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SNAKEBITE COAGULOPATHY: CONTROVERSIES IN UNDERSTANDING AND MANAGEMENT

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Professor Senaka William Bibile

Senaka William Bibile was born on the 13th of February 1920, 97 years ago at Kathaluwa, in the Southern Province. Prof Bibile received his primary and secondary education at Trinity College, Kandy and graduated from the University of Colombo with first class honours at the final MBBS with distinctions and gold medals in Medicine and Surgery. He obtained his Ph.D. from the University of Edinburgh for research on steroid hormones. On his return to Sri Lanka, he was appointed as the Professor of Pharmacology at the Colombo Medical Faculty.

He was the first Dean of the Faculty of Medicine at Peradeniya from 1967 to 1970. Prof Bibile was also instrumental in initiating the idea of building the Peradeniya Teaching Hospital and played a key role in establishing the State Pharmaceuticals Corporation. Perhaps most notably,

Professor Bibile was the founder of Sri Lanka's national drugs policy, which was used as a model for development of policies on rational pharmaceutical use in other countries as well. He played the leading role in developing a rational pharmaceutical policy which ensured drugs at an affordable cost to impoverished people.

The content of this oration focuses on snake envenoming and antivenoms, particularly in relation to Sri Lanka. It will highlight the irrational use of pharmaceutical products related to snake envenoming without proper scientific evidence and key shortcomings of the pharmaceutical policy in our country.

Burden of snakebite

Approximately 400 thousand to 1.8 million people are bitten by snakes worldwide each year [1]. The highest burden has been reported in south Asia and south-east Asia followed by sub-Saharan Africa. Over 100 thousand snakebites are reported from the south and south east Asian region. Annual, global snakebite death toll is over 100,000 [1]. India has the highest number of deaths due to snake envenoming, which exceeds 10,000 per year. Until recently, the World Health Organization classified snakebites as a neglected tropical disease. This suggests that snake envenoming is among the most neglected of tropical diseases.

As a tropical country with an agricultural

economy, Sri Lanka has a significant burden of snake envenoming. Based on hospital data, Sri Lanka's annual health bulletin reports an average of 40,000 snakebites and 100 deaths in the island annually [2,3]. However, a recent community based study reported over 80,000 snakebites, 40,000 envenomings and 500 fatalities per year[4]. The discrepancy between the two data sources highlights the neglected nature of this tropical disease and the intensity of the burden.

Pathophysiology of snake envenoming

Snakebite can manifest as a number of different clinical syndromes. Coagulopathy is the commonest, and also the leading cause of death following any snakebite in the world[5]. Snake venom acts on the human clotting cascade, and either activates or inhibits a specific reaction in the pathway. Secondly, snake venom blocks neuro transmission at synapses, leading to neurotoxicity[6,7]. Hemorrhagic effects of venom are expressed as a result of increasing vascular permeability by hemorrhagic toxins. Some venoms destroy skeletal muscle membrane, leading to myotoxicity[8,9]. Renal injury occurs due to either direct renal damage by the toxins or secondarily due to myotoxicity[8]. Local effects of envenoming are common and sometimes life threatening. These vary from mild local pain to extensive tissue necrosis, which could lead to amputations[10].

When a person is bitten by a snake, venom is injected either subcutaneously or deep intra-muscularly. The local effects of venom around the site of bite begin immediately. Venom is absorbed into the central compartment and enters the circulation via lymphatics. Blockage of lymphatics, delaying venom absorption, is a novel approach in the field of envenoming research[11]. The toxins that act on the clotting cascade react immediately after they reach the circulation[5]. Therefore, coagulopathy starts sooner than later following a snakebite. Similarly, toxins acting on the vascular endothelium also begin their hemorrhagic effects immediately. Based on these factors, antivenom should be administered within the first few minutes after envenoming in order to prevent coagulopathy and hemorrhagic effects. In contrast, the development of other peripheral toxic effects such as myotoxicity, renal injury and neurotoxicity, takes time. Toxins have to be distributed to these peripheral sites and the timing depends on many other regional tissue factors.

