ANTISNAKE VENOM SERUM (ASVS)

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ABSTRACT
Snakebite is a common medical emergency in the tropical region causing multisystemic involvement. Treatment with antisnake venom (ASV) is life saving yet dangerous. ASV usage remains a very risky, because the serum being heterologous and liable to cause sensitivity reactions in occasional patients. The world of ASV production is currently a gloomy place to visit. The methodology of antivenom serum preparation has not advanced much since its discovery; procedures of immunisation and purification have been improved using modern technology. The improved purification of antivenom by using immunoglobulin fragments has leads to increased tolerance, efficiency and safety of antivenom. The future of immunotherapy seems to be good. This article gives prominence to ASV production, purification and quality control, dose and administration of ASV, incidence of adverse reactions and its proposed treatment.

1. INTRODUCTION:

ANTIVENOM
Antivenom (or antivenin or antivenene) is a biological product used in the treatment of venomous bites or stings. Antivenom is created by injecting a small amount of the targeted venom into an animal such as a horse, sheep, goat, or rabbit, the subject animal will undergo an immune response to the venom, producing antibodies against the venom's active molecule which can then be harvested from the animal's blood and used to treat envenomation. Internationally, antivenoms must conform to the standards of Pharmacopoeia and the World Health Organization (WHO).

TERMINOLOGY
The name antivenin comes from the French word venin, meaning venom, and historically antivenin was predominant around the world. In 1981, the World Health Organization (WHO) decided that the preferred terminology in the English language would be "venom" and "antivenom" rather than "venin/antivenin" or "venen/antivenene".

The first antivenom for snakes (called an anti-ophidic serum) was developed by Albert Calmette, a French scientist of the Pasteur Institute working at its Indochine branch in 1895, against the Indian Cobra (Naja naja). Vital Brazil, a Brazilian scientist, developed in 1901 the first monovalent and polyvalent antivenoms for central and South American Crotalus, Bothrops and Elaps genera, as well as for certain species of venomous spiders, scorpions, and frogs. They were all developed in a Brazilian institution, the Instituto Butantan, located in Sao Paulo, Brazil.

In the U.S. the only approved antivenom for pit viper (rattlesnake, copperhead and water moccasin) snakebite is based on a purified product made in sheep known as CroFab. It was approved by the FDA in October, 2000. U.S. coral snake antivenom is no longer manufactured, and remaining stocks of in-date antivenom for coral snakebite will expire in the fall of 2009 leaving the U.S. without Coral snake antivenom at this time (January, 2009). Efforts are being made to obtain approval for coral snake antivenom produced in Mexico which would work against U.S. coral snakebite, but such approval remains speculative. In the absence of antivenom, all coral snakebite should be treated in a hospital by elective endotracheal intubation and mechanical ventilation until the effects of coral snake neurotoxins abate. It is important to remember that respiratory paralysis in coral snakebite can occur suddenly, often up to 12 or more hours after the bite, so intubation and ventilation should be employed in anticipation of respiratory failure and not after it occurs, when it may be too late.

The venoms of snakes, rich in varied components, have not yet been used successfully for vaccinations of humans. However, antivenoms remain the only specific therapy for envenoming. Although the methodology of
antivenom preparation has not advanced much since its discovery, procedures of purification and modes of utilisation have changed considerably.

The principle of antivenom is based on that of vaccines, developed by Louis Pasteur; however, instead of inducing immunity in the patient directly, it is induced in a host animal and the hyperimmunized serum is transfused into the patient. The majority of antivenoms (including all snake antivenoms) are administered intravenously; however, stonefish and redback spider antivenoms are given intramuscularly. The intramuscular route has been questioned in some situations as not uniformly effective4.

Antivenoms bind to and neutralize the venom, halting further damage, but do not reverse damage already done. Thus, they should be administered as soon as possible after the venom has been injected. Sheep are generally used in preference over horses now, however, as the potential for an adverse immunological response in humans from sheep-derived antibodies is generally somewhat less than that from horse-derived antibodies.

2. Antivenom classification

- Monovalent (when they are effective against a given species venom).
- Polyvalent (when they are effective against a range of species, or several different species at the same time).

Availability of Antivenoms:

- 1. Spiders
- 2. Acarids
- 3. Insects
- 4. Scorpions
- 5. Marine animals
- 6. Snakes

3. Natural and acquired immunity

Although individuals can vary in their physiopathological response and sensitivity to animal venoms, there is no natural immunity to them in humans. Some ophiophagic animals are immune to the venoms produced by some species of venomous snakes, by the presence of antihemorrhagic and antineurotoxic factors in their blood. These animals include King snakes, opossums, mongooses and hedgehogs. It is quite possible to immunize a person directly with small and graded doses of venom rather than an animal. However, unlike a vaccination against disease which must only produce a latent immunity that can be reused in case of infection, to neutralize a sudden and large dose of venom requires maintaining a high level of circulating antibody (a hyperimmunized state), through repeated venom injections (typically every 21 days). The long-term health effects of this process have not been studied. For some large snakes, the total amount of antibody it is possible to maintain in one human being is not enough to neutralize one envenomation. Further, cytotoxic venom components can cause pain and minor scarring at the immunization site. Finally, the resistance is specific to the particular venom used; maintaining resistance to a variety of venoms requires multiple monthly venom injections.

