by Venom of the Snake Bothrops Jararaca.” Mediators of Inflammation 11 (6): 351–57.

Zingali, R. B., M. Jandrot-Perrus, M. C. Guillin, and C. Bon. 1993. “Bothrojaracin, a New Thrombin Inhibitor Isolated from Bothrops Jararaca Venom: Characterization and Mechanism of Thrombin Inhibition.” Biochemistry 32 (40): 10794–802.

Zuliani, Juliana P., Cristina M. Fernandes, Stella R. Zamuner, José M. Gutiérrez, and Catarina F.P. Teixeira. 2005. “Inflammatory Events Induced by Lys-49 and Asp-49 Phospholipases A2 Isolated from Bothrops Asper Snake Venom: Role of Catalytic Activity.” Toxicon 45 (3): 335–46.

Nom, Prénom: **COSTET Juliette**

TITLE: **INFLAMMATORY RESPONSE TO NATURALLY OCCURING TIGER SNAKE ENVENOMATION IN DOGS, WITH A SPECIAL EMPHASIS ON IL-6**

ABSTRACT:

Tiger snakes are the most common snake species implicated in dogs' snake envenomation in Australia. Clinical signs, biochemical and haematological parameters have been studied but the systemic inflammatory response to snakebite in dogs is poorly documented. Antivenom administration could further trigger a venom induced inflammatory response.

Ten dogs presented at the University of Melbourne Veterinary Hospital (UMVH) for snake envenomation were enrolled in the study. Clinical signs, snakebite data and clinicopathological parameters were recorded. An inflammatory marker, interleukin-6 (IL-6), was determined in dog plasma at different times after admission.

Snakebite caused mild clinical and biochemical signs and variable degrees of coagulopathy. IL-6 was indictable for all control cases. For the affected dogs, IL-6 levels widely ranges from 0 pg/mL to 260 pg/mL. IL-6 concentration was markedly influenced by time and was significantly increased after antivenom administration.

A systemic inflammatory response naturally occurs in dogs envenomed by tiger snake, possibly exacerbated by antivenom administration. Further clinical research including a larger population and additional inflammatory markers is warranted to better understand the inflammatory response to snakebite in dogs and to consider new therapeutic avenue.

KEYWORDS: Interleukin-6, Envenomation, Tiger snake, Elapid, Dog, Immune response, Inflammation

TITRE: **LA REPONSE INFLAMMATOIRE SYTEMIQUE DES CHIENS SUITE AUX ENVENIMATIONS NATURELLES PAR LE SERPENT TIGRE. Etude particulière de l'interleukine-6.**

RESUME:

Le serpent tigre est l'espèce de serpent la plus fréquemment rencontrée en Australie. Bien que certaines études aient publié des résultats sur les signes cliniques et les paramètres biochimiques et hématologiques les plus fréquents, il existe très peu de données bibliographiques sur la réponse inflammatoire suite aux morsures ophidiennes, et plus particulièrement en ce qui concerne cette espèce de serpent. L'administration d'antivenin pourrait potentiellement exacerber la réponse inflammatoire induite par le venin lui-même.

Dix chiens admis à l'Hôpital Universitaire Vétérinaire de Melbourne suite à une envenimation ophidienne ont été inclus dans l'étude. L'anamnèse, les commémoratifs, les signes cliniques, et certains paramètres biochimiques et hématologiques ont été enregistrés. Nous avons quantifié l'interleukine-6 (IL-6) plasmatique, marqueur de l'inflammation, grâce au kit quantikine standard, à différents temps après admission.

Les morsures ophidiennes par des serpents tigres sont à l'origine de signes cliniques et biochimiques modérés ainsi que de troubles de la coagulation d'intensité variable. L'IL-6 plasmatique était indétectable chez tous les chiens témoins. Chez les chiens envenimés, les concentrations plasmatiques en IL-6 variaient fortement en fonction des individus, avec des valeurs extrêmes comprises entre 0 à 260 pg/mL. La concentration en IL-6 était significativement plus élevée après l'administration d'antivenin par rapport à l'admission et aux cas témoins.

Une réponse inflammatoire systémique est présente chez les chiens envenimés par des serpents tigres et une exacerbation de cette réponse immunitaire par l'antivenin ne peut être exclue. D'autres études portant sur un plus grand nombre de chien et incluant d'autres marqueurs de l'inflammation seraient souhaitables afin de mieux caractériser la réponse inflammatoire systémique des chiens aux morsures par des serpents tigres et d'adapter le traitement.

MOTS-CLES: Interleukine-6, Envenimation, Serpent tigre, Elapidé, chien, Réponse immunitaire, Inflammation