Snake antivenom

Snake antivenom was first discovered in 1894 in France, by Albert Calmette. Even after a century of its first clinical use, antivenom is considered as the mainstay of treating snake envenoming. Antivenoms are polyclonal immunoglobulins raised against one or more species of snake venom. They are produced by a host animal, commonly horses. There are three-forms of immunoglobulins used as antivenoms: whole IgG, $(Fab')_2$ fragments and small Fab fragments[12]. All three-forms have their own pharmacodynamic and pharmacokinetics properties. Mechanism of action of antivenom against snake venom is simple, an antigen-antibody binding reaction, preventing the active-site interaction of the antigen with target tissue.

Controversies on coagulopathy following snake envenoming

Many controversies exist on the events following snake envenoming, particularly on coagulopathy. Identity of some of the venomous snakes is indistinct and understanding of snake-venom induced coagulopathy is not well established.

Clinical and hematological spectrums of some Sri Lankan snake envenoming are poorly understood. Diagnosis of envenoming and coagulopathy are essential for the management. However, there is no well-established scientific basis for the diagnosis and monitoring of coagulopathy. Sri Lanka has used Indian-snake antivenoms for more than 4 decades without establishing their efficacy or effectiveness. Some clinicians use fresh-frozen plasma (FFP) for coagulopathy following snake envenoming[3,13]. It is important to search for scientific evidence to use FFP for snakebite coagulopathy. Many books and research papers have described disseminated intravascular coagulation following snake envenoming[14], but there is a notable lack of evidence for this concept. Therefore accurate, bedside diagnosis of envenoming is critical to decide antivenom treatment and prevent systemic envenoming. Lack of such tests has worsened the outcomes of envenoming.

Snakebite coagulopathy

Snake venom has two types of coagulant toxins. Pro-coagulant toxins activate the clotting cascade, and anti-coagulant toxins inhibit the clotting cascade. Pro-coagulant toxicity is far more common than anti-coagulant toxicity[5]. All Sri Lankan viper venoms are composed of pro-coagulant toxins. The action of pro-coagulant toxins on the clotting cascade is different inside a test tube and within the circulation of humans. When snake venom is added to a sample of blood in a test tube, it clots immediately. In contrast to that, when a person is bitten by a snake, there is no extensive clot formation within the circulation[15]. Otherwise, patients should frequently present to the hospital with ischemic strokes, myocardial infractions, massive pulmonary embolism and multiple thrombotic events. But this does not happen.

When the human clotting cascade is exposed to toxins, toxins activate the clotting cascade. Activation of the clotting pathway utilizes the clotting factors and consumes them[5]. The actions of the anti-clotting pathway, fibrinolytic pathway and other factors in live humans prevent extensive clot formation within the vasculature. Utilization of clotting factors ends with little or no clotting factors in the circulation. Therefore, snake envenoming leads to consumption coagulopathy, correctly termed as venom-induced consumption coagulopathy or VICC[15,16]. Even-though the venom activates the clotting pathway, the end result looks like anti-clotting in nature.

Russell's viper venom is well known to contain factor X and V activators. Activation of factors X and V converts pro-thrombin in to thrombin and fibrinogen in to fibrin[5]. Generation of thrombin activates many positive feedback loops and subsequently triggers all clotting factors. Saw-scaled viper venom is known for activation of prothrombin[15]. Activation of prothrombin triggers the clotting pathway. Our current experiments indicate that hump-nosed viper venom contains thrombin-like enzymes. Toxins cleave fibrinogen into fibrinopeptides and make an unstable fibrin clot. The action of toxin on a single reaction in a clotting cascade triggers the consumption of all clotting factors, leading to coagulopathy[5].

When factor consumption occurs within the vasculature, the patient expresses a wide range of clinical features. Frequently, patients do not express clinically detectable bleeding or hemorrhages. Lack of clinical signs could also be possible even with no detectable fibrinogen in the circulation[17,18,19]. Venom-induced consumption coagulopathy is clinically expressed as gum bleeding, hematemesis, hemoptysis, hematuria, and bleeding per

rectum or bleeding in to internal organs such as brain[17,20,21].

Hump-nosed viper envenoming is the leading cause of snake bite in Sri Lanka[1]. Although the bites frequently cause no systemic effects, coagulopathy is the commonest systemic effect[10]. A descriptive study was conducted to explore the concentrations of clotting factors following hump-nosed viper envenoming. All cases were confirmed by the detection of hump-nosed viper venom in the circulation. Of 80 patients, none exhibited clinical features of coagulopathy. PT/INR and aPTT were slightly elevated and close to the values of a patients who were being treated with warfarin. Further, hump-nosed viper envenoming led to reduced levels of factors V, VIII and fibrinogen[22]. The 20 minute Whole blood clotting time is the commonest test that is used to detect coagulopathy of snake envenoming. However, mild coagulopathy following hump-nosed viper envenoming cannot be detected by Whole blood clotting time 20[22].