4. Composition and toxicity of venoms

Venoms are a complex mixture of substances, mainly proteins, produced by a specialised sero-mucous gland and inoculated by a specialised apparatus. In snakes, the injection apparatus comprises modified teeth (fangs) which permit the injection of venom under pressure into the tissues.

4.1. Composition of snake venoms

Classically, substances of snake venoms are divided into several series of components:

- Toxins of the neuro-muscular system, the most important of which are the presynaptic neurotoxins that possess often a phospholipasic activity and the postsynaptic curare-like neurotoxins.
- Toxins which bind cell membrane receptors inducing a cytolysis.
- Haemorrhagins, that causes damage to the vascular endothelium.
- Factors acting on the blood coagulation, numerous, but largely dominated by the thrombin-like enzymes that convert fibrinogen in fibrinopeptides.
- Enzymes, possessing various structures and activities but showing generally a reduced toxicity compared with neurotoxins.
Because some molecules are present in two or three categories, we propose another category based on pharmacokinetic properties and directly applicable in antivenomous immunotherapy. Substances present in venoms can be divided into two groups: Toxins and enzymes.

Toxins are proteins of variable molecular weight, but generally less than 30 kDa. They have target specific receptors, mostly on cell membranes. The specificity of toxins can be neurological, cardiovascular, muscular or not differentiated according to the anatomical distribution of recognised receptors. The pharmacological effect is proportional to the quantity of introduced toxin and to the quantity of receptors. It is considered as dose-dependent.

Enzymes are also proteins but their molecular weight is generally higher than those of the toxins. Their catalytic properties, which distinguish them from toxins, have two major consequences. First, the product of degradation even if toxic has, in principle, no immunogenic property for the envenomed organism. Secondly, pharmacological effects depend more on the time of the enzymatic reaction cycle than on the initial quantity of enzymes. Pharmacological effects are therefore mainly time-dependent. Venoms of *Viperidae* and *Crotalidae* are rich in enzymes.

### 4.2. Variability of venoms

Variations in venoms are due to both different concentrations and the biochemical differences of certain venom components. Consequently, an antivenom prepared from a venom will be able to present different specificity against venoms of individuals of the same species but of different origin. The mixture of venoms from various populations of snakes is a precaution that has appeared insufficient, since the WHO has been brought in to create a collaborating centre on venom research, retaining eight venomous species of medical importance.

### 5. Availability of antivenoms

Antivenoms have been developed for the venoms associated with the following animals:

<table>
<thead>
<tr>
<th>Antivenom</th>
<th>Species</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyvalent snake antivenom</td>
<td>South American Rattlesnake <em>Crotalus durissus</em> and fer-de-lance <em>Bothrops asper</em></td>
<td>South America</td>
</tr>
<tr>
<td>Polyvalent snake antivenom</td>
<td>Saw-scaled Viper <em>Echis carinatus</em>, Russell's Viper <em>Daboia russelli</em>, Spectacled Cobra <em>Naja naja</em>, Common Krait <em>Bungarus caeruleus</em></td>
<td>India</td>
</tr>
<tr>
<td>Death adder antivenom</td>
<td>Death adder</td>
<td>Australia</td>
</tr>
<tr>
<td>Taipan antivenom</td>
<td>Taipan</td>
<td>Australia</td>
</tr>
<tr>
<td>Black snake antivenom</td>
<td><em>Pseudechis spp.</em></td>
<td>Australia</td>
</tr>
<tr>
<td>Sea snake antivenom</td>
<td>Sea snakes</td>
<td>Australia</td>
</tr>
<tr>
<td>Vipera tab</td>
<td><em>Vipera spp.</em></td>
<td>USA</td>
</tr>
<tr>
<td>Antivenom</td>
<td>Species</td>
<td>Country</td>
</tr>
<tr>
<td>----------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Polyvalent crotalid antivenin</td>
<td>North American pit vipers (all rattlesnakes, copperheads, and cottonmouths)</td>
<td>USA</td>
</tr>
<tr>
<td>(CroFab - Crotalidae Polyvalent Immune Fab (Ovine))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antielapidico</td>
<td>Coral snakes</td>
<td>Brazil</td>
</tr>
<tr>
<td>Soro anti-elapidico</td>
<td>Coral snakes</td>
<td>Brazil</td>
</tr>
<tr>
<td>SAIMR echis antivenom</td>
<td>Boomslang</td>
<td>South Africa</td>
</tr>
<tr>
<td>SAIMR polyvalent antivenom</td>
<td>Mambas, Cobras, Rinkhalses, Puff adders (Unsuitable small adders: B. worthingtoni, B. atropos, B. caudalis, B. cornuta, B. heraldica, B. inornata, B. peringueyi, B. schneideri, B. xeropaga)</td>
<td>South Africa</td>
</tr>
<tr>
<td>Pan-American serum</td>
<td>Coral snakes</td>
<td>Costa Rica</td>
</tr>
<tr>
<td>Anticoral</td>
<td>Coral snakes</td>
<td>Costa Rica</td>
</tr>
<tr>
<td>Antimicruru</td>
<td>Coral snakes</td>
<td>Argentina</td>
</tr>
<tr>
<td>Soro antibotropicocrotalico</td>
<td>Pit vipers and rattlesnakes</td>
<td>Brazil</td>
</tr>
</tbody>
</table>