Investigating the venoms and clinical characterization of three species of hump-nosed vipers was critically important. A fatal case of *Hypnale zara* envenoming reported that the patient had developed severe coagulopathy and acute kidney injury, which led to death[23]. This case report was directed for further investigation of the species-specific toxic effects of hump-nosed viper venom. Analysis of hundreds of confirmed cases revealed that all three species of hump-nosed vipers can cause similar fatal envenoming. Investigation of venoms from all three species showed closely similar venom profiles and toxic effects which concluded that envenoming by all three species of hump-nosed viper could lead to similar and severe clinical effects[10,23,24].

A study about the in-vivo effects of hump-nosed viper venom using a mice model was helpful to understand the venom induced organ damage. The study confirmed closely related lethal, hemorrhagic and necrotic effects in all three venoms. Further, the same study explained many organ-specific toxic effects of hump-nosed viper venom, including the kidney, liver, lung and gastro-intestinal tract[25].

No toxins have been isolated from hump-nosed viper venom. The results of initial experiments reveal that this venom acts like thrombin, and hence is classified as snake-venom thrombin-like enzymes that cleave fibrinogen into fibrinopetides. Isolation and characterization of the coagulant toxin of hump-nosed viper venom is particularly important. The Malayan pit viper, *Calloselasma rhodostoma*, which inhabits the Malayasian peninsula, is genetically the closest species to hump-nosed vipers. Ancrod, a fibrinolytic toxin[26], has been isolated from Malayan pit viper venom. Phase III clinical trials are now in progress to establish the effect of Ancrod for the treatment for ischemic stroke and other thromboembolic diseases. The coagulant toxin in Sri Lankan hump-nosed viper venom is expected to have closely similar biochemical properties to Ancrod.

Details of Sri Lankan saw scaled viper coagulopathy has not been well documented. Saw scaled vipers are widely distributed in the Asian and African continents, and are the leading cause of snake bite induced deaths in the world. Research on saw scaled viper envenoming in Sri Lanka was actually hindered by the war due to inaccessibility to this area. The study showed that 92% of confirmed saw scaled viper bites led to coagulopathy, with a few cases of spontaneous bleeding[20].

Russell's viper is the challenge of Asian snake envenoming. Coagulopathy is the commonest, and also the leading, cause of death in Russell's viper envenoming.

confirmed the importance of abdominal pain as a predictor of development of coagulopathy[21].

Effectiveness of antivenom for snakebite

Table 1. The median, interquartile range (IQR) and range of the minimum (Factors I, II, V, VII, VIII, IX, X) or maximum (PT/INR, aPTT, D-Dimer) factor concentrations/clotting times measured for the 147 patients during their hospital admission.

Factor Concentration or Clotting times	Normal Range	Median	Interquartile range	Range
Prothrombin time (PT) sec	9-14	69	36 – 180	12 – 180
INR	0.9 – 1.3	6.8	3.7 – >13	1.3 – >13
aPTT (s)	25 – 35	180	91.3 – 180	29 – 180
Fibrinogen (g/L)	2 – 4	< 0.01	<0.01 – 0.9	<0.01 – 3
Factor II (%)	70 – 120	60	49 – 74	10 – 120
Factor V (%)	70 – 120	< 5	<5 – 4	<5 – 61
Factor VII (%)	70 – 120	63	43 – 123	15 – 1203
Factor VIII (%)	70 – 120	24	10 – 41	1 – 335
Factor IX (%)	70 – 120	88	66 – 109	2 – 860

In these viper bites potent factor X and V activating toxins, activate and consume almost all clotting factors in the circulation, resulting in factor deficiency[15,19].