5.1. Antivenom sources

The following groups assist in locating antivenoms:

- **Africa**: South African Institute for Medical Research, Johannesburg, Republic of South Africa.
- **Asia**: Haffkine Biopharmaceutical Corporation, Parel, Mumbai, India.
- **Australia**: CSL Limited, Parkville, Victoria.
- **Brazil**: Instituto Butantan, São Paulo
- **Costa Rica**: Instituto Clodomiro Picado, San José
- **Mexico**: Instituto Bioclon
- **USA**: The Antivenom Index is a joint project of the Association of Zoos and Aquariums and the American Association of Poison Control Centres; They maintain a website to help locate rare antivenoms.[2]
- **USA, Colorado**: Poisindex central office in Denver, Colorado, USA (1-800-332-3073).
- **USA, Miami, Florida**: The Miami-Dade Fire Rescue Antivenom Bank: Emergency: 1-786-336-6600 available 24 hours. A list of available antivenoms and more information about the bank is available at [3].
- **Pakistan**: National Institute of Health (NIH), Islamabad Produces polyvalent antivenom.

6. THE MANUFACTURE OF ANTIVENOM

The product is prepared from hyperimmunised equines against the venoms of the four most commonly encountered poisonous snakes.
Plasma obtained from the hyperimmunized equines is enzyme refined, purified and concentrated. Each ml. of the reconstituted Anti Snake Venom Serum neutralizes not less than the following quantities of standard venoms when tested in white mice.

<table>
<thead>
<tr>
<th>Snake</th>
<th>Quantity (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobra</td>
<td>0.6</td>
</tr>
<tr>
<td>Common Krait</td>
<td>0.45</td>
</tr>
<tr>
<td>Russell's Viper</td>
<td>0.6</td>
</tr>
<tr>
<td>Saw Scaled Viper</td>
<td>0.45</td>
</tr>
</tbody>
</table>

### 6.1. Animal Immunisation

Inoculation of the crude venom provides the highest titre; however, whole venom is often badly tolerated by the animal. As a result, toxoids have been prepared by biological detoxification of the venom which preserves its immunogenicity. The most usual toxoiding procedures are complexing with an aldehyde such as formalin or glutaraldehyde. The venom preparation used for immunisation is often associated with an adjuvant. The precise role of the adjuvant has not been elucidated but it is thought that it acts in a controlled manner by decreasing the rate of release of venom and thus stimulating further the immunological response. The most commonly used adjuvants are Freund's, bentonite, aluminium hydroxide and sodium alginate. The immunisation protocol depends on the toxicity and the immunogenicity of the venom, the animal model used for the immunisation and the quality of the immune response of the animal. The optimum dose for immunisation is generally obtained by trial and error to obtain a sufficient antibody titre. Ten to fifty injections performed over a period of 3 to 15 months may be necessary to obtain hyperimmunisation. When a suitable antibody titre is reached, the blood of the animal is collected into an appropriate anticoagulant (e.g. sodium citrate), purified, treated and then ampouled for marketing, either in liquid form or, more rarely, as a lyophilised preparation. The preferred animal for immunisation is the horse because of the large blood volume available, but other species can also be used.

Antivenom is monospecific if only one venom is used, or polyspecific if the immune animal receives a pool of venoms from several different species. The choice of either type depends on a range of considerations. In principle, monospecific antivenom is more efficient for treating envenomation by the corresponding species. Consequently, polyspecific antivenom obtained from a horse immunised with both venoms gives better protection. In some cases, it also appears that a pool of venoms may act synergistically to induce the optimum immunological response. Lists of antivenom producers are regularly updated.