Not all patients develop systemic effects after Russell's viper bites. Exploring the prediction of development of coagulopathy based on some non-specific clinical features at an early stage is important. A study explored the correlation between abdominal pain and the future development of coagulopathy and renal injury. The association between abdominal pain and coagulopathy was strong, and the study

coagulopathy

Nature of clotting times and factor concentrations following Russell's viper envenoming have not yet been studied in detail. The key question is how depleted clotting factors recover after antivenom treatment. Patients with Russell's viper envenoming (147 proven cases) were recruited for the study. Serial serum and plasma samples were collected and analyzed for venom concentrations, clotting time tests and factor levels (Table 1)[19].

Russell's viper envenoming causes prolonged PT/INR and aPTT. The reason of prolonged clotting time could be simply

explained by the long time taken to make a clot in these two tests. The reason for taking a long time is explained by the results in the latter part of the table 1. These patients have no fibrinogen, no factor V, and low factors X and VIII. The lack of many important factors in the clotting pathway has led to prolonged clotting times. The lack of clotting factors is due to consumption of factors after the activation of the clotting pathway by pro-coagulant toxins in Russell's viper venom. The excessive d-dimer levels reflect massive fibrinolysis[19].

After the initial dose of antivenom, the venom concentration has dropped to zero and remained almost undetectable. The minimum fibrinogen concentration was reported immediately before antivenom administration. Even-though the venom is not present after antivenom administration, the recovery of fibrinogen took more than 24 hours. The recovery of INR and aPTT has taken over 48 hours. Similarly, factors X and V have taken 24 and 48 hours to return to normal. The recovery of clotting parameters takes 24-48 hours even after the sufficient neutralization of venom in the circulation[19].

This explains the potential and limitations of antivenom administration for coagulopathy. Antivenom remains in the central circulation, binds with venom and neutralizes its toxic effects. But antivenom plays no role in the restoration of depleted fibrinogen and other clotting factors. Restoration of clotting factors depends on the synthesis of these factors by the liver[5]. It takes time, and administration of massive doses of antivenom is not justifiable and does not present a scientific basis. Antivenom is critical to neutralise the free venom in the circulation and stop the ongoing factor consumption. But recovery of clotting parameters should not be expected by administration of repeated

doses of antivenom. Importantly, patients may be in coagulopathic situations such as no fibrinogen and factor levels, for up to 48 hours after the initial dose of antivenom.

Diagnosis and monitoring of coagulopathy

Current guidelines discourage treatment until the patient develops clinical features of coagulopathy. Two factors suggest this may be unwise. First, many patients have coagulopathy in their circulation despite lacking clinically detectable signs and symptoms[18,22]. Secondly, delaying antivenom treatment might worsen the outcomes of all systemic effects including coagulopathy. The Whole Blood Clotting Test 20 (WBCT20) is used in many parts of the world to diagnose coagulopathy. The sensitivity and specificity of WBCT20 has been debated over many decades. Based on the results of clotting parameters, as you have seen, the appropriate tests to diagnose coagulopathy include PT/ INR, aPTT, fibrinogen, and Factor X or V. Point of care PT/INR devices have no place in the detection of envenoming-induced coagulopathy due to false positive results produced by snake venom toxins in the patient's blood. Not all hospitals are equipped with PT/INR, aPTT or fibrinogen assays. As envenoming is prevalent in rural settings, developing an inexpensive simple test is important.

A study was conducted to explore the sensitivity and specificity of WBCT20 for Russell's viper envenoming. WBCT20 was performed by routine hospital staff and PT/INR assessed using the same blood sample to explore correlation. Patients who had normal INR had positive WBCT20 and an approximately equal number of patients who had very high INR had negative WBCT20. This study explained the low sensitivity and specify of WBCT20 in the

detection of Russell's viper coagulopathy. Not only that, false negative WBCT20 had also delayed administration of anitivenom[27].

In the ward, WBCT20 is usually performed by hospital staff without a standard procedure. Most of the time, the test is performed in a used penicillin bottle, with the bottle being shaken every few minutes to check whether the sample is clotted. For this reason, another study was conducted after standardisation of WBCT20 by trained research assistants, using specialised glass tubes. The results showed that the majority of patients with positive WBCT20 also had elevated INR. The study indicated good sensitivity of WBCT20 in the detection of Russell's viper coagulopathy, but with the standardized test procedure, using a clean, previously unused glass test tube, 2 ml of venous blood, kept at room temperature for 20 minutes with no disturbance[28].