### 6.2. Purification of Antivenom and Quality Control

Although crude serum was originally used for therapy, for many years antivenom has been purified by successive steps in order to reduce anaphylactic reactions. After elimination of the cellular elements by centrifugation, non-immune proteins and especially the albumin are discarded by precipitation with ammonium sulphate. The immunoglobulins are digested using either pepsin to produce F(ab')2, or papain to produce a smaller F(ab) fragment. Before final packaging, the antivenom is submitted to various controls: bacteriological culture in an appropriate medium, toxicological studies using animal inoculation for pyrogenicity and immunological studies to measure the neutralising efficiency of the product. The first two types of tests are well established, but the last one is not yet fully standardised. The first immunological verification of immunogenicity is usually carried out by gel immunoelectrophoresis in gel or by Western blot analysis. The serum should ideally react with all the protein fractions of the venom after electrophoretic separation. It is thus possible to compare both the antisera obtained from different animals and that produced by different manufactures. Cross-reactivity with other venomous species can also be detected using these techniques. The process of standardisation of antivenoms necessitates checking their neutralising activities, their specificity and the stability of these characteristics. Both in vivo and in vitro tests are currently used to verify the neutralising efficiency of the antivenom. The principle of all these tests consists of measuring the decrease of venom toxic effects, lethality or particular biological activities, in relation to increasing concentrations of antivenom.
In vivo tests. Mixtures are prepared with variable proportions of venom and antivenom and injected to the animal to evaluate the effect of the antivenom on the lethality or on a particular biological activity. Usually a definite quantity of venom (specified as number of LD$_{50}$) vs variable quantities of antivenom is used, but the test could also be performed using a defined quantity of antivenom vs increasing quantities of venom. The mixture is incubated at 37°C for 15 or 30 min before administration to the animal, generally by the intravenous route for evaluation of lethality. Animals are observed over 24 or 48 h. The median effective dose (ED$_{50}$) of the antivenom is measured and the quantity of venom neutralised by the volume of antivenom used in the mixture is calculated. This quantity is often expressed as number of LD$_{50}$ per ml of antivenom, but it is preferable to express it in mg of dry venom per unit of volume because the LD$_{50}$ expression is related to the animal used for the titration and not to human or other animal species.

In human envenomation, the inoculation of venom, the administration of antivenom, obviously precedes at varying time intervals. Sequential procedures and other parameters have been used for evaluation, such as the FTL (fatal time limit) and the LMT (last mortality time). The sequential procedures are suitable for toxin-type venoms, because they can evaluate both the affinity of antivenom for the antigens and the antigen extraction capacity of antivenom. They are less adapted to enzyme-type venoms, because the penetration of venom induces a chain of reactions evolving to their own account once begun, even after the neutralisation of enzymes. Using other parameters, one observes, on the one hand, that the necessary quantities of antivenom to neutralise the lethality of the venom increase when the administration of the antivenom approaches the FTL and, on the other hand, that it no longer becomes possible to protect all individuals when this period is close to the FTL.

However, the incubation of venom/antivenom mixtures remains the most frequently used method for the determination of the antivenom neutralising potential. It is possible to establish a neutralisation curve of the antivenom according to increasing quantities of venom. If the curve is linear, each venom dose being neutralised by a proportional quantity of antivenom, it can be concluded that only one toxic antigen is responsible for the toxicity, or that other toxic components, if they exist, are neutralised efficiently. Normally, the curve is not linear, suggesting that a toxic component with poor antigenicity induces only low levels of neutralising antibodies. Alternatively a toxic component present already in large quantities may be a poor antigen.

Whichever procedure is used, statistical calculations are carried out in the same manner as for the determination of the LD$_{50}$.

In vitro tests. These permit the evaluation of a particular biological activity of the venom and its neutralisation after mixing the venom with the antivenom. The neutralisation of the haemolytic activity of venom, tested on the red cells of rabbit, sheep or human seems correlated with the neutralisation of lethal activity in mice in some venoms. The fibrinolytic activity is evaluated on sheep or ox fibrin plates obtained by fresh plasma coagulation. Proteolytic activity is estimated by the action of the venom on various substrates, generally casein. The main problem can be to define a reproducible technique. However, it appears that testing the capacity of antivenom to neutralise each individualised toxin is better, especially for neurotoxins and myotoxins.

Quantitative ELISA tests can also compete usefully with in vivo and in vitro assays. Microtitration plates are coated by venoms used for the preparation of the antivenom. The latter is incubated at different concentrations in the microtitration plate and the presence of antigen–antibody complexes is revealed by an antibody/anti-immunoglobulin reagent conjugated to an enzyme. The coloured reaction, depending on the concentration of antivenom, is obtained after addition of the enzyme substrate and can be compared to ED$_{50}$ or to the number of neutralised LD$_{50}$. It can also be used in the same manner with the toxic fraction or the major toxin of venom. This type of test can be used only if significant correlation between in vivo assays and ELISA are recorded.

In practice, the standardisation of antivenom will be improved by using as wide a range as possible of both in vivo and in vitro tests. The choice will depend on the individual properties of each venom.

### Procedures used in Manufacture of Antivenom

| (1) Animal immunisation (Horse) |
• Toxoid preparation

• Adjuvant utilisation

• Repeated administration (increasing doses)

• Serum sampling and venom neutralisation titration

(2) Concentration of neutralising fraction

• Elimination of contaminated cells by centrifugation

• Precipitation of γ-globulins using ammonium sulphate

• Enzymatic cleavage of IgG

• Fc coagulation by heat (57°)

• Elimination of denatured protein by centrifugation or filtration

• Purification of γ-globulins with higher concentration of ammonium sulphate

• Dialysis to remove salts

• Lipid remove by adsorption on aluminium hydroxide

• Purification by chromatography

(3) Control and standardisation tests

• Titration (neutralising potential)

• Stability

• Specificity (precipitating potential)

• Control tests (bacteriology-pyrogenic substances)

7. Future of Immunotherapy

The efficiency and safety of immunotherapy can be improved as follows.

7.1. Improvement in the manufacturing process

The quality and type of venoms used for immunisation must be rigorously supervised\(^2\). The venoms used for the preparation of antivenom should both contain a great number of components and present a weak toxicity. The possibility of producing the venom in vitro, from venom gland cell culture remains at experimental Stage\(^2\). The used of isolated toxic fractions could increase the protective titre of the antivenom and reduce the quantity of antivenom administered, while avoiding the formation of antibodies against non-toxic proteins\(^2\). The cloning of toxins of Hydrophidae or Crotalidae\(^2\) may be a first step in the production of new antivenom for therapy. The use of more immunogenic recombinant variants is a possibility, but is also something for the future\(^2\). Immunisation procedures need to be improved. Besides the preparation of toxoids using various physicochemical processes\(^2\) or molecular techniques, other new procedures have been proposed. Venom has been incorporated into stabilised sphingomyelin–cholesterol liposomes. The administration of such preparations, through injection or oral route\(^2\) is followed by a rapid increase of specific protective antibodies\(^2\). The subcutaneous route appears to be the most efficient, due to improved presentation of the antigens to tissue macrophages. The slow liberation of antigens from the liposomes and their distribution via lymphatic routes allows progressive stimulation of the immune system.
7.2. Improvement in the quality of antivenom
Several techniques should be proposed.
Affinity purification of antivenom enables the preservation of specific antibody only. The process is performed
on gels to which venom antigens are bound chemically28. Proteins which do not react with the gel are eluted,
while specific antibodies are retained. The latter are then retrieved from the column after a modification of the
pH which separates antibody from antigen. Antibodies thus collected are specific to the venom and should
therefore possess a higher neutralising activity. Theoretically they permit smaller doses to be used in therapy,
thus reducing the incidence and severity of adverse reactions. On the other hand, the process increases the cost
of the antivenom.

Other Species than horses can be used to produce therapeutic antivenom. It should thus be possible to avoid
allergic reactions due to exposure to horse sera.
Sheep have been recommended by some authors29 because they do not produce the highly allergenic IgG. Some
commercial antivenoms30 are already prepared using sheep (anti-Crotalus durissus terrificus, anti-Vipera
russelli). However, non conventional communicable agents (NCCA) present in sheep may pose problems for
antivenom production. The transmission modes of some of these agents remain unknown, so it may be unwise
to recommend the use of such antivenom. Some antivenins are prepared in goats (anti-Bungarus multicinctus,
anti-Calloselasma rhodostoma, anti-Vipera latastii), or from the rabbit (anti-Naja naja). In this particular context
of NCCA, the possibility of obtaining neutralising antibody from the hen merits attention31. Hen antibodies
have a high protective power and do not react with human complement; this would result in a decrease in the
prevalence of adverse reactions. Nevertheless, chicken IgG is strongly allergic and the volume of material is
much less than from the horse32.

The utilisation of monoclonal antibodies offers at first glance an alternative to the polyclonal antibodies
currently used in immunotherapy. A monoclonal antibody directed against the Naja nigricollis postsynaptic
neurotoxin induces the dissociation of the complex toxin-receptor which is replaced by a complex toxin–
receptor–antibody, and neutralises the action of the toxin in the rabbit33. Monoclonal antibodies may be useful
for treating neurotoxic elapid envenoming; it would be difficult to develop a cocktail of monoclonal antibodies
for neutralising viperine venoms. Because rat antibodies may induce immune response in man, it has been
proposed to ‘humanise’ the antibodies34. Apart from the fact that these techniques are expensive, the utilisation
of monoclonal antibodies in human therapy is avoided because they originate from tumour cells.