Snake antivenom for coagulopathy

Antivenom is considered the gold standard treatment for snakebite. However, a detailed mechanism of action of snake antivenom is essential to understand its potential and its limitations. Intravenously administered polyclonal immunoglobulins bind with snake venom in the circulation. Binding of antibodies blocks the active site interactions between the toxins and target tissues. Secondly, antivenom-venom complexes remain in the circulation due to massive molecular mass and block the distribution of toxins to the peripheral tissues. Thirdly, the production of antivenom-venom complexes enhances the elimination of toxins from the circulation through the reticular-endothelial system[12]. Early administration of antivenom is thus important to stop the distribution of venom to peripheral sites.

Over one hundred different antivenoms are used for coagulopathic snake envenoming worldwide. Despite its therapeutic potential, antivenom can cause life-threatening anaphylaxis[29]. Many deaths still occur due to antivenom, but are poorly reported for a variety of reasons. Therefore, exploring the available evidence to establish the effect of antivenom is crucial.

According to the pyramid of evidence-based practice of medicine, expert opinions and case reports are considered as the basic level of evidence that support the practice. However, in certain locations management of snakebite is based on expert opinions than recommendations. On the other side of the spectrum, randomized controlled trials, systematics reviews, especially Cochrane Systematic reviews, provide collective evidence for or against specific clinical interventions. Cochrane systematic reviews explore all studies published on a particular intervention for a specific disease and summarize the collective effect of the intervention.

Cochrane Systematic Review was conducted to explore the published evidence of antivenom for venom-induced consumption coagulopathy. All published and ongoing placebo-controlled randomized trials of antivenom for venom induced consumption coagulopathy (VICC) in the cited databases from 1947 onwards were screened. After screening over 6000 abstracts, 35 full-texts were selected to check eligibility. Except for one ongoing study, all 34 other studies were excluded with valid reasons. There is one ongoing placebo randomized trial of antivenom for North American copperhead envenoming. Due to a lack of placebo controlled randomized evidence; no studies were available to conduct a meta-analysis. Cochrane reviews concluded that there is a lack of placebo controlled randomized evidence to give antivenom for VICC[5].

Exploration of the results published in randomized comparative trials on antivenom for VICC is important in clinical practice. There were 25 randomized comparative trials published on antivenoms for VICC. These trials compared 2 or 3 antivenoms, two or more different doses of antivenoms, and antivenom with heparin for snake coagulopathy. Conclusions from the studies are 2 or 3 antivenoms are equally effective for VICC, different doses of antivenom are nearly equally effective for VICC, antivenom alone and antivenom plus heparin are equally effective for VICC[5,30]. Equal effectiveness of all different tests arms could be interpreted as equal ineffectiveness due to lack of a control group.

Therefore, randomized comparative trials were insufficient to establish the effectiveness of antivenom for VICC. Due to strong belief of the effectiveness of antivenom for VICC, no study has investigated the effect with placebo.

Two interesting observational studies provided useful evidence on the use of antivenom for VICC. A group of saw-scaled viper bite patients had been treated with the appropriate antivenom, while another group had not received antivenom treatment for various reasons. Researchers analyzed the recovery of fibrinogen in the two groups. The results clearly showed that fibrinogen recovers within 48 hours when antivenom has been administered but remains low for weeks in the absence of antivenom. This observational study provides valuable piece of evidence to support the effectiveness of antivenom for VICC for saw-scaled viper envenoming[31].

The other study was on Australian Elapid snake envenoming. Recovery of INR was compared in two groups of patients, one of which had received antivenom during the first 6 hours after the bite and other at some time after 6 hours of the bite. The recovery of INR showed no difference between the two groups[15]. The effectiveness of antivenom for VICC thus appears to vary among different snake species, and this needs to be taken into account when planning treatment.

Indian antivenom used in Sri Lankan snake envenoming

Indian Polyvalent antivenom have been used for Sri Lankan snake envenoming over the past 50 years or so, manufactured mainly by the companies VINS and BHARAT. These polyvalent antivenoms have been developed against the four major venomous snakes in India: Cobra, Indian krait, saw scaled viper and Russell's viper. These antivenoms are used in Sri Lanka based on the assumption that the venoms of the Indian snakes are identical to those of the Sri Lankan snakes that bear the same scientific names. But in fact the taxonomy of these snakes is still at a fairly primitive stage, lacking robust molecular studies.

There are many differences in the venom and clinical profiles of envenoming between the Indian and Sri Lankan snakes bearing the same name. No laboratory or properly designed clinical trials have been conducted to explore the efficacy and effectiveness of Indian antivenoms for Sri Lankan snake envenoming. The extremely high rate of reactions, between 43 and 81%, is the key issue in Indian antivenoms[32,33,34,35]. Indeed, it appears that a substantial proportion of Sri Lankan snakebite fatalities are caused by reactions to the antivenom rather than to the effects of the venom itself. Based on all the known facts, there is an urgent need to develop antivenoms for Sri Lankan's venomous snakes, including hump-nosed vipers, which account for over half of all reported bites. As a result, Peradeniya medical faculty has developed a polyvelant

snake antivenom for Sri Lankan snakes including hump-nosed vipers. This collaborative project is being led by Prof. Indika Gawarammara and the Institute of Clodomiro Picado in Costa-Rica. Currently, a clinical trial is in progress to establish the clinical effectiveness and safety of this new antivenom.

As the efficacy of Indian antivenom not valuated in Sri Lanka, several batches of VINS and BHARAT antivenom were investigated to explore inter-batch variation and several vials from each batch were analyzed to examine variation within each batch (Table 2).

Table 2. The dry powder weight of antivenom, the percentage of proteins per mg of antivenom and the amount of protein per vial in VINS and BHARAT antivenoms.

Vial Type	Dry powder wt. (mg/vial)	Protein %	Protein content/ vial (mg)
VINS2000	800	30.7%	246
VINS2008	614	26.4%	162
VINS2010[*]	658 (535 – 815)	31.6% (23 – 36)	201 (157 – 238)
VINS2011	801	40.9%	328
VINS2012	798	62.7%	500
BHARAT2011[*]	433 (155 – 510)	25.2% (23 – 27)	109 (39 – 125)

* The median value and range is reported for VINS2010 and BHARAT2011 based on testing 10 vials from each batch.

Table 2 shows the variation of dry powder weight, and the quantity of proteins in each vial of antivenom. The fraction of proteins indicates the active ingredient, immunoglobulins. It is obvious that, the protein content varies widely between batches. The effect of antivenom depends on the dose of proteins injected into the patient. The administration of 10 vials from the VINS 2012 batch delivers 5g of proteins to the patient. But the administration of 10 vials from the BHARAT 2011 batch delivers only 1 g of protein to the patient. The difference is 5 times between two manufacturers and even between two batches from the same manufacturer. Both antivenoms bind to all four Sri Lankan venoms, but their binding abilities vary grossly between manufacturers, batches and even within the same batch. Both antivenoms neutralize the coagulant an effect of Russell's and saw scaled viper venoms, but their efficacies show a very wide variation[36].

The efficacy of VINS and BHARAT antivenoms for various toxic effects are shown in the table 3. In general, the study highlighted that BHARAT antivenom is less effective for coagulopathy of Sri Lankan Russell's and saw-scaled viper venoms. After many decades, this is the first time a detailed investigation has been conducted on Indian antivenoms for Sri Lankan snake venom. There are numerous brands for a given generic preparation. The actual facts about the efficacies and effectiveness of each one do not get published in journals.

This is a single example for what Prof Bibile understood 45 years ago. Having a scientific pharmaceutical testing institute is an urgent need for Sri Lanka. However, this is a routine practice in many developed countries before they use drugs in clinical settings.

Table 3. Efficacy and venom binding of VINS and BHARAT antivenoms for various toxic effects.

Toxic effect	Snake venom	VINS antivenom	BHARAT antivenom
Neurotoxicity	Common krait	Efficacious	Not efficacious
	Cobra	Less efficacious	Less efficacious
	Russell's viper	Not efficacious	Not efficacious
Coagulopathy (Hemotoxicity)	Russell's viper	Efficacious	Less efficacious
	Saw scaled viper	Efficacious	Less efficacious
Venom-antivenom binding	Russell's viper	Good binding	Poor binding
	Saw scaled viper	Good binding	Good binding
	Cobra	Poor binding	Good binding
	Common krait	Good binding	Poor binding

Consumption of clotting factors by venom is the key pathology in snake envenoming. But it is very clear that antivenom plays no role on the restoration of depleted clotting factors in the circulation. Whether FFP can restore the clotting factors in snake coagulopathy remains a doubt. Introducing additional clotting factors that can be consumed by the unneutralized venom in the circulation may worsen the coagulopathy. This resembles pouring fuel on the fire[5].

This hypothesis was checked on Australian Elapid envenoming and it was concluded that FFP speeds up the recovery of VICC[37]. However, there is no evidence to support the use FFP for Russell's viper envenoming. A randomized controlled trial to test the effect of adding FFP was conducted. One group received 20 vials of antivenom and other group had 10 vials of antivenom and 4 packs of FFP. The time to recover INR < 2 was the primary outcome of the study. There were no differences of recovery of fibrinogen, factor V, factor X and d-dimer concentration between the two groups. The study concluded lack of any difference in the recovery of coagulopathy in the two groups [38].

Snakebite is listed as a cause for disseminated intravascular coagulation (DIC) in many textbooks and research papers. Recently published this study uncovered the difference between DIC and VICC on snake envenoming.

Snake envenoming does not cause DIC. The differences are, there is no microthrombi formation, end organ failure, and other system involvement in VICC, but all these are associated with DIC. Snake envenoming is a rapid-onset process that spontaneously recovers within 24 hours and initiated by activation of any reaction of clotting cascade[16]. But DIC is a relatively long process. It takes many days to recover and is triggered by activation of tissue factor pathway. Therefore pathological process

termed DIC does not occur in snake envenoming.

Reappearance of venom in the circulation after its initial neutralization is a phenomenon described in many studies over past 4 decades[39,40].

The phenomenon has been explained by many hypotheses including slow absorption of venom from the bite site, dissociation of venom-antivenom complexes, and the mismatch of venom and antivenom pharmacokinetics. The question was whether this recurrence of venom actually worsens the coagulopathy and other toxic effects. So we designed a study to explore the association of venom recurrence and the worsening of coagulopathy.

Recovery of fibrinogen and INR between a group of Russell's viper envenomed patients with no venom recurrence and patients with venom recurrence were compared. There was no difference in the recovery of fibrinogen in these two groups[41]. The study concluded that the recurrence of venom in the circulation was not associated with worsening of coagulopathy. This raised many queries on the mechanism and the purpose of venom recurrence in the circulation. What then, is this recurrence? How is the venom reappearing in the circulation? Why is the recurrence of venom not toxic, worsening the coagulopathy?

The results of the experiments demonstrated that recurrence of venom in the circulation was not in fact free Russell's viper venom, but that the Enzyme immuno assay detects antivenom-bound venom as free venom in the circulation. Detection of venom in patients' blood is achieved by binding of venom to the coated antibodies in an ELISA plate. Bound venom is detected by enzyme-linked secondary antibodies and finally by the development of color in the positive

well[41]. When the patient does have antivenom, the venom molecules are fully covered. Completely bound venom cannot bind to the ELISA plate and hence expresses as no venom in the circulation. When the venom molecules are partially covered by the antivenom, probably at small doses of antivenom, partially bound venom can still bind to the ELISA plate and express as positive venom. Series of different immunological experiments confirmed this venom recurrence phenomenon as detection of partially bound venom in ELISA[42]. This partially bound venom in not biologically active: its active epitopes are blocked by the antivenom, which is why the recurrence of venom is not associated with the worsening of coaguopathy.

Pharmacokinetics of antivenom

True recurrence of coagulopathy is, however, reported from North American Rattlesnake envenoming. True recurrence of coagulopathy, is explained by the mismatch of venom- antivenom pharmacokinetics. Rattlesnake antivenom is aFab antivenom, which is the smallest-sized antivenom, and hence undergoes rapid renal clearance. After the lapse of 7 days no antivenom remains in the circulation, but venom can be slowly absorbed to the circulation from the bite site once the antivenom is completely cleared. Therefore, investigation of the pharmacokinetics of antivenom is crucial to determine the initial dose and the repetition of the doses [12].

The Indian antivenoms used for Sri Lankan snake envenoming involve Fab_2 antibodies, which are larger than Rattlesnake antivenom. Detailed studies of antivenom pharmacokinetics have not been conducted globally. The very few studies published are based on fewer than 10 patients. I carried out a study on Indian antivenom pharmacokinetics to analyze all basic and

derived pharmacokinetic parameters and, for the first time, data were mathematically modelled to apply to the population to predict the exact behavior of antivenom following intravenous administration. The study concluded that Fab_2 antivenoms have a bi-exponential disposition in the tissue compartments, with rapid initial half-life and prolonged elimination half-life [43].

Diagnosis of envenoming

Many snakebites are 'dry', involving no venom, and the treatment of a dry bite with antivenom can carry its own risks. Therefore, when a patient presents with snakebite it is vital to know whether he or she has actually been envenomed. Currently, envenoming is diagnosed based on the patient's history, the availability of the specimen that is suspected of causing the bite, clinical features of envenoming, and abnormal laboratory results.

The early detection of venom in the circulation is important for the administration of antivenom to prevent the development of irreversible peripheral effects such as neurotoxicity. Detection of snake venom in patients' blood is difficult, complex, time consuming and not available except in few laboratories in the world[44]. But the confirmation of envenomation is critical for prompt diagnosis and treatment. The time between venom entering into the circulation and development of peripheral toxic effects is the available narrow window for venom detection. Administration of antivenom during that time traps the venom in circulation and stop further toxic effects.

There is a common snake-venom toxin found in almost all venomous snakes, which is called phospholipase 2 or PLA_2. The detection of PLA_2 is not complex: it is quite similar to our pancreatic PLA_2, though not identical. Detection of elevated PLA_2 activity in patients' blood could indicate the presence of venom. The detection PLA_2 activity in the early period of envenoming would be sufficient to decide antivenom administration to prevent peripheral toxic effects.

Samples from 5 major venomous snakes were investigated. All envenomed patients had significantly higher PLA2 activity compared to non-envenomed patients. Not only that, PLA2 activity correlated well with the actually measured snake venom concentrations. The study concluded that PLA2 activity mirrored the presence of venom in the circulation [44]. With the availability of polyvalent antivenom, the identity of the assailant snake becomes less important: what we need to know is whether the patient has been envenomed. The confirmation of venom in the circulation is sufficient to administer antivenom. Following the publication of this work in the Journal Nature Scientific Reports in 2014, studies to identify the possibility of using this test to detect envenoming by other types of snakes has been initiated by several other countries.

The second phase of the clinical study is currently underway. The target is to develop a rapid, sensitive, affordable, venom-detection device for bedside use. This would be in the form of a lateral flow immune chromatographic device similar to the commonly used urine hCG strip. But the development of such test will clearly indicate envenoming at the bed side and facilitate the early administration of antivenom, hopefully even before the patient develops clinical features, thus preventing unnecessary antivenom treatment and all the reactions and deaths induced by the antivenoms.

Conclusions

Recovery from coagulopathy following antivenom treatment is slow, taking 24 to 48 hours. Therefore, repeated doses of antivenom at short intervals is not indicated. Properly conducted WBCT20 has good sensitivity for the detection of Russell's viper coagulopathy. The efficacy of Indian antivenoms vary widely and doubtful for envenoming by Sri Lankan snakes. The published evidence is insufficient to establish the effectiveness of antivenom for coagulopathy. Administration of FFP does not speed up recovery from Russell's viper coagulopathy. Snake envenoming leads to venom-induced consumption coagulopathy, but not disseminated intravascular coagulation. The development of a diagnostic test for the presence of venom promises to revolutionize the treatment of snakebite in the future.

At present, Missions to Mars have been successfully implemented. Genes are modified to treat serious inherited diseases. Thousands of new medications are discovered each year. But antivenom, which was first invented a century ago, revolutionizing the treatment of snakebites remains the treatment of choice to this day. It is, however, just an antigen-antibody reaction. Nevertheless, about a million of people suffer due to snakebite each year. Scientists and clinicians are battling against a powerful evolutionary tool called, venom. The treatment appears to be simple, antivenom to neutralize venom. But the consequences of envenoming and its treatment with antivenom are yet to be explored in depth.

Indian antivenom was used in Sri Lanka during the past 50 years even without analyzing the quantity of proteins in the vial. Despite using these antivenoms to treat thousands of victims annually, even simple tests of efficacy were not conducted. It is accepted that antivenom remains the only option of treatment for envenoming. But as Professor Senaka William Bibile himself raised in 1971. "Provide correct scientific information relating to the medications used on patients", in the field of snakebite, it is a pity that, we have to this day, failed to heed his advice.

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