8. Clinical Pharmacology35
Symptoms and signs of snakebite depend on species, size and age of the biting snake, location and number of
bites, depth of fang penetration, period of snake hangs on and volume of venom injected. They also depend on
age, size and general health of the victim. Some snake species are neurotoxic, others are hemotoxic therefore,
the clinical picture shows a wide range of symptoms and signs.

Grades of severity:
Grade 0 (no) = no local or systemic manifestations.
Grade I (minimal) = local swelling – no systemic manifestations – normal laboratory results.
Grade II (moderate) = local swelling – one or more systemic manifestations – Abnormal laboratory results.
Grade III (severe) = marked local and systemic manifestations with significant changes in laboratory results.

Envenomation is a highly dynamic process which means that grade I state can very rapidly progress to grade III
state. The blood peak level is delayed up to 8 hours after intramuscular injection therefore; the intravenous route
is preferred especially for moderate and severe envenomation and it is mandatory in venom induced shock. If
adequate dose is given, cardiovascular effects respond within 10-20 minutes, spontaneous systemic bleeding
stops within 15-30 minutes, blood coagulability is restored within about 6 hours and neurotoxic signs respond
slowly after several hours.

9. Dosage and Administration
The antivenom should be injected as soon as possible after the bite. It is given either intramuscularly or by
intravenous drip according to severity of the condition. However, the subcutaneous route may be used in case
of absence of anti-shock measures or an expert physician.
The dose is neither age nor weight dependent however; it depends on severity of the condition with no
recommended maximum dose. The total required dose is the amount needed to neutralize the venom as
determined by cessation of progression of all components of envenomation (initial control).

Grade 0: No treatment is required as the drug should never be administrated prophylactically in
asymptomatic patients.

Grade I: The recommended initial dose is 20-40 ml i.e. contents of 2-4 vials given by intramuscular route
into a large muscle mass preferably the gluteal area, at different sites, with care to avoid injury of nerve trunks.

Grades II and III: The recommended initial dose is 4 –6 vials, given by intravenous drip after diluting
the product with 5-10 times 0.9% sodium chloride or 5% dextrose. The product should be infused slowly for the
first 10 minutes at a rate of 25 –50 ml /h, with careful observation of any allergic reaction. If no reaction occurs, the infusion rate should be increased to the full 250 ml /h until completion.

10. Treatment of snake bite

10.1. Associated Treatment Including First Aid

All snake bites should be treated immediately. All measures taken to meet the emergency should be quick and positive. Patient should be removed to a well ventilated and quiet place. Assurance to the patient is important to overcome shock which is mainly psychogenic. Ligation - If the patient presents within an hour of a snake bite, a ligature should be tied at a moderate distance above the bitten part to prevent the venom being absorbed into the upper part of the limb. A rubber ligature is by far the best. It should be released every half an hour for about 5 to 10 seconds and then again tightened. Clean the wound and apply antiseptic dressing without rubbing. Immobilize the bitten part as you would for a fracture.

In case of viper poisoning, small doses of benzodiazepines and / or analgesics such as paracetamol may be given for relief of pain and anxiety. In case of collapse, strychnine, pituitrin and stimulants like coramine are of special value. The use of corticosteroids is restricted to treat and minimize allergic reactions to serum. Appropriate antibiotics should be given to combat local sepsis. Treatment of complications - Shock should be treated along empirical lines. Proper hydration, judicious use of furosemide, mannitol etc. is effective in preventing renal failure. Respiratory paralysis should be treated by tracheostomy and artificial respiration. Digoxin, diuretics, sedatives, salt and fluid restrictions are indicated for treatment of cardiac failure. Anti Tetanus injection may be given. All patients should receive a potassium poor diet for the first 48 hours.

10.2. Specific Serum Treatment

1. Immediate neutralisation of the circulating venom is of utmost importance.
2. For fast effect, the AntiSnake Venom Serum should be injected intravenously as soon as possible after the bite. As a first dose, at least 20 ml. of the reconstituted serum should be injected intravenously very gradually (for every 1 ml per minute).
3. The second dose should be repeated two hours after the first dose or even earlier. If the symptoms persist. If the symptoms, which vary with different snake venoms, indicate persistence of venom action, further doses should be repeated after every six hours until the symptoms disappear completely.
4. At present, there is no simple method to measure the amount of circulating venom in the body, therefore the antivenin dose cannot be accurately recommended. It has been found that the clotting time returns to normal about two hours after the neutralisation of venom therefore repeated testing of clotting time after the antivenom therapy is necessary. Close monitoring of the patient's condition with urine output, BP, pulse, respiration and urea and electrolyte estimations must be done. Local pain and necrosis at the site of bite may need attention, but is usually not very serious except in special circumstances such as a bite on the digit which can become gangrenous.

Suitable dosage schedule is recommended depending on clotting time is as follows:

### Antivenin Administration

<table>
<thead>
<tr>
<th>No. of hrs. after administration</th>
<th>Clotting time (Lee-White method)</th>
<th>Lyophilised Antivenin 1vial=10ml</th>
<th>Polyvalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Hour</td>
<td>Normal</td>
<td>No Treatment</td>
<td></td>
</tr>
<tr>
<td>1 Hour</td>
<td>More than 10 mins.</td>
<td>2 vials of antivenin in 100 to 500ml fluid infused in 2 hrs.</td>
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<tr>
<td>3 Hour</td>
<td>More than 10 mins.</td>
<td>2 vials of antivenin in 100 to 500ml fluid infused in 3 hrs.</td>
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</tr>
<tr>
<td>6 Hour</td>
<td>More than 10 mins.</td>
<td>2 vials of antivenin in 100 to 500ml fluid infused in 3 hrs.</td>
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</tbody>
</table>
5. In case of Viper bite, some of Anti Snake Venom Serum should also be injected around the site of the snake bite additionally to prevent gangrene which is one of the more destructive effects of localised Viper Venom on tissue.

6. First Aid treatment should never be relaxed even when the serum is administered. Intravenous injection of a reconstituted Anti Snake Venom Serum in equine serum sensitive subjects can produce very severe serum reactions and even acute anaphylaxis. Further care should be taken to prevent these reactions.

7. Intravenous injection is the most effective but if expert medical aid is not available the serum may be administered by a subcutaneous or through intramuscular route. It is more efficacious to dilute the serum 5-10 times with normal or glucose saline and be administered as slowly as possible.

11. Adverse Reactions

The serum being heterologous is liable to cause sensitivity reactions in occasional patient. Adverse reactions are due to the administration of foreign proteins, to preliminary sensitisation of the patient to horse serum, or to the presence of immune complex. The first are non-antivenom specific reactions of type I hypersensitivity (HS) and are proportional to the quantity of injected proteins. They appear a few minutes after the administration of antivenom. The sensitisation to horse proteins corresponds to a response of type III or type IV HS according, respectively, to immediate reactions (less than 12 h) or delayed reactions (1 to 3 weeks).

**Type I - Early anaphylactic reactions** (within 5-180 min of starting antivenom): It may manifest as itching, urticaria, dry cough, abdominal colic, fever, nausea, tachycardia, hypotension, bronchospasm, and angioedema. Few may develop life threatening anaphylactic shock. In most cases, these reactions are not truly “allergic”. They are not IgE-mediated type I hypersensitivity reactions to horse or sheep proteins. Complement activation by IgG aggregates or residual Fe fragments or direct stimulation of mast cells or basophils by the proteins in ASV is likely mechanisms for these reactions.

**Type II Pyrogenic reactions** (within 1-2 hours of treatment): The patient may have rigors, fever, vasodilatation and fall in BP. These reactions are caused by pyrogen contamination during the manufacturing process.

**Late (Serum sickness type) reactions** (within 1-12 days after treatment): The clinical features include fever, nausea, vomiting, diarrhoea, itching, recurrent urticaria, arthralgia, myalgia, lymphadenopathy, periarticular swellings, mononeuritis multiplex, proteinuria with immune complex nephritis and rarely encephalopathy. This reaction is less likely to develop in patients who had early reactions and are treated with antihistamines and corticosteroids.

11.1. Treatment of early anaphylactic and pyrogenic ASV reactions

ASV administration is temporarily stopped at the earliest sign of a reaction. Adrenaline (0.1% solution) is given intramuscularly (into the deltoid muscle or the upper lateral thigh) in an initial dose of 0.5 mg for adults, 0.01 mg/kg body weight for children. Severe, life threatening anaphylaxis can evolve very rapidly and so adrenaline should be given at the very first sign of a reaction, even when only a few spots of urticaria have appeared or at the start of itching, tachycardia or restlessness. The dose can be repeated every 5-10 minutes if the patient’s condition is deteriorating. In addition to adrenaline, H1 antagonist such as chlorpheniramine maleate (adults - 10 mg, children - 0.2 mg/kg) is given intravenously over a few minutes followed by intravenous hydrocortisone (adults - 100 mg, children - 2 mg/kg body weight). The corticosteroid is unlikely to act immediately, but may prevent recurrent anaphylaxis. Further H2 antagonists such as cimetidine (adults - 200 mg, children - 4 mg/kg) or ranitidine (adults - 50 mg, children - 1 mg/kg) diluted in 20 ml of isotonic saline, is given by slow i.v. injection over 2 minutes.

In pyrogenic reactions the patient must also be cooled physically and with antipyretics (e.g. paracetamol p.o. or suppository). Intravenous fluids should be given to correct hypovolemia. Treatment of late (serum sickness) reactions Late (serum sickness) reactions usually respond to a 5-day course of oral antihistamine (chlorpheniramine maleate: adults - 2 mg sixth hourly, children - 0.25 mg/ kg /day in divided doses). Patients who fail to respond with in 24-48 hours should be given a 5-day course of prednisolone (adults - 5 mg sixth hourly, children - 0.7 mg/kg/day in divided doses for 5-7 days).

11.2. Prevention of Serum Reaction

Before injection of Anti-Snake Venom Serum, it is necessary to enquire from the patient:

1. Whether he has had injections of serum (eg. anti-tetanus or anti-diphtheria serum) before.
2. Whether there is personal or familial history of allergy, i.e. asthma, eczema or drug allergy.
3. A skin test should be performed prior to administration of Antivenom. Inject 0.1- 0.2 ml (1:10 dilution) of antivenom intradermally. A positive reaction occurs within 5-30 minutes manifested by a wheal with or without surrounding erythema accompanied by increased risk of systemic reactions in sensitive patients.
4. If history is positive for allergy and the test is positive, administration may be dangerous especially if the test is accompanied by systemic allergic manifestations. In such instances, the benefit of administration must be weighed against the risk of withholding the product keeping in mind that severe envenomation can be fatal.

5. If history is negative for allergy and the result of test is negative, administrate the drug; however, these do not rule out the possibility of an immediate reaction as 10% of false negative reactions have been reported.

6. The necessity of skin test is controversial however, it is preferable to be performed and interpreted prior to administration of antivenin in order to be ready for early interference and close observation.

7. In case of severe symptoms, it is not recommended to wait for 30 minutes to observe the skin test result therefore; it is advisable to initiate serum therapy with simultaneous injection of 0.5 - 1 ml of 1:1000 adrenaline subcutaneous and parallel infusions of hydrocortisone and antihistamine to decrease the acute allergic reactions.

NOTE: Use of larger amount of skin test dose, increases the likelihood of false positive reactions.

12. Directions for Use

Reconstitution of Lyophilised Serum:

1. Remove the metallic disc in the cap over the diaphragms of the vials of antivenin and diluent. Sterilize the rubber diaphragm with alcohol.

2. Draw 10 ml. of sterile water for injection in a sterile syringe.

3. Transfer the sterile water from the syringe to the serum vial and shake well till the contents dissolve.

4. Let the vial stand for one minute for the serum to clear. The reconstituted serum will become crystal-clear and ready for injection. Froth and undissolved particles, if any, should be left in the vial.

5. For the second and subsequent injections, you will have more time to dissolve the lyophilised serum. For these add 10 ml. sterile water for injection to the serum vial and rotate it between the palms of your hands until the serum is fully dissolved, and let the vial stand for serum to clear.

Special note:

In severely envenomated patient, initial dose of 10 to 20ml of Antivenin may be administered in bolus, provided the patient is not sensitive to antivenin. The above regimen is to be continued 3 hrs. Thereafter till clotting time is less than 10 min. and the clot is firm. After completing the above regimen, 2 vials of Antivenin are infused in 500ml fluid in a period of 24 hrs. In case of viper-bite, some of the Antivenin may be infiltrated around the site of bite with extreme caution. In all cases of snake bites, Antivenin is never discontinued till all the signs of envenomation disappear and clotting time is less than 10 mins.

Storage:

Liquid serum is very unstable at room temperature. It requires storage at 0° to 4°. Even then it deteriorates, and 2 years from the date of manufacture, the serum becomes unfit for use. In India, proper cold-storage facilities are not freely available and, therefore, liquid serum may only be stored at the risk of very rapid deterioration. Lyophilised serum obviates this difficulty. It is many times more stable than liquid serum. It should retain its potency for 5 years even if stored in any cool dark place. Thus Anti Snake Venom Serum can be made available for use far away from cold-storage facilities. It can be safely kept at rural dispensaries and even carried in a haversack if an occasion demands it. However, it is preferable to store it in a refrigerator if one is available.

Packing:

One dose vial of lyophilised Anti-Snake Venom Serum with 10 ml. ampoule of sterile water for injection.

Presentation:

10 ml vial (liquid)/ box.

10 ml diluent + vial for lyophilized powder/ box.

Conclusion

The annual incidence of snakebite in the world is estimated at five million with about 40000 deaths. The only specific medically-approved treatment is the antivenom. Regarding the purification of sera, which are separated from other undesirable proteins such as albumin and complement, it should be preferable for immunotherapy. The efficiency and safety of immunotherapy can be improved by the quality and type of venoms used for
imunisation. The dose of antivenom depends on the type of snake and on the amount of venom injected by the snake; the dose necessary should be evaluated depending on the clinical state of the patient and the evolution of the symptoms. The most recent improvement is the use of ELISAs to determine antivenom dosage.

References:


