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REVIEW

The management of snakebites in South Africa

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Snake bites are common in southern Africa especially in the rural and remote areas. Although all snake bites are not venomous, people bitten by venomous snakes require urgent medical attention and many will require antivenom. In the healthcare facility, the type of toxin may be identified by the wound and presenting clinical features which then should be managed appropriately.

Keywords: snakebite, venomous snakes, envenoming, antivenom

Introduction

Snakebite envenomation (poisoning) is a potentially life-threatening condition that results from toxins (venom) being injected through bite or sprayed into the eyes by spitting snakes.[1] Although there are more than 3 000 species of snakes globally, approximately 250 are potentially harmful. Snakebite envenoming is an important health problem in rural areas of tropical and sub-tropical countries where communities depend on agriculture or subsistence hunting and gathering, live in poorly constructed housing and have limited access to education and health care.[2]

Besides death there can be permanent physical damage due to tissue necrosis, venom-ophthalmia, persistent nerve damage and psychological consequences. Because of issues relating to treatment costs, loss of earning capacity and ongoing disability, the economic impact of snakebites can be considerable.[3,4]

The World Health Organization (WHO) estimates that about five million snakebites occur annually, resulting in 2.7 million envenomings.[2,4] Snakebite envenoming causes as many as 400 000 amputations and other permanent disabilities. As a result, WHO added snakebite envenoming to its priority list of neglected tropical diseases (NTDs) in June 2017.[2,4]

In sub-Saharan Africa about a million people are reportedly bitten annually, with estimated 7 000–20 000 deaths. Many victims delay or fail to seek medical care timeously.[5]

In South Africa the hospital admission rate is 30–80 per 100 000 persons per year. About 20% show no signs of envenomation. Hospital mortality varies from 0–5%. Necrosis at the bite site occurs in about 10% of patients, with permanent morbidity in 2–3%.[6]

Three major families of venomous snakes

Elapidae (cobra, king cobra, krait, and coral snake): These snakes have similar width of the head and neck, have grooved fangs that are short, fixed, and covered by mucous membrane, and

Figure 1. Mozambican cobra[3]

Figure 2. Rattle snake[3]

Figure 3. Hydrophiinae[2]

therefore cannot bite through clothes. They usually deliver sub-lethal venom doses.[2,3,7]

Viperidae: Vipers have a triangular head that is wider than the neck and laureal shields. Their fangs are long, movable, and canalised.[2,3,7]

Hydrophidae (sea snake): They have a small head and a flattened tail that helps them swim. Though venomous, they seldom bite.[2,3,7]

Young children (under five years) and pregnant women who are at risk of haemorrhage and miscarriage following a venomous snakebite suffer higher case fatality.[2,3,6,7] Other factors contributing to severity and outcome in snakebite are listed in Table I.

Table I. Factors contributing to severity and outcome in snakebite[7-]

Factor	Effect on outcome
Size of victim	Bigger body size results in better outcomes due to lower toxin concentration
Comorbidity	Increases to harmful effect
Body part bitten	Bites on the trunk, face, and directly into bloodstream have a worse prognosis
Activity	Activity enhances systemic absorption of toxin hence poor outcome
Individual sensitivity	Individual sensitivity to venom modifies the clinical picture
Bite characteristics	Bite number, depth, wetness, cushion, amount of venom injected, condition of fangs and duration snake clings to the victim all affect outcome
Snake species	Different species have different lethal dose, lethal period, and aggressiveness
Secondary infection	Presence of pathogenic organisms in the mouth of the snake
Treatment	Nature of first aid given and time to antivenom administration

Classification of snake bites

Venomous snakes in southern Africa can be divided into three groups: cytotoxic, neurotoxic and haemostatic toxic effects with significant overlap of the cytotoxicity and neurotoxicity effects.[3] Five main clinical syndromes of snake envenoming are recognised in southern Africa. Identifying the correct clinical syndrome will assist the clinician in following a syndromic approach in the majority of cases.

Marked local pain and progressive swelling associated with prominent cytotoxic skin changes with coagulable blood

The toxins of cytotoxic snake venom are digestive hydrolases (proteolytic enzymes and phospholipases) and polypeptides that destroy cell membranes, skeletal muscle and other tissues. These effects increase the permeability of the vascular endothelium, which leads to local swelling, blistering and oedema. Necrosis/gangrene may result.[3,7]

Snakes responsible for this syndrome include:

The major adders (puff adder, gaboon adder), spitting cobras (Mozambique and black-necked spitting cobra) and the rinkhals (*Hemachatus haemachatus*).[3,5]

Clinical features of cytotoxic snake bites:

Swelling begins early, often within 10–30 minutes and may become extensive, involving the entire limb and adjacent areas of the trunk, especially in children. Regional lymph nodes become enlarged and painful within 30–60 minutes. The aggressive and progressive cytotoxic nature of envenoming is evident within hours of the bite. Blisters and bullous skin lesions, fluid or blood filled, and ecchymosis develop, at first near the fang marks, but may later extend beyond the bite site within 6–24 hours.[3,5]

Skip lesions (areas of necrosis separated by strips of apparently normal skin caused by proximal spread of venom in lymphatic vessels) are characteristic of spitting cobra bites.[3,5]

Extravasations of plasma causes hypovolaemia, which may lead to shock, especially in children. The local cytotoxic effects progress to necrosis, with spontaneous sloughing of dead tissue. Compartment syndrome may develop, especially involving the anterior tibial compartment after bites of the feet and ankles, or forearm. This complication may lead to ischaemic necrosis of the compartmental muscles and nerve damage. Late (2–3 days post bite) haemostatic disturbances, especially thrombocytopaenia, in puff adder and gaboon adder bites.[3,5]

Gaboon adder bites may be accompanied by cardiovascular abnormalities, including hypotension, cardiac dysrhythmias and shock.[3,5]

Antivenom is available (SAIMR Polyvalent Snakebite Antiserum SAVP).[3,5]

Progressive paralysis (neurotoxicity), with negligible or minor local swelling

The venoms of neurotoxic cobras contain polypeptides that compete with acetylcholine for binding at post-synaptic nicotinic receptors at skeletal muscle nerve junctions, leading to a curare-like paralysis. Neurotoxins that block muscarinic receptors have also been described in mamba venom.[3,7]

Snakes responsible for this syndrome include:

Neurotoxic cobras (Anchieta's Egyptian cobra, banded or snouted cobra and Cape cobra) and Mambas (black mamba, common, eastern green, white mouthed mamba and green mambas).[3,5]

Clinical features of neurotoxic snake bites:

Neurotoxicity causes progressive, descending flaccid paralysis. Early symptoms and signs include transient paraesthesia of the tongue and lips, blurred and double vision and ptosis, pupillary abnormalities (e.g. dilated pupils), external and internal ophthalmoplegia and paralysis of facial muscles and other muscles innervated by the cranial nerves, leading to dysarthria, dysphonia, and dysphagia. There is an increase in oropharyngeal secretions due to difficulty in swallowing. This is followed by

progressive, descending paralysis, and finally respiratory failure. As respiratory distress increases, the patient becomes anxious, sweaty and cyanosed and will die unless given ventilatory support. Neurotoxic snakes can cause life-threatening paralysis and death within 1–8 hours. Respiratory failure is usually the cause of death.[3,5]

In addition, patients bitten by mambas may present with skeletal muscle fasciculations and signs of autonomic nervous system stimulation. Early features are vomiting, chest and limb pains and excessive salivation. Cardiac dysrhythmias have also been described in mamba bite victims.[3,5]

Antivenom is available for these snake bites (SAIMR Polyvalent Snakebite Antiserum SAVP).[3,5]

Incoagulable blood, with negligible to mild local swelling

Venom of these snakes has potent pro-coagulant effects by activating factors II (prothrombin), X and possibly also IX. Severe consumptive coagulopathy may lead to multiple organ failure.[3,7]

Snakes responsible for this syndrome include:

Boomslang, south-eastern Savanna vine/bird/twig snake and Oate's savanna vine snake.[3,5]

Clinical features of this syndrome are:

Patients may present with nausea, vomiting, abdominal pain, headache, dizziness and fainting. Persistent oozing of blood from fang punctures or other wound sites may occur. Although bleeding may occur within 6–24 hours after a bite, systemic haemostatic symptoms and signs may be delayed for more than 24 hours after the bite. Bleeding usually manifests as gingival bleeding, epistaxis, purpura, haematemesis, melaena, haematuria, extensive ecchymosis, and in severe cases, subarachnoid or intracerebral haemorrhage.[3,5]

Antivenom is available for boomslang bite (SAIMR Boomslang Snakebite Antiserum SAVP). No antivenom is available for vine/bird twig (Thelotornis) bites.[3,5]

Moderate to marked local swelling, associated with neurotoxicity

Phospholipase A2 neurotoxins are responsible for the toxic effects of these snake venoms. The neurotoxins act presynaptically, initially releasing acetylcholine, followed by an interference with or blockade of its release.[3,7]

Snakes responsible for this syndrome are:

Berg adder and other small/dwarf adders.[3,5]

Clinical features of this syndrome are:

After initial pain and the development of local swelling, paraesthesia of the tongue and lips, blurring of vision and the loss of the sense of smell (anosmia) and taste, and dysphagia develop, often within 2–3 hours of the bite. External and internal ophthalmoplegia are characterised by ptosis, fixed dilated pupils and loss of eye movements and accommodation. Muscle weakness and respiratory failure are common complications and typically develop late (6–36 hours after the bite), often at a stage when not expected.[3,5]

Hyponatraemia, attributable to a natriuretic hormone-like toxin present in the venom, is also a frequent complication. It typically develops late (24–36 hours). If undetected this may lead to unexpected convulsions. Ophthalmoplegia and anosmia may take months to resolve.[3,5]

The local effects include moderate to marked local swelling. Swelling may involve more than half the bitten limb. Blistering and necrosis may develop in the region of the bite site. Extensive cytotoxic skin changes and compartment syndrome are not expected to develop.[3,5]

No antivenom is available for these bites.[3,5]

Mild to moderate swelling, with negligible or absent systemic symptoms

Snakes responsible for this syndrome include:

Night adder, burrowing asp, Natal black snake and some dwarf adders, e.g. horned adder.[3,7]

Clinical features of this syndrome are:

Symptoms and signs include local pain, regional lymphadenopathy and fever. Swelling rarely involves more than half of the bitten limb. Blistering and necrosis may develop at the bite site.[3,5,7]

Minor envenoming by spitting cobras and major adders should be considered in the differential diagnosis.[3,5]

No antivenom is available for these snake bites.[3,5]

Management of snake bites

Prevention and control

As venomous snakes coexist with humans and play important roles in ecosystems, including the natural biological containment of agricultural pests (e.g. rodents), it is not possible to completely eliminate snakebite envenoming.[5]

Prophylactic measures are:

- Avoiding handling seemingly dead snakes as rinkhals and elapids sham death.
- Sleeping in a snake-proof dwelling.
- Keeping rubble, wood piles, chicken coops and dense vegetation far from houses.
- Wearing shoes and using a torch when walking at night.[1]

Treatment

Early access and providing emergency treatment in a health facility capable of diagnosing and managing snakebite envenoming is critical.[1,3,7]

First aid management

First aid procedures, informing the receiving medical facility and transport of the patient should be done urgently.[3]

Reassure and immediately move the victim away from the area.[2,3,7]

Remove constricting clothing, rings, bracelets, bands, shoes, etc. from the bitten limb/area.[3]

Immobilise the patient and splint the limb to keep it still.[2,3,7]

Avoid potentially harmful traditional treatments such as cauterisation, local incision or excision, tattooing, immediate prophylactic amputation of the bitten digit, suction by mouth or vacuum pumps or 'venom-ex' apparatuses, instillation of chemical compounds such as potassium permanganate, application of petrol, ice packs, 'snake stones' and electric shocks. In suspected neurotoxic cobra or mamba bite, especially if the patient is far from medical help, apply a tight crepe bandage over and proximal to the bite site. This procedure may reduce rapid distribution of the venom. The classic 'pressure-immobilisation technique' demands special equipment and training and is considered not practicable for general use in South Africa.[2,3]

A tight arterial tourniquet should never be used. Tourniquets may lead to ischaemia and gangrene.[1,3]

In suspected neurotoxic snake bites, the patient should be assessed regularly (e.g. every 10–15 minutes) for the development of complications of neurotoxicity.[2,3]

Cardiopulmonary resuscitation (CPR) may be needed. This includes clearance of the airway, oxygen administration by face mask or nasal catheters, and establishment of intravenous access. Shocked, hypotensive patients should be given intravenous fluids. Pressor agents, such as dopamine or phenylephrine may be required.[1,2,3]

Give analgesia by mouth if required: paracetamol or paracetamol/codeine combinations are preferred. Aspirin and other nonsteroidal anti-inflammatory agents should be avoided. When using parenteral opioids in neurotoxic snake bite, respiratory function should be monitored closely.[3]

In cases where the snake has not been identified it is recommended that asymptomatic patients be admitted to a medical facility for observations for 12–24 hours.[3,8]

Nurse the patient on the left side with mouth turned down to avoid airway obstruction and aspiration of vomitus.[3]

Hospital care

As identification of the snake is usually difficult, unless a dead snake is brought, descriptions of the snake and the circumstances of the bite may suggest a species.[2,3,6]

In most cases of snake bite appropriate clinical management requires reliable identification of a distinctive clinical syndrome based on epidemiological, clinical and laboratory data. A syndromic approach is, therefore, recommended in the majority of cases.[3,7]

Emergency care department

Evaluation should begin with the assessment of the airway, breathing, circulatory status, and consciousness.[3,5,6,7,8]

Urgent resuscitation will be needed in those in shock (cardiovascular toxicity), those with respiratory failure (neurotoxin), and in those who have had cardiac arrest (due to hypoxia, cardiac toxicity, or hyperkalaemia from rhabdomyolysis).[2,3,6,7,8]

Oxygen should be administered to every envenomed patient and a large-bore intravenous line inserted. A bolus of normal saline or Ringer's lactate should be given to all patients with suspected envenomation. The patient may then receive specific treatment.[2,3,5,6]

In cases of berg adder bite, hyponatraemia should be treated by means of a titrated infusion of hypertonic saline. The administration of normal saline may prove useful as a means of partially meeting both fluid and salt requirements.[3]

History

Attempts should be made to determine if a venomous snake has actually bitten the patient and the severity of the bite. Questions should be asked to determine the time elapsed since the snakebite and a brief medical history should be obtained (e.g. date of last tetanus immunisation, use of any medication, presence of any systemic disease, history of allergy and if the patient has received antivenom before as this has an increased risk of anaphylaxis). Specific inquiry should include time of the bite, what part of the body was bitten, description of the snake and symptoms experienced.[2,3,5,6,7]

Physical examination

The bite site should be examined for signs of local envenomation (oedema, petechiae, bullae, oozing from the wound, etc.) and for the extent of swelling. The bite site and at least two other, more proximal, locations should be marked and the circumference of the bitten limb should be measured every 15 min thereafter, until the swelling is no longer progressing. The extremity should be placed in a well-padded splint for at least 24 hours. Lymph nodes draining the bite should be palpated and the presence of lymphangitic lines noted.[2,3,5,6,7]

Distal pulses should be checked and monitored if there is presence of gross swelling. The presence of a pulse does not rule out compartment syndrome however, and compartment pressure should be measured directly if there is concern that a compartment syndrome is developing. The diagnosis is established if the compartment pressure, measured directly by inserting a 22G IV cannula and connecting it with manometer, is raised above 55 cm water/saline. Direct measurement is necessary before resorting to fasciotomy since compartment syndrome is rare in snakebite victims and fasciotomy done without correction of haemostatic abnormality may cause the patient to bleed to death.[2,3,5,6,7]

Severe snake envenomation indicated by:

- Venomous snake identified
- Rapid early extension of local swelling from the site of the bite
- Early tender enlargement of local lymph nodes
- Early systemic symptoms
- Early spontaneous systemic bleeding
- Passage of dark brown urine[7]

Ancillary treatment

Although most local effects of snakebite are attributable directly to cytolytic and other activities of the venom itself, the bite may introduce pathogenic bacteria. The risk of local infections greatly increases if the wound has been incised with an unsterile instrument or tampered with in some other way. The wound should be cleaned with an antiseptic. Blisters and tense bullae should be aspirated only if rupture seems imminent. Snake-bitten limbs should be nursed in the most comfortable position but should not be elevated excessively if there is tense swelling or suspicion of incipient compartment syndrome, as this increases the risk of ischaemia. Debrided tissue, serosanguinous discharge and pus should be cultured and the patient treated with appropriate antimicrobials.[3,7]

Supportive therapy

The ICU will be required for patients with signs of severe envenomation (coma, respiratory paralysis, hypotension, pulmonary oedema, and history of syncope). Patients with presence of fang marks, moderate pain, minimal local oedema, erythema, ecchymosis, and no systemic reactions can be treated in the ward under close monitoring. Supportive therapy is required to buy time while the damaged organs recover.[2,3,7,8]

Coagulopathy with bleeding

Coagulopathy usually reverses after antivenom treatment. If there is severe bleeding or when urgent surgery is necessary, restoration of coagulability can be accelerated by giving fresh frozen plasma, cryoprecipitate (fibrinogen, factor VIII), fresh whole blood, or platelet concentrates.[2,3,7,8]

Neurotoxic symptoms and anticholinesterase therapy as an option for neurotoxic cobra bite

Antivenom treatment alone cannot be relied upon to save the life of a patient with bulbar and respiratory paralysis. Once there is loss of the gag reflex, failure to cough, or respiratory distress, endotracheal intubation and initiation of mechanical ventilation is indicated.

Anticholinesterase drugs can have a useful effect in patients with neurotoxic envenomation, especially in those bitten by cobras. Although anticholinesterase may assist in management, this should not replace antivenom therapy and should also not take priority over respiratory support. Neostigmine is the drug of choice in South Africa. The administration of anticholinesterases requires the co-administration of an anticholinergic drug to block potentially serious muscarinic effects, such as bradycardia, bronchospasm and an increase in secretions. Glycopyrrolate is the preferred anticholinergic.[3,7]

Compartment syndrome

There may be severe pain, tense swelling, cold cyanosed skin, pain on passive stretching of the muscles and apparently absent pulses. However, these appearances are usually misleading. When the intracompartmental (tissue) pressure is measured directly (e.g. with a Stryker monitor) pressures are usually found to be below the threshold of danger for ischaemic necrosis of the intracompartmental muscles. Should conservative treatment fail, full-length fasciotomy should be performed, providing there is no coagulopathy or gross thrombocytopaenia. Provided that adequate antivenom treatment is given as soon as possible after the bite, fasciotomy is rarely needed. Necrotic tissue should be debrided. Skin grafts may be necessary.[2,3,7]

Haemostatic abnormalities

Recovery of normal haemostatic function may be accelerated by giving fresh whole blood, fresh frozen plasma, cryoprecipitates or platelet concentrates. Heparin and antifibrinolytic agents should never be used.[2,3,7]

Renal dysfunction

Acute renal failure may be caused by haemorrhage, ischaemia resulting from hypotension, effects of blood clotting abnormalities, renal vasoconstriction, pigment nephropathy caused by haemoglobinuria or myoglobinuria, direct nephrotoxicity and immune complex glomerulonephritis caused by serum sickness reactions to antivenom. If the urine output falls below 400 ml in 24 hours, central venous pressure should be monitored and a urethral catheter inserted. Cautious rehydration with isotonic fluids can be followed by a high dose of furosemide. If these measures fail, dialysis may be indicated.[2,3,7]

Snake venom ophthalmia

The spitting elapid species in southern Africa can cause intense conjunctivitis and bullous corneal erosions, complicated by secondary infection, anterior uveitis, corneal opacities and permanent blindness.

First aid treatment consists of irrigating the eye or other affected mucous membrane as soon as possible, using large volumes of water or any other available bland fluid such as milk. A single application of local anaesthetic eye drops to overcome blepharospasm may be used to facilitate irrigation. Topical or systemic antivenom treatment should not be applied or given. Corneal abrasions can be excluded by fluorescein staining/slit lamp examination. If there are no abrasions, treat with antibiotic eye ointment and an eye pad. Resolution should occur within 24–48 hours. If corneal erosions are present, antibiotic eye drops/ointment, mydriatics and an eye pad should be applied. Daily slit lamp examinations are recommended until resolved. An ophthalmologist should be consulted in all cases.[3]

Investigations

Specific investigations

(a) The 20-min whole blood clotting test (20 WBCT): The 20 WBCT is a simple bedside test of coagulopathy to diagnose viper

envenomation and rule out elapid bite. It requires a new clean, dry test tube made up of simple glass that has not been washed with any detergent. A few millilitres of fresh venous blood is drawn and left undisturbed in the test tube for 20 min; the tube is then tilted gently. If the blood is still liquid after 20 min, it is evidence of coagulopathy and confirms that the patient has been bitten by a viper.[2,7,8]

(b) Enzyme-linked immunosorbent assay (ELISA): ELISA tests are now available to identify the species involved, based on antigens in the venom. These tests are expensive and not freely available.[2,7,8]

Non-specific investigations

i. Full blood count[1]: May show transient elevation of haemoglobin level due to haemoconcentration (because of the increased capillary leak) or may show anaemia (due to haemolysis, especially in viper bites). Presence of neutrophilic leucocytosis signifies systemic absorption of venom. Thrombocytopaenia may be a feature of viper envenomation.

ii. Serum creatinine: This is necessary to rule out renal failure.

iii. Serum amylase and creatinine phosphokinase (CK): Elevated levels of these markers suggests muscle damage (caution for renal impairment).

iv. Prothrombin time (PT) and activated partial thromboplastin time (aPTT): Prolongation may be present in viper bite.

v. Fibrinogen and fibrin degradation products (FDPs): Low fibrinogen with elevated FDP is present when venom interferes with the clotting mechanism.

vi. Arterial blood gas (ABG) and electrolyte determinations: These tests are necessary for patients with systemic symptoms.

vii. Urine examination: Can reveal haematuria, proteinuria, haemoglobinuria, or myoglobinuria. (ABG and urine examination should be repeated at frequent intervals during the acute phase to assess progressive systemic toxicity).

viii. Electrocardiogram (ECG): Nonspecific ECG changes such as bradycardia and atrioventricular block with ST-T changes may be seen.

ix. Electroencephalogram (EEG): EEG changes have been noted in up to 96% of patients bitten by snakes. Sixty-two percent showed grade I changes, 31% cases manifested grade II changes (moderate to severe abnormality), and the remaining 4% showed severe abnormality (grade III). These abnormal EEG patterns were seen mainly in the temporal lobes.[7]

The first blood drawn from the patient should be typed and cross-matched, as the effects of both venom and antivenom can interfere with later cross-matching.[7]

4.5 The use of antivenom

Snake antivenoms are effective to prevent or reverse most of the harmful effects of snakebite envenoming. They are included in the WHO Essential Medicines List and should be part of any primary healthcare package where snake bites occur.[2,4]

Administered early, antivenoms are not just life-saving, but can also spare patients some of the suffering caused by necrotic and other toxins in snake venom, leading to faster recovery, less time in hospital and a more rapid transition back to a productive life in their communities.[1,2,3,5,6,7]

Available snakebite antivenoms

- Polyvalent antivenom (SAIMR Polyvalent Snakebite Antiserum SAVP) is effective against: puff adder, gaboon adder, rinkhals, green mamba, Jameson's mamba, black mamba, Cape cobra, forest cobra, snouted cobra and Mozambique spitting cobra. Polyvalent antivenom is ineffective and should not be used in treatment of bites caused by the berg adder, other dwarf adders, night adders, the burrowing asp and back-fanged snakes (boomslang and vine snake).[1,2,3,5,6,7]
- Boomslang antivenom (SAIMR Boomslang Snakebite Antiserum SAVP) is effective against the venom of boomslang, but not against the venom of the vine snake (bird or twig snake).
- Antivenom is not always necessary: some patients are bitten by non-venomous snakes and 10 to 50% of those bitten by venomous snakes are not envenomed (so called 'dry bites').[1,2,3,5,6,7]

Indications for antivenom treatment

- Neurotoxicity.
- Abnormal blood clotting parameters, incoagulable blood and/or spontaneous systemic bleeding.
- Rapidly progressive and/or extensive swelling involving more than half the bitten limb within a few hours after the bite.
- Cardiovascular abnormalities such as hypotension, shock and cardiac arrhythmias.[2,3,7]

Precautions

Skin testing for sensitivity is not recommended, since it is unreliable.[3]

Administration of antivenom may be associated with acute life-threatening adverse reactions such as anaphylaxis, pyrogenic reactions, or late immune complex disease (serum sickness). Most acute/severe allergic reactions occur during the first hour after antivenom administration.[3]

There is no absolute contraindication to antivenom treatment when a patient has life-threatening systemic envenoming. However, patients with an atopic history and those with a history of previous reactions to equine antisera have an increased risk of severe antivenom reactions. In these cases, pretreatment with subcutaneous adrenaline is justified to prevent or diminish the reaction. Patients in whom adrenaline is relatively contraindicated include those with a history of ischaemic heart disease or stroke, uncontrolled hypertension and tachyarrhythmias.[3]

Premedication with antihistamines may dampen minor allergic reactions but will not prevent serious allergic reactions. Hydrocortisone takes several hours to act and is ineffective as a prophylactic agent against acute reactions. Slow infusion of the antivenom reduces serious antivenom reactions.[3]

Dose and methods of administration

Since snakes inject the same amount of venom into adults and children, the same dose/volume of antivenom must be administered to children as in adults.[3]

Antivenom should be given as soon as possible. Although the polyvalent antivenom is more effective when given early it may be administered up to 24–48 hours or later in serious envenomations – it is never too late to give antivenom.[3]

Antivenom is most effective when given intravenously. Do not inject antivenom into or around the wound.[3]

Response to antivenom treatment

Neurotoxic signs improve slowly after 2–6 hours, but often unconvincingly. The administration of polyvalent antivenom in the acute phase of neurotoxic snake envenoming will usually not prevent progression of neurotoxic effects notably respiratory paralysis, and consequently the patient will not survive without life support. However intravenous administration of adequate doses of antivenom will decrease the time course of muscle paralysis. Similarly, in cytotoxic envenoming, administration of polyvalent antivenom will not reverse but may limit further tissue damage. However, in boomslang bite the haemostatic effects are rapidly reversed by boomslang antivenom at any time after the bite.[3]

Treatment of antivenom reactions

Early serious reactions may begin 3–60 minutes after starting intravenous administration. Adrenaline could be given intramuscularly in a dose of 0.5–1.0 ml for adults and 0.01 mg/kg for children. This should be followed by a slow intravenous injection of an H_1 antagonist (antihistamine) such as promethazine. It is contraindicated in children < 2 years of age.

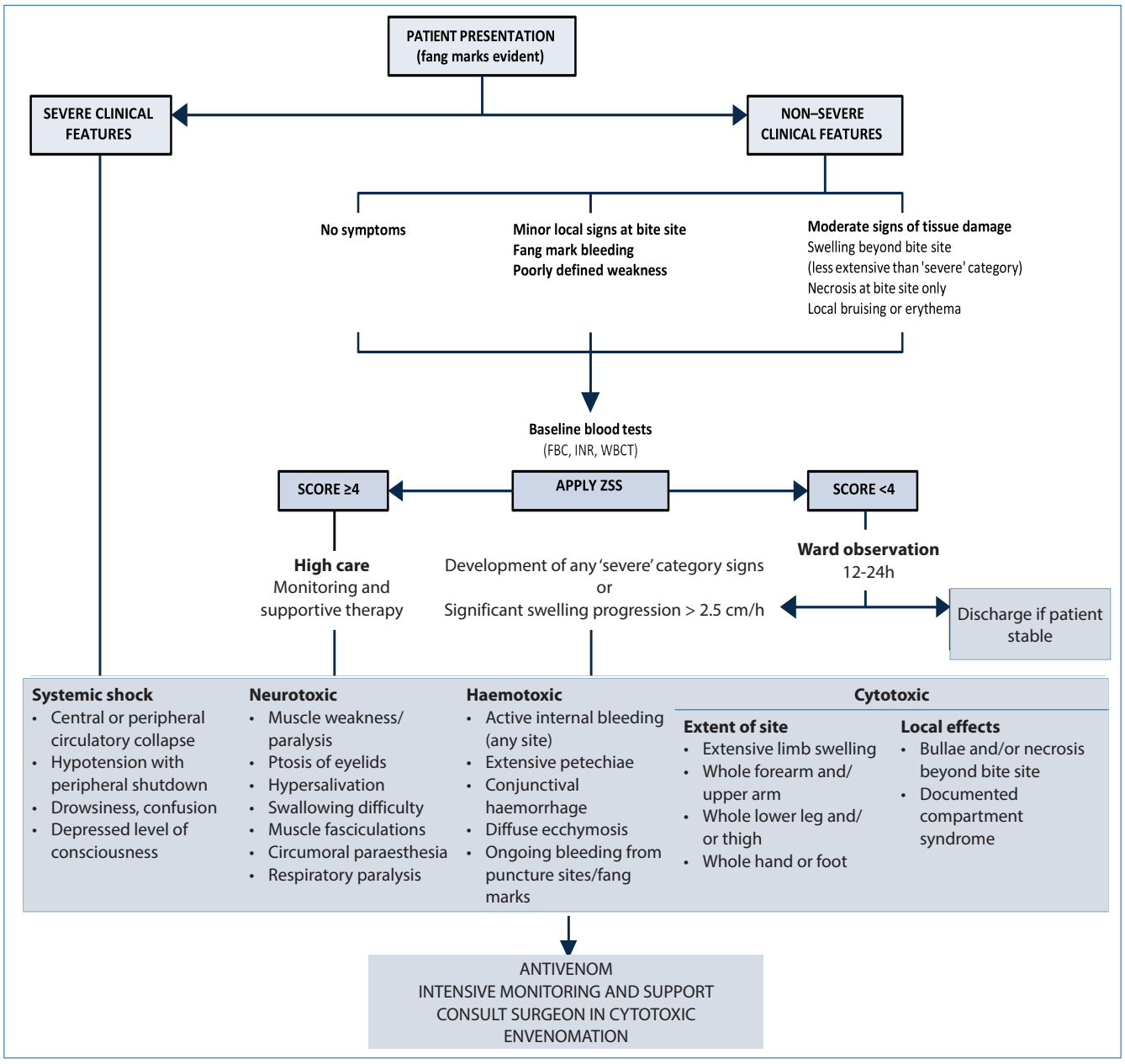

Figure 1. Algorithm for the management of snake bite according to the six point scale score[8]

Source: Wood D, Sartorius B, Hift R. Classifying snakebite in South Africa: Validating a scoring system. SAMJ 2017;107(1):46-51.

Table II. Six point scale snake bite score (ZSS)[8]

Risk predictors	Allocated score
Children < 14 years	1
Duration > 7 hours	1
White cell count > $10x10^9$/L	1
INR > 1.2	1
Platelets < $92x10^9$/L	1
Haemoglobin < 7.4 g/dL	1

In children 5–10 years old the dose of promethazine is 6.25–12.5 mg and in children 10–16 years of age 12.5–25 mg (or 0.125–0.5 mg/kg).[3]

Late (serum sickness type) reactions occur 5–24 (average 7) days after treatment. It presents with itching, urticaria, fever, arthralgia, peri-articular swellings, proteinuria and sometimes neurological symptoms. Antihistamines are used for milder attacks, but in severe cases a short course of prednisone should be given.[3]

Snake bite in pregnancy

The foetus may be hypoxic while the mother is not as there may be uterine vasoconstriction while the mother is normotensive.[6]

Ensure adequate oxygenation and fluid replacement.[6]

Beware of the supine hypotension syndrome of the third trimester of pregnancy.[6]

Most maternal and foetal deaths occur in the haemotoxic syndrome, thus administer adequate antivenom therapy.[6]

4.7 Snakebite treatment algorithm

A simple and effective guide to the management of snakebite is to allocate a score to certain predictors of severity of the bite, and then by following the algorithm according to the score, as can be seen in Table II and Figure 1.

Conclusion

Snake bite with envenomation remains a major cause of morbidity and potential mortality especially in highly vulnerable children and pregnant women. Appropriate timeous intervention at a facility with capacity to manage all types of envenomation is critical in preventing debility and saving life. Ongoing education of the public and medical personnel is important in ensuring appropriate response and improved outcomes.

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The identification and syndromic management of snakebite in South Africa

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Keywords: snake bite, antivenom

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Introduction

The identification of snakebite injury is uncertain, especially in the 40% of patients who do not see the offending snake, unless there are paired fang marks or typical findings of an envenomation syndrome. The differential diagnosis would include a thorn prick, spider bite or scorpion sting. Thorn pricks are not associated with the onset of progressive swelling or systemic illness within minutes. Swelling following dermonecrotic spider bites is slow in onset, whilst significant button (widow) spider bites and scorpion stings are associated with muscle spasticity which is not a feature of snakebites. [1, 2, 3]

Where snakes and humans abound, encounters between the two are not uncommon, with bites leading to 30 – 80 hospital admissions per 100,000 persons per year.[4-7] Snakebites are most common in the summer months, from late afternoon to early evening, affecting males and females roughly equally, depending on daytime activity. Most occur on the foot or leg during the first three decades of life. Finger and hand bites are far more prone to necrosis than bites elsewhere. Multiple bites on any body part may occur in sleeping patients. The venom to mass ratio is larger in children, resulting in a higher mortality rate than adults. [4-7]

The syndromic approach to snakebite

Snakebite presents as minor mechanical trauma, allergy to venom (rare) and/or an evenomation syndrome. Simplified, three main clinical envenomation syndromes in snakebite should be identified, namely:

- **Painful progressive swelling (PPS)**
- **Progressive weakness (PW)**
- **Bleeding (B)**

This article follows the syndromic approach which is logical and effective, whether the species of the snake is known or not. (Algorithm 1). For easy reference, the three syndromes have been colour-coded in the text and in the algorithms.

First aid

There is no good first aid measure for all snakebites. These measures attempt to denature venom (topical applications, electrotherapy, cryotherapy), remove venom (incision and suction, excision) or retard its absorption (various types of tourniquet, cryotherapy). These measures in most cases are either ineffective per se or the venom is absorbed too rapidly (mambas) or deposited too deeply (adders) for them to be effective. The vast majority of snakebites lead to PPS and here tourniquet use would aggravate or precipitate necrosis and compartment syndromes. The pressure immobilisation (Sutherland) technique is useful for non-spitting cobra bites where the dominant neurotoxin(s) is lymphatically transported and an arterial tourniquet is effective against the bites of the same snakes and mambas but is extremely uncomfortable and should not be left on for more than 90 minutes. Venom in the mouth should be washed out with water or another bland solution. Venom on the skin should be wiped or washed away. Venom ophthalmia may be complicated by corneal erosions which require repeated slit lamp examinations, specific treatment and follow-up by the practitioner. (See Algorithm 2)

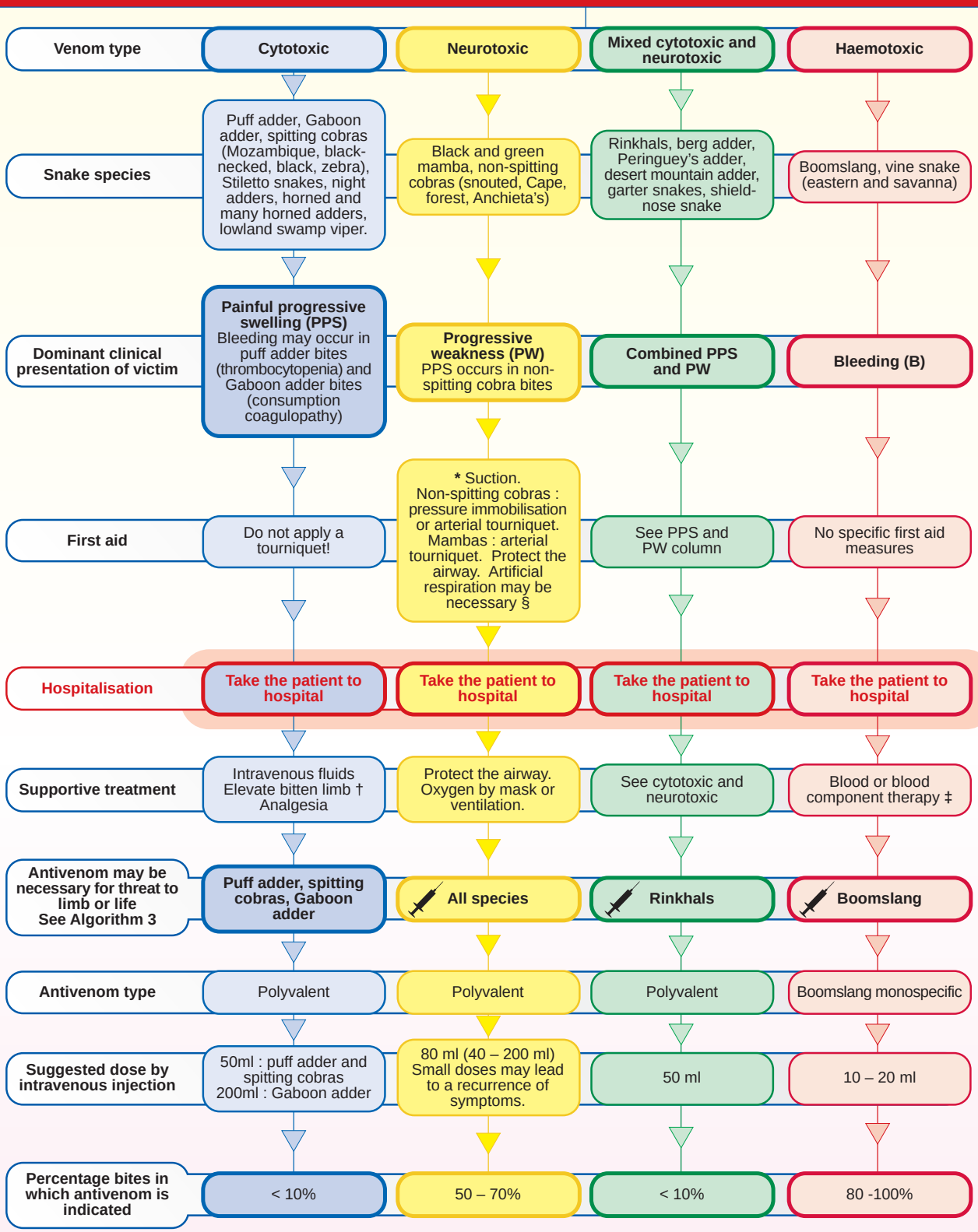
Antivenom

The suggested indications are for threat to limb or life, whether potential or established. (See algorithm 3.) Antivenom is best given in a hospital setting as anaphylaxis may occur, the latter being best prevented or treated with adrenaline.[8]

Take to Practice Messages:

- There is no first aid measure effective against all snake bites.
- Tourniquets are not recommended if the snake species is unknown.
- Syndromic management of snakebite without knowing the species of the snake is logical and effective.
- The majority of poisonous snakebites may be managed without the use of antivenom.
- Antivenom is best administered in a medical setting.
- Antibiotic use is not necessary unless there is bite site necrosis or iatrogenic interference.
- Puff adder bites are responsible for most cases of bleeding.
- Heparin should not be used for a consumption coagulopathy.
- True compartment syndromes are uncommon.

Algorithm 1: Guidelines for the Management of Snakebite



* Mechanical suction device if HIV status is not known (Suction is of minimal benefit but reassuring to the patient.).
† Some authorities prefer keeping the bitten limb at heart level.
‡ Heparin, antifibrinolytics and thrombolytics are of no value and may be dangerous.
§ Polyvalent antivenom for the triad of perioral paraesthesia, excessive salivation and sweating or metallic taste, within a few minutes of the bite (mambas) OR difficulty in breathing.
Polyvalent antivenom is effective against the bites of the mambas, cobras, rinkhals, puff adder and Gaboon adder only.
A test dose of antivenom is not indicated.

Antivenom Unit (SAVP (Pty) Ltd: (011) 386 6000 Fax (011) 386 6016
For emergencies: Contact Dr Roger Blaylock at 083 652 0105

South African manufactured antivenom is recommended as venom from South African snakes is used during manufacture, which negates geographic venom variation. Undiluted antivenom administered intravenously over 10 minutes is as safe as diluted antivenom over 30 minutes[9] and ensures that a medical practitioner is at the bed side should an acute allergic reaction occur. A test dose of antivenom for acute adverse reactions does not predict the response to the main dose and may be omitted. Appropriate administration of antivenom can stop progression of swelling, prevent or reverse an inability to breathe (not the latter in Cape cobra bites) and stop bleeding. Antivenom is efficacious whilst venom is still active as shown by continued deterioration of the patient which in some cases may last several days. Indications for antivenom arise sooner and more frequently in children due to high venom-to-mass ratio which counteracts the increased morbidity and mortality in this age group.

Antivenom is effective and readily available from the SAVP (Pty) Ltd (Antivenom Unit) at Tel 011-386-6000 and Fax 011-386-6016

Antibiotics

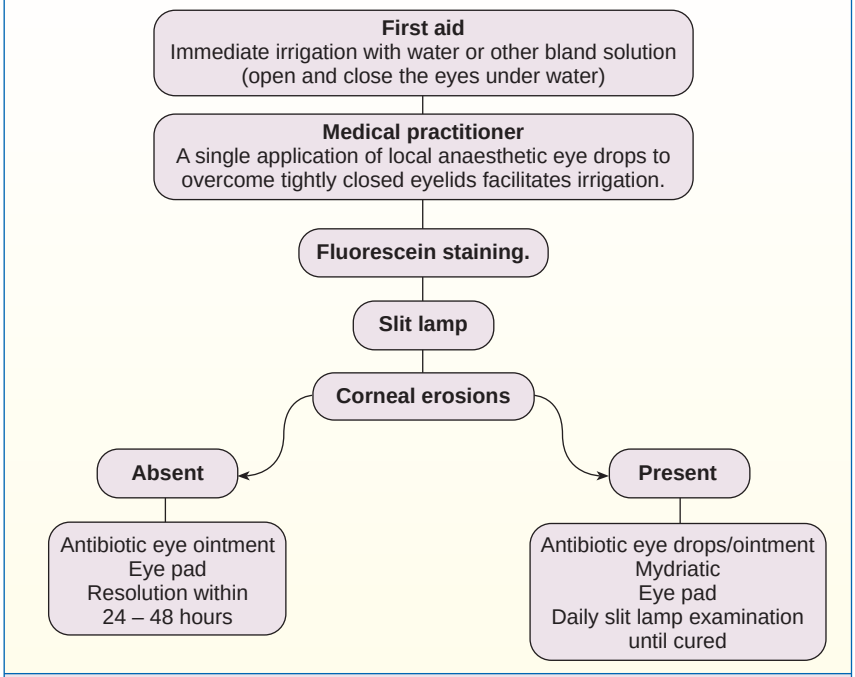
In general, antibiotics are usually unnecessary as bacterial infection is uncommon unless secondary to necrosis or iatrogenic bite site interference.[10] There is a paucity of bacteria in snake mouths which are mainly Gram negative enterobacteriacae and venom has antibacterial properties.[11,12] Steroids are of no value and interfere with the venom / antivenom reaction

Painful progressive swelling (PPS)

Clinical presentation

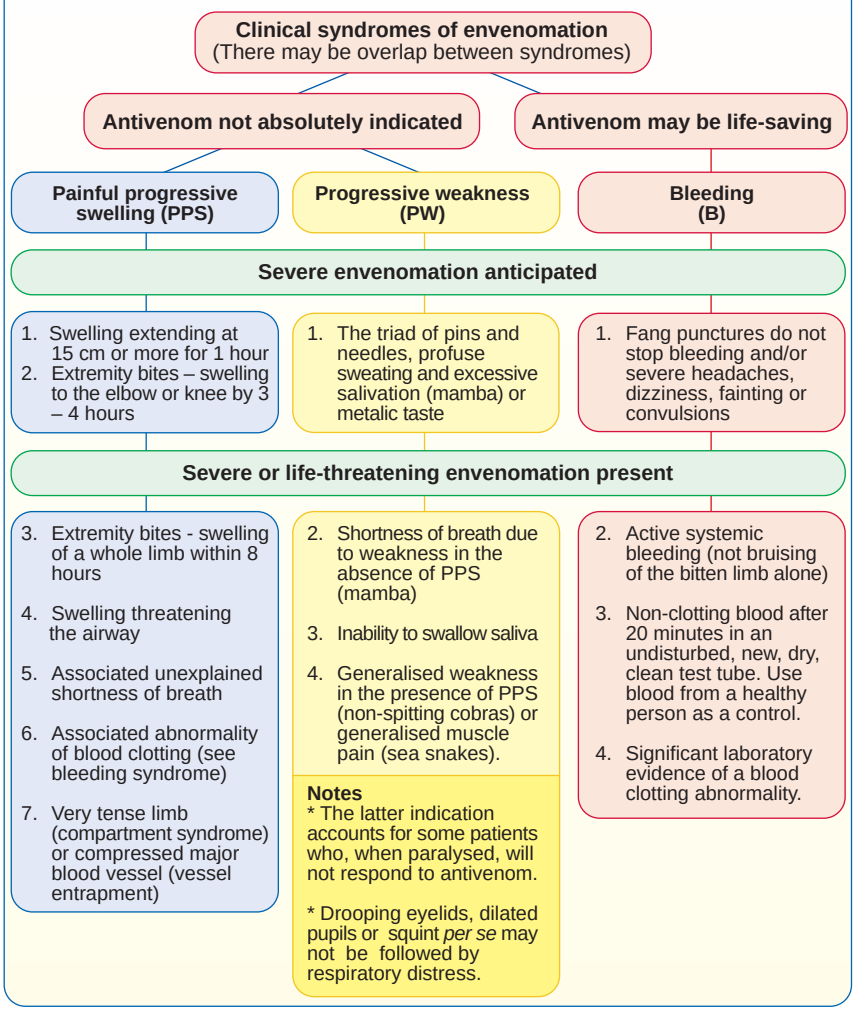
This is by far the most common presentation comprising about 90% of all envenomations and is due to cytotoxic venom. Swelling commences around the bite site within a few minutes and spreads mainly in a proximal direction. It is painful, often tender, warm to hot and indurated. The duration and rate at which it spreads is mainly snake species dependent. It is quicker with the adders at 5 – 10 cm or more per hour whilst that due to stiletto snake and spitting cobra bites spreads at about 1 - 2 cm per hour. It spreads faster soon after the bite and slows before stopping. Within 1 – 2 hours of the bite there is usually painful regional lymphadenopathy.

Algorithm 2: Management of venom ophthalmia



Antivenom topically (dilute) or systemically not indicated.
Steroids (topical or systemic) are contraindicated.

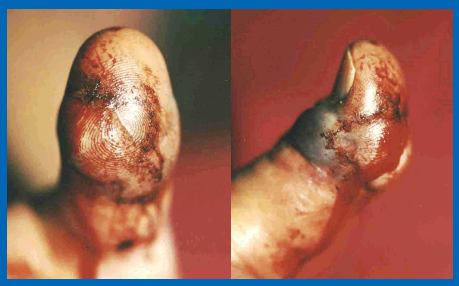
Algorithm 3: Indications for antivenom



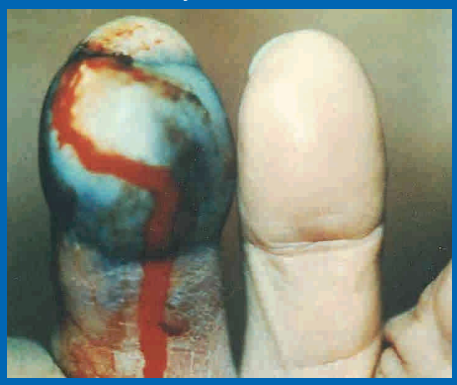
Painful progressive swelling (PPS)

Puff adder bite

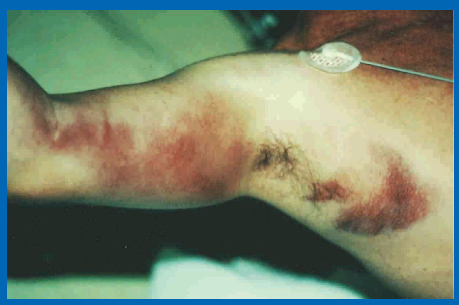
Antivenom was not administered. Platelets 28 x 10^9/l at 4 h 10 min. Blistering does not necessarily equate to necrosis, but did in this case.



Day of the bite



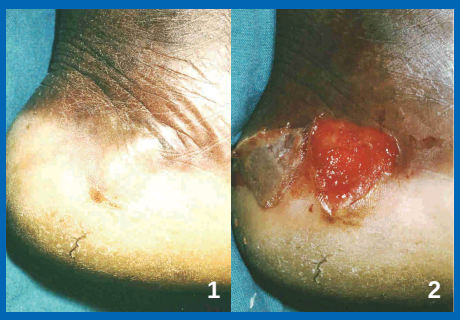
Second day after bite - Note continuous ooze of blood



Second day after bite - Purpura

Common night adder bite

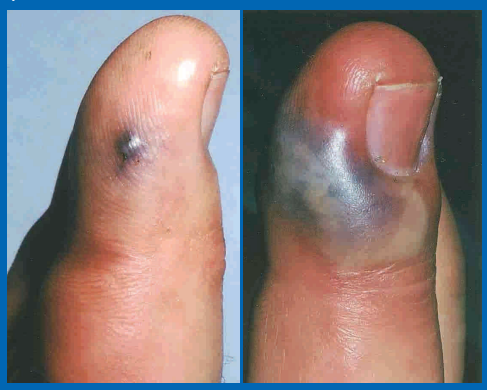
Antivenom is ineffective and should not be used. Necrosis has not been recorded following a night adder bite.



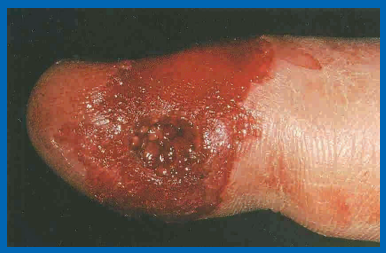
1. Seventh day after bite – Blister formation
2. Seventh day after bite – Deroof of blister

Stiletto snake bite

Antivenom is ineffective and should not be used. There is initial blanching at the bite site. A blister equates to necrosis, which occurs in 25% of bites.



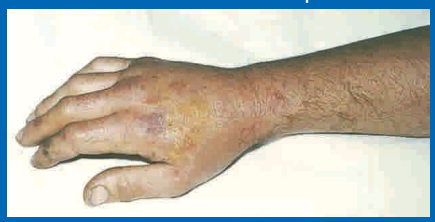
17 hours after bite 5 days after bite



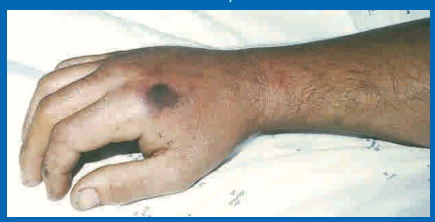
Day five following debridement

Mozambique spitting cobra bite

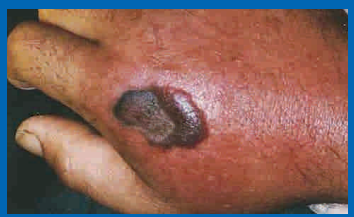
Necrosis occurs in the majority of bites and is usually surrounded by a peripheral blister 4 to 6 days after the bite. Antivenom does not prevent necrosis.



Discoloured area 6,5 hours after bite



Second day after bite – discoloured area larger and more obvious



Seventh day after bite – peripheral blistering. Area of underlying necrosis much larger than appears on the surface.

Complications of PPS may be classified as:

- Local, such as bite site blister, haematoma or necrosis (10%).
- Regional, such as compartment syndrome, nerve and vessel entrapment and deep vein thrombosis (uncommon).
- Systemic, due to loss of fluid and blood components into the swollen area which may lead to hypotension, anaemia, hypoalbuminaemia and hypofibrinogenaemia with prolongation of the PTT and INR. This occurs mostly in those cases where swelling rapidly spreads to the trunk and is most common following puff adder bites. Cardiotoxicity and pulmonary oedema due to circulating venom have only been described following Gaboon adder bites.[13]

Snakes responsible for PPS include:

- Puff adder *(Bitis arietans)*
- Spitting cobras *(Naja mossambica, N .nigricollis sp.)*
- Stiletto snake *(Atractaspis bibronii)*
- Night adders *(Causus sp.)*.

Gaboon adder *(B. gabonica)* and other small adder bites are less common. Although bites by all these snakes lead to PPS there are clinical peculiarities which identifies offending snake species. Bite site necrosis may result.[14-16]

Management (Algorithm 1,3,4)

Elevation is analgesic and diminishes swelling. Intravenous fluids replace what has been lost into the swollen area. Analgesia is important. This triad of elevation, intravenous fluid and analgesia is all that is required for the majority of snake bites. Should there be necrosis, surgery is best left for 5 – 7 days, as, prior to this time, the junction between dead and dying tissue may not be well defined. This procrastination does not prejudice the patient in any way.

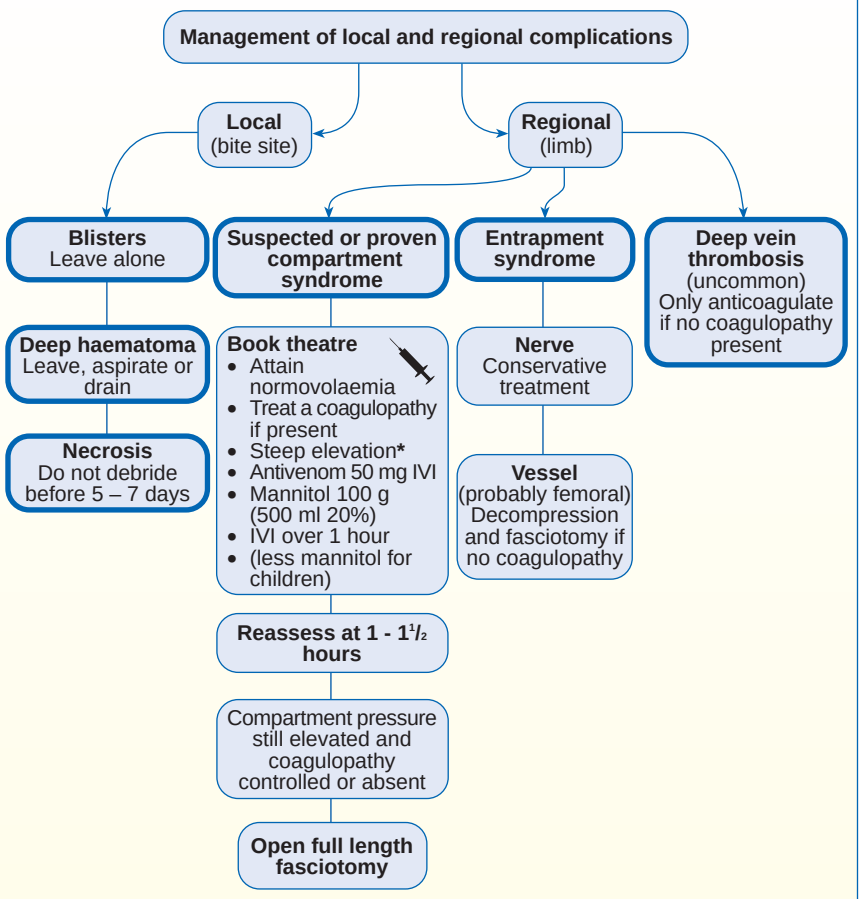
Compartment syndrome of a limb is uncommon but requires urgent attention. Snake bitten limbs may present like compartment syndrome but, on measuring intra-compartmental pressures, most are not.[17] Compartment syndromes of hands and feet self-decompress via the bite site. Compartment syndromes of limbs may be successfully managed conservatively for an hour by elevating the limb, administering intravenous mannitol (reduces swelling and helps prevent renal failure) and intravenous antivenom which, in an appropriate dose, stops progression of swelling. Conservative treatment must be aggressively policed or nothing other than elevation and a drip will have been achieved during this time. Should conservative treatment fail, providing there is no significant coagulopathy, open full-length fasciotomy should be performed.

Carpal tunnel syndrome is not uncommon if bitten on a hand or finger. It is self-limiting and responds to elevation.

Vessel entrapment syndrome of the femoral vessels beneath the inguinal ligament and the axillary vessels at the thoracic outlet lead to limb ischaemia. A blister covered pulseless limb suggests the diagnosis.[18]

Deep vein thrombosis is not common, is usually diagnosed late when swelling persists for several days and should not be anticoagulated if a coagulopathy is still present.

Algorithm 4: Painful Progressive Swelling



Progressive weakness (PW)

Clinical Presentation

Injected neurotoxic venom produces striated muscle dysfunction:

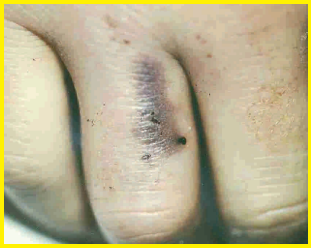
- Venom of the non-spitting cobras (Cape *(Naja nivea)*, snouted (*N. annulifera)*, forest *(N. melanoleuca)* and Anchieta's *(N. anchietae)*) contain curare-like post-synaptic toxins.
- Mamba *(Dendroaspis spp.)* bites lead to excessive circulating levels of acetylcholine
- Some of the small adders, i.e. berg adder *(Bitis atropos)*, Peringuey's adder *(B. peringueyi)*, desert mountain adder *(B. xeropaga)* contain pre-synaptic toxins.

The different mechanisms of producing paresis lead to different symptomatology. Black mamba *(D. polylepis)* bites may be associated with minor swelling which is neither painful nor tender whilst that caused by non-spitting cobras, the rinkhals

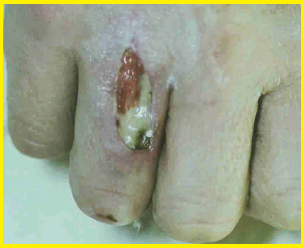
Progressive weakness (PW)

Rinkhals bite

Progressive weakness may occur and necrosis is uncommon. The patient was unconscious within one minute due to venom-induced anaphylaxis.



40,5 hours after bite



10 days after bite

Black mamba bite (juvenile snake)

5 hours after the bite. Puncture wounds were not visible. The patient was ventilated from 3 hours 25 min for 2 hours 5 min. 60 ml polyvalent antivenom IVI was administered during ventilation. Patient discharged the following day. If the patient had been bitten by an adult snake, death would have occurred within half an hour due to high venom-to-mass ratio.

Photographs © RS Blaylock

(Haemachatus haemachatus) and adders is appreciable in extent, painful, tender and bite site necrosis may result.

Management

A clear airway and adequate oxygenation are all important. Ventilation may be required, in which case sedation is mandatory to prevent the patient overhearing disturbing conversations. There is little place for muscle relaxants except during intubation of a struggling hypoxic patient. The approach to neurotoxic bites is depicted in Algorithm 1 and 3.

Bleeding (B)

Clinical Presentation

Injected haemotoxic venom may lead to a bleeding diathysis. Boomslang *(Dispholidus typus)* and vine snake *(Thelotornis spp.)* venom contains procoagulant toxins which activate factors II and X leading to a consumption coagulopathy with all the possible attendant complications and mortality.[19,20] These bites are uncommon.

Puff adder bites are far more common and these snakes are responsible for the majority of patients with bleeding due to snake bite. In this case a whole limb may be swollen, which does not occur with boomslang and vine snake bites.

Gaboon adder bites may lead to a severe consumption coagulopathy as well as significant swelling.[13] These snakes are placid and only found around St Lucia in South Africa making bites decidedly uncommon.

Management

Antivenom has the greatest benefit should a patient have a severe coagulopathy with active bleeding.[21] There is no place for heparin, fibrin stabilising drugs, fibrinolytics or thrombolytics. Venom-induced 'thrombin' is far less susceptible to heparin than physiological thrombin.[22]

(See Algorithm 1 and 3 for further management.)

Referral guidelines

- If the patient's condition is outside of local expertise and resources.
- While awaiting referral, give supportive care (algorithm 1).
- The ability to intubate and ventilate during transfer is essential

Prevention of snakebite

Being sensible is most important. Wearing shoes and using a torch at night are helpful. Do not handle "dead" snakes as some elapid species, particularly the rinkhals, feign death. Sleep in a zip-up tent or tuck a mosquito net under the mattress when on camping trips.

See CPD Questionnaire, page 41

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Low-Dose Adrenaline, Promethazine, and Hydrocortisone in the Prevention of Acute Adverse Reactions to Antivenom following Snakebite: A Randomised, Double-Blind, Placebo-Controlled Trial

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Abstract

Background: Envenoming from snakebites is most effectively treated by antivenom. However, the antivenom available in South Asian countries commonly causes acute allergic reactions, anaphylactic reactions being particularly serious. We investigated whether adrenaline, promethazine, and hydrocortisone prevent such reactions in secondary referral hospitals in Sri Lanka by conducting a randomised, double-blind placebo-controlled trial.

Methods and Findings: In total, 1,007 patients were randomized, using a 2×2×2 factorial design, in a double-blind, placebo-controlled trial of adrenaline (0.25 ml of a 1:1,000 solution subcutaneously), promethazine (25 mg intravenously), and hydrocortisone (200 mg intravenously), each alone and in all possible combinations. The interventions, or matching placebo, were given immediately before infusion of antivenom. Patients were monitored for mild, moderate, or severe adverse reactions for at least 96 h. The prespecified primary end point was the effect of the interventions on the incidence of severe reactions up to and including 48 h after antivenom administration. In total, 752 (75%) patients had acute reactions to antivenom: 9% mild, 48% moderate, and 43% severe; 89% of the reactions occurred within 1 h; and 40% of all patients were given rescue medication (adrenaline, promethazine, and hydrocortisone) during the first hour. Compared with placebo, adrenaline significantly reduced severe reactions to antivenom by 43% (95% CI 25–67) at 1 h and by 38% (95% CI 26–49) up to and including 48 h after antivenom administration; hydrocortisone and promethazine did not. Adding hydrocortisone negated the benefit of adrenaline.

Conclusions: Pretreatment with low-dose adrenaline was safe and reduced the risk of acute severe reactions to snake antivenom. This may be of particular importance in countries where adverse reactions to antivenom are common, although the need to improve the quality of available antivenom cannot be overemphasized.

Trial registration: http://www.ClinicalTrials.gov NCT00270777

Please see later in the article for the Editors' Summary.

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Abbreviations: BP, blood pressure; OR, odds ratio

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Introduction

Globally an estimated 421,000 envenomings and 20,000 deaths occur each year due to snakebite, although the incidence may be as high as 1,841,000 envenomings and 94,000 deaths [1]. Populations with the highest burden (in rural areas of South Asia, Southeast Asia, and sub-Saharan Africa) experience high morbidity and mortality because of poor access to often suboptimal health services; scarcity of antivenom, which is the only specific treatment for snakebite, may also be a problem [2]. The incidence of snakebite in Sri Lanka (based on hospital data) is about 200 per 100,000 individuals per year [1,3], one of the highest in the world. In the North-Central and North-Western Provinces of the country, which have the highest incidence of bites by highly venomous snakes, three regional hospitals reported 1,851 snakebite admissions, with 11 deaths due to snakebite during 2000 [4].

Antivenom is the mainstay of treatment for snakebite. Adverse reactions to the snake antivenoms available in Sri Lanka and other countries in South Asia, which contains equine proteins, are common: both acute (anaphylactoid or pyrogenic) and delayed (serum sickness type) reactions occur [5]. Acute reactions cause the greatest problem: in most cases, symptoms are mild (urticaria, nausea, vomiting, headache, and fever), but in up to 40% of cases, severe systemic anaphylaxis may develop, including bronchospasm and hypotension [6–9]. In Sri Lanka, only Indian-manufactured polyvalent antivenoms are available. The rates of adverse reactions to these antivenoms are high, ranging from 43% to 81% [10–12]. Increasing the safety of treating individuals with snakebite using antivenom therefore has a high priority.

Prophylactic use of hydrocortisone and antihistamines before infusion of antivenom is widely practised, although the theoretical basis for this treatment is unclear and there is limited evidence of efficacy. Subcutaneous adrenaline (epinephrine) significantly reduced the incidence of acute adverse reactions in one prospective study [10], but this study was of inadequate size to establish the safety of pretreatment with adrenaline [13]. A retrospective study in Papua New Guinea suggested that adrenaline pretreatment significantly reduced acute adverse reaction rates to antivenom but that promethazine or hydrocortisone had no effect [14]. This study has subsequently been criticised for its poor design [15]. Other studies investigating the use of pretreatment with hydrocortisone or promethazine have failed to demonstrate any clear benefit [12,16]. In view of this uncertainty about the safety and efficacy of pretreatment to reduce or prevent adverse reactions to antivenom, we conducted a large randomized, placebo-controlled, double-blind trial to determine whether low-dose adrenaline, promethazine, and hydrocortisone, alone and in all possible combinations, are significantly better than placebo in preventing acute adverse reactions to antivenom in snakebite victims.

Methods

Subjects and Procedures

The study was developed for secondary referral hospitals in areas in Sri Lanka with a high incidence of snakebite (Text S1). It was initiated in March 2005 at Anuradhapura, Kurunegala, and Polonnaruwa hospitals. Polonnaruwa and Kurunegala hospitals participated throughout the study to its conclusion in April 2008. The study was terminated in Anuradhapura in June 2005. Recruitment was subsequently moved to Embilipitiya hospital for the period November 2005 to May 2006, and thereafter to Hambantota hospital until the conclusion of the trial. These changes were made for a combination of administrative reasons and poor recruitment rates, and were approved at each step by the ethics review committee that approved the study. At any given time, no more than three hospitals participated in the study.

All patients who presented after snakebite were screened for eligibility by attending clinical staff (Table 1). Those over age 12 y requiring antivenom were eligible for randomisation. All participants provided written informed consent; for those unable to give consent or less than 16 y of age, a relative provided written informed consent.

The primary aim was to determine whether low-dose adrenaline (0.25 ml of a 1:1,000 solution subcutaneously; i.e., 250 micrograms), promethazine (25 mg intravenously), or hydrocortisone (200 mg intravenously), alone or in combination, given as pretreatment, significantly reduced severe adverse reactions to antivenom compared with placebo (0.9% NaCl) up to and including 48 h. All time points relate to time after starting the antivenom infusion. Adverse reactions to antivenom were predefined as mild, moderate, and severe based on an international classification of anaphylaxis reactions [17] (Table 2). We also assessed the safety of the pretreatment medication, looking specifically for complications that might be caused by adrenaline: arrhythmias, increased systolic blood pressure (BP) (>30 mm Hg increase), and intracerebral haemorrhage.

Patients were randomized with equal probability to one of eight different treatments in a 2×2×2 factorial blinded design, using a triple-dummy technique (Figure 1). Stratified block randomization was done by hospital site. For each site, computer-generated random allocation sequences were prepared independently by the trial statistician. All trial medications were prepared at the Clinical Trials Unit, Faculty of Medicine, University of Kelaniya, and packaged in identical sealed envelopes. Syringes containing adrenaline and adrenaline placebo were clearly marked to ensure that they were not administered intravenously. The envelopes, with unique, centre-specific identification numbers, were stored on site.

Patients were seen by ward doctors within 10 min of admission and examined. Baseline investigations, such as electrocardiography and assessment of blood clotting, were done as indicated. Randomization occurred after clinical assessment by ward doctors and after written informed consent had been obtained. Patients remained under the care of consultant physicians following management protocols based on current treatment guidelines that had been approved by the study team. The ward team made all clinical decisions relating to patient care and administered the pretreatment medication and antivenom. Monitoring for acute reactions was carried out independently by groups of three medically qualified clinical research coordinators dedicated to each site who were blind to the interventions. Participants were observed continuously for the first 2 h and then reviewed at 4-h intervals until 48 h.

Patients were given ten vials of antivenom dissolved in 500 ml of isotonic saline as an intravenous infusion over 1 h. Antivenom treatment was repeated as deemed necessary by attending clinicians, according to clinical judgement. However, patients were not given further doses of trial medication, even if antivenom was repeated. Patients were monitored using a clinical observation protocol developed jointly by consultant physicians and study coordinators for acute adverse reactions to antivenom and any adverse reactions to the study drugs. Study-related patient information was recorded in standardised clinical record forms.

Patients were kept in hospital for at least 96 h after the infusion of antivenom. If a reaction developed during infusion, or if a patient developed cardiac arrhythmias, ischaemic changes on the electrocardiogram, a rise in BP (for systolic, an increase of

Table 1. Inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
Patients above 12 y of age	Patients who are pregnant or nursing
Patients admitted to hospital after snakebite in whom antivenom is indicated	Patients who are currently taking beta- or alpha-adrenoceptor antagonists, or tricyclic antidepressants
Patients who give informed consent	Patients in whom adrenaline may be contraindicated (this may include patients with the following): (1) history of ischaemic heart disease or stroke, (2) uncontrolled hypertension, (3) tachyarrhythmias

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>30 mm Hg, or for diastolic, an increase of >20 mm Hg, from pretreatment level), a fall in BP (for systolic, a decrease of >20 mm Hg, or for diastolic, a decrease of >10 mm Hg, from pretreatment level), or anaphylaxis after the study drug and antivenom, appropriate treatment ("rescue medication") was given solely at the discretion of the attending clinicians. Reactions to antivenom were treated by stopping the antivenom infusion temporarily, and giving, alone or in any combination, 0.25 ml (mild reactions) or 0.5 ml (moderate and severe reactions) of 1:1,000 adrenaline intramuscularly, 25 mg of promethazine intravenously, or 200 mg of hydrocortisone intravenously (rescue medication).

Ethics committee approval was received from the Ethics Review Committee, Faculty of Medicine, University of Kelaniya. An independent data monitoring committee was provided with interim analyses when information from groups of 200 new patients became available. In the light of these analyses and the results of any other new relevant information, the data monitoring committee was instructed to advise the principal investigator if, in the committee's view, there was proof beyond reasonable doubt that the data showed that any part of the protocol under investigation became clearly indicated or contraindicated, either for all participants or for a specific subgroup of trial participants, or if it appeared that no clear outcome would be obtained. However, no data monitoring committee–driven changes to protocol were made as a result of interim analyses.

Statistical Analysis

Sample size calculations. We estimated that acute adverse reactions would occur in about 40% of patients who received antivenom and that a reduction of over 25% in the rate of acute adverse reactions would correspond to a substantial benefit. Using the proposed design, a sample size of 1,000 gave 80% power to detect a 25% relative reduction in adverse reactions from the current reaction rate by any one treatment, at $p<0.01$.

Analysis. The prespecified primary outcome measure was the frequency of severe reactions to antivenom up to and including 48 h after antivenom administration in those allocated to each treatment compared to those not allocated to that treatment. Secondary outcomes were rates of severe reactions within 1 or 6 h, rates of any adverse reactions up to and including 48 h, and acute adverse reactions to study treatments separately (prespecified as arrhythmias, intracerebral haemorrhage, or an increase in systolic BP>30 mm Hg). The 2×2×2 factorial design used for this trial facilitates primary analyses to determine the main effects of the three treatments, and allows investigation of two-way and three-way interactions.

Analyses were undertaken on an intention-to-treat basis using logistic regression, and took into account clustering by trial site. The final model included the three trial medications and all three two-way interaction terms. Odds ratios (ORs) and 95% confidence intervals for the effects of each treatment and the two-way interactions were calculated. This superseded our original intention to compare event rates for those who received a particular drug versus those not given that drug, and to repeat these analyses with stratification by other treatments administered to check for interactions between trial medications. This change was made on the advice given by the statistical reviewer for the journal.

No allowance was to be made for multiple comparisons in the primary analyses but for secondary and, particularly, for tertiary comparisons, allowance was made for multiple hypothesis testing, taking into account the nature of the events (including timing, duration, and severity) and evidence from other studies.

Results

From March 2005 to April 2008, 4,677 patients who presented after snakebite to trial hospitals were screened, and 1,007 eligible patients were randomized (53 at Anuradhapura, 16 at Embilipitiya, 152 at Hambantota, 353 at Kurunegala, and 433 at Polonnaruwa) (Text S2). The main reason for exclusion was lack of clinical indication for antivenom. Recruitment was stopped when the target sample size of 1,000 was reached in April 2008. All the randomized patients completed the study and were evaluated; there were no protocol deviations.

Table 3 shows the baseline demographic and other clinical characteristics in the three treatment groups and shows good balance between the groups. The median time from snakebite to administration of antivenom was similar at the different hospitals (median time ranged from 3.9 to 4.6 h). More than 70% of patients were transferred from smaller rural hospitals. Some of them had received antivenom (20% of all study patients), hydrocortisone (25% of all study patients), or promethazine (9.7% of all study patients) before transfer to a trial hospital. None of the patients had been given adrenaline. This did not have a significant effect on the trial outcomes. The biting snake species was identified in only 25% of the cases.

In total, 752 patients (75%) developed acute reactions to antivenom within 48 h of administration (Table 4), of which 667 reactions (almost 90%) occurred in the first hour (Figure 2). Of these, 9% were mild reactions, 48%, moderate, and 43%, severe; 83% of severe reactions occurred in the first hour. After the first hour the category of reaction changed in 128 patients (12.7%); this change in reaction category took place before the end of 6 h

Table 2. Classification of acute adverse reactions to antivenom.

Mild	Moderate	Severe
Facial oedema	Abdominal pain	Drowsiness or altered consciousness
Pruritus	Nausea	Systolic BP < 80 mm Hg
Urticaria	Vomiting	Cyanosis
Fever[a]	Bronchospasm	Confusion
Rigor[a]	Stridor	

[a]Not in original classification [17] but added to capture all of the systemic reactions.

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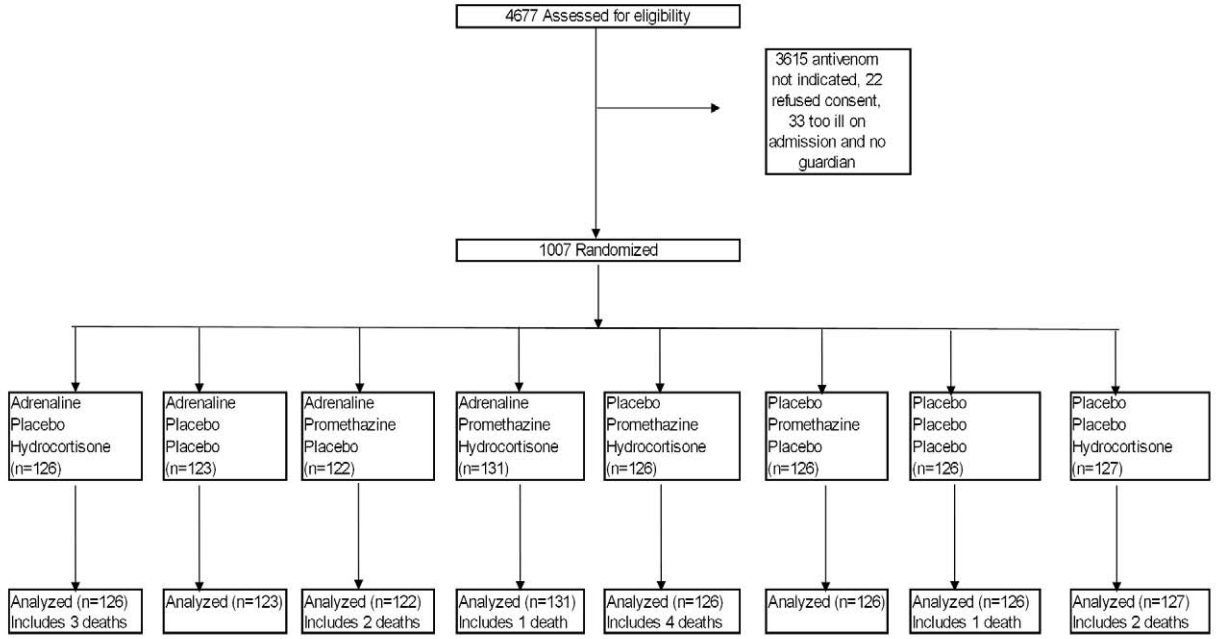

Figure 1. Trial profile.
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in 93 of these 128 patients (Figure 2). There was a change in reaction category after 6 h in only 35 patients, and this included the one patient whose reaction category changed from moderate to severe during the second 24 h of observation. Patients were given rescue medication at the discretion of the attending clinicians and managed as clinically indicated. In all, 40% of patients received rescue medication within the first hour: 27% of all patients with mild or no acute reactions, 47% of all patients with moderate reactions, and 50% of all patients with severe reactions.

Adrenaline reduced the rate of severe adverse reactions compared with placebo at 1 h by 43% (OR 0.57, 95% CI 0.43–0.75; $p<0.001$); and by 38% over 48 h (OR 0.62, 0.51–0.74; $p<0.001$) (Tables 5 and 6). Neither hydrocortisone nor prometh-

Table 3. Patient baseline characteristics by treatment allocation.

Characteristic	Adrenaline		Hydrocortisone		Promethazine		Total ($n=1,007$)
	Yes ($n=502$)	No ($n=505$)	Yes ($n=510$)	No ($n=497$)	Yes ($n=505$)	No ($n=502$)	
Male, *n* (%)	392 (78.1)	384 (76.0)	388 (76.1)	388 (78.1)	383 (75.8)	393 (78.3)	776 (77.1)
Age in years, mean (standard deviation)	36.0 (13.6)	37.1 (13.5)	36.0 (13.4)	37.1 (13.7)	36.8 (13.8)	36.3 (13.4)	36.5 (13.6)
Time between bite and antivenom in hours, median (interquartile range)	4.3 (2.8–6.8)	4.3 (2.9–6.8)	4.3 (2.8–6.7)	4.3 (3.0–7.2)	4.2 (2.8–7.9)	4.4 (3–6.9)	4.3 (2.9–6.8)
Direct admission, *n* (%)	136 (27.1)	134 (26.5)	141 (27.7)	129 (26.0)	155 (30.7)	115 (22.9)	270 (26.8)
History of previous snakebite, *n* (%)	51 (10.2)	54 (10.7)	54 (10.6)	51 (10.3)	60 (11.8)	45 (9.0)	105 (10.4)
Snake identified (%)	135 (26.9)	124 (24.6)	126 (24.7)	133 (26.7)	124 (24.6)	135 (26.9)	259 (25.7)
Antivenom given before transfer, *n* (%)	102 (20.3)	103 (20.4)	96 (18.8)	109 (21.9)	95 (18.8)	110 (21.9)	205 (20.4)
Hydrocortisone given before transfer, *n* (%)	127 (25.3)	128 (25.4)	131 (25.7)	124 (25.0)	117 (23.2)	138 (27.5)	255 (25.3)
Promethazine given before transfer, *n* (%)	47 (9.4)	51 (10.1)	45 (8.8)	53 (10.7)	46 (9.1)	52 (10.3)	98 (9.7)
History of allergy, *n* (%)	37 (7.4)	45 (8.9)	39 (7.7)	43 (8.7)	42 (8.3)	40 (8.0)	82 (8.1)
History of bronchial asthma, *n* (%)	25 (5.0)	32 (6.3)	32 (6.3)	25 (5.0)	30 (5.9)	27 (5.4)	57 (5.7)

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Table 4. Outcomes during first hour and 48 h by treatment allocation.

Outcome	Reaction	Adrenaline		Hydrocortisone		Promethazine		Total ($n=1,007$)
		Yes ($n=502$)	No ($n=505$)	Yes ($n=510$)	No ($n=497$)	Yes ($n=505$)	No ($n=502$)	
Reaction during first hour	None, *n* (%)	185 (36.9)	155 (30.7)	170 (33.3)	170 (34.2)	182 (36.0)	158 (31.5)	340 (33.8)
	Mild, *n* (%)	43 (8.6)	41 (8.1)	39 (7.7)	45 (9.1)	29 (5.7)	55 (11.0)	84 (8.3)
	Moderate, *n* (%)	154 (30.7)	161 (31.9)	164 (32.2)	151 (30.4)	167 (33.1)	148 (29.5)	315 (31.3)
	Severe, *n* (%)	120 (23.9)	148 (29.3)	137 (26.9)	131 (26.4)	127 (25.2)	141 (28.1)	268 (26.6)
	Any reaction, *n* (%)	317 (63.1)	350 (69.3)	340 (66.7)	327 (65.8)	323 (64.0)	344 (68.5)	667 (66.2)
	OR[a] (95% CI) for severe reaction	0.57 (0.43–0.75)		0.86 (0.60–1.24)		0.81 (0.51–1.30)		
	OR[a] (95% CI) for any reaction	0.76 (0.64–0.91)		1.04 (0.85–1.28)		0.81 (0.65–1.02)		
Reaction during 48 h	None, *n* (%)	135 (26.9)	120 (23.7)	126 (24.7)	129 (26.0)	128 (25.4)	127 (25.3)	255 (25.3)
	Mild, *n* (%)	29 (5.8)	37 (7.3)	30 (5.9)	36 (7.2)	22 (4.4)	44 (8.8)	66 (6.6)
	Moderate, *n* (%)	184 (36.7)	180 (35.6)	188 (36.9)	176 (35.4)	197 (39.0)	167 (33.3)	364 (36.2)
	Severe, *n* (%)	154 (30.7)	168 (33.3)	166 (32.5)	156 (31.4)	158 (31.3)	164 (32.7)	322 (32.0)
	Any reaction, *n* (%)	367 (73.1)	385 (76.3)	384 (75.3)	368 (74.0)	377 (74.6)	375 (74.7)	752 (74.7)
	OR[a] (95% CI) for severe reaction	0.62 (0.51–0.74)		0.80 (0.53–1.21)		0.87 (0.50–1.52)		
	OR[a] (95% CI) for any reaction	0.85 (0.71–1.00)		1.07 (0.87–1.32)		1.00 (0.74–1.35)		
Rescue medication during first hour		206 (41.0)	191 (37.8)	186 (36.5)	211 (42.5)	194 (38.4)	203 (40.4)	
		$X^2=1.09$; $p=0.30$		$X^2=3.77$; $p=0.052$		$X^2=0.43$; $p=0.51$		
Time (min) to rescue medication, mean (standard error)		30.7 (2.2)	25.9 (1.6)	31.1 (2.1)	25.4 (1.7)	30.7 (2.1)	25.8 (1.7)	
		$t=1.78$; $p=0.08$		$t=2.13$; $p=0.03$		$t=1.86$; $p=0.06$		

All time points relate to time after starting the antivenom infusion.

[a]For predictors of severe reaction, ORs were calculated using the main effects and all two-way interactions of the trial medications; for predictors of any reaction, ORs were calculated using only the main effects of the trial medications because there were no significant interactions.

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azine had any significant effect on the risk of severe adverse reactions at 1 h or 48 h (Tables 5 and 6). The same pattern was observed at 6 and 24 h (Tables S1 and S2, respectively). There was some evidence that the effect of allocation to hydrocortisone in addition to adrenaline negated the benefit of adrenaline (OR 1.50, 95% CI 1.09–2.07; $p=0.013$). Furthermore, adrenaline, but neither hydrocortisone nor promethazine, reduced the rate of all reactions, especially at 1 h (Table 4).

Adrenaline and promethazine seemed to be safe (Table 7): only 13 (1.3%) patients died. All deaths were considered by the supervising physician to be consequences of envenoming or complications that developed during intensive care treatment for envenoming (one death from pneumonia, four from sepsis, three from shock, three from acute renal failure, and two from respiratory failure). There were significantly more deaths among those who received hydrocortisone compared to no hydrocortisone (ten [2%] versus three [0.6%]; OR 3.3, 95%CI 1.28–8.52; $p=0.014$) (Table 8). In all, 261 patients had a significant rise in BP (increase in systolic BP of >30 mm Hg and/or increase in diastolic BP of >20 mm Hg) within 48 h, but there was no significant association between rise in BP and trial medications, individually or combined at 30 or 60 min (Table 7). No patient had an intracerebral haemorrhage or arrhythmia. There was no significant difference in the use of rescue medication between the treatment groups.

Discussion

Reactions to antivenom present considerable challenges to clinicians treating snakebite. The frequency of early reactions varies markedly between individual antivenoms and between different batches of antivenom from the same manufacturer, occurring with a frequency that ranges from less than 0.5% up to 87%, although only a small proportion of reactions are life threatening [7]. The high reaction rates of 75% observed in this study are in line with the rates of between 43% and 81% that were observed in three previous Sri Lankan studies [10–12].

Given such high rates of antivenom reactions in some settings, it is not surprising that pharmacological prophylaxis has been advocated to reduce acute adverse reactions to antivenom. Before this study, only the routine use of adrenaline was supported by any evidence. Low-dose subcutaneous adrenaline given immediately before antivenom to snakebite victims significantly reduced the incidence of acute adverse reactions to the antivenom from 43% to 11% [10]. However, the study included only 102 participants, primarily observed for the first hour after infusion, and could not

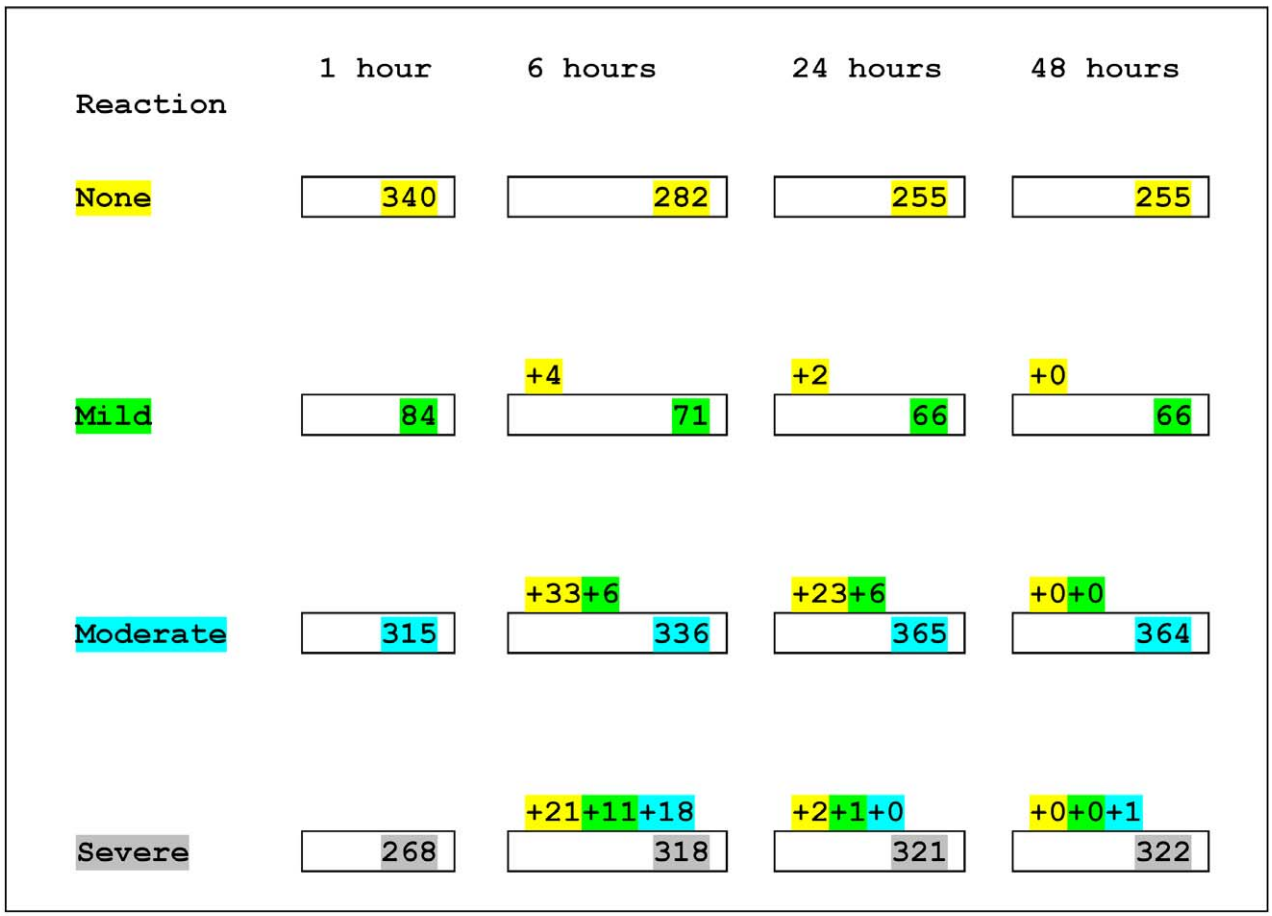

Figure 2. Progression of type of reaction over 48 h. Numbers within boxes indicate the number of patients according to the highest category of reaction they had experienced by that time. Numbers above the boxes indicate the number of patients who experienced a higher category of reaction during the preceding interval. Those who changed from no reaction to a reaction category are indicated by numbers highlighted in yellow. Those who changed from mild reaction to a higher category are indicated by numbers highlighted in green. Those who changed from moderate to severe reaction are indicated by numbers highlighted in turquoise. For example the above numbers can be interpreted as follows. At 1 h, there were 315 patients with moderate reaction, and by 6 h, 336 patients were classified as moderate reactions; 33 patients who had had no reaction in 1 h had a moderate reaction during this interval, six patients who had had mild reaction in 1 h had a moderate reaction during this interval, and 18 patients who had had moderate reaction in 1 h had a severe reaction during this interval: 336 = (315+33+6) − 18.
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Table 5. Risk of severe reaction during the first hour by treatment: main effects and two-way interactions adjusted for clustering by trial site.

Treatment	Severe Reaction			Logistic Regression Model, Main Effects and Two-Way Interactions		
	Yes	No	Total	OR	95% CI	*p*-Value
Adrenaline	28	95	123	0.57	0.43–0.75	<0.001
Hydrocortisone	39	88	127	0.86	0.60–1.24	0.430
Promethazine	37	89	126	0.81	0.51–1.30	0.378
Adrenaline and hydrocortisone	33	93	126	1.50	1.09–2.07	0.013
Adrenaline and promethazine	25	97	122	1.17	0.85–1.61	0.327
Hydrocortisone and promethazine	31	95	126	0.97	0.64–1.47	0.896
Adrenaline, hydrocortisone, and promethazine	34	97	131			
Triple placebo	41	85	126			
Total	268	739	1,007			

There were no three-way interactions. Data are from five hospitals. All time points relate to time after starting the antivenom infusion.
doi:10.1371/journal.pmed.1000435.t005

Table 6. Risk of severe reaction up to and including 48 h by treatment: main effects and two-way interactions adjusted for clustering by trial site.

Treatment	Severe Reaction			Logistic Regression Model, Main Effects and Two-Way Interactions		
	Yes	No	Total	OR	95% CI	*p*-Value
Adrenaline	33	90	123	0.62	0.51–0.74	<0.001
Hydrocortisone	41	86	127	0.80	0.53–1.21	0.296
Promethazine	43	83	126	0.87	0.50–1.52	0.629
Adrenaline and hydrocortisone	43	83	126	1.76	1.24–2.50	0.002
Adrenaline and promethazine	33	89	122	1.16	0.80–1.69	0.441
Hydrocortisone and promethazine	37	89	126	1.00	0.65–1.55	0.999
Adrenaline, hydrocortisone, and promethazine	45	86	131			
Triple placebo	47	79	126			
Total	322	685	1,007			

There were no three-way interactions. Data are from five hospitals. All time points relate to time after starting the antivenom infusion.
doi:10.1371/journal.pmed.1000435.t006

establish safety, a major concern regarding the use of adrenaline as a prophylactic agent [13], particularly the risk of intracerebral haemorrhage [18,19]. Although a recent study from Papua New Guinea suggested that adrenaline pretreatment was effective [14], the retrospective design, lack of standardised definitions, and a selective statistical analysis that did not correct for multiple comparisons make it difficult to draw firm conclusions from this study.

Prophylactic use of hydrocortisone and antihistamines before infusion of antivenom is widely implemented. However, one small randomized controlled trial demonstrated no benefit from the routine use of antihistamines [16]. Hydrocortisone takes several hours to act and may be ineffective as a prophylactic against acute adverse reactions that develop almost immediately after antivenom treatment. One small study (52 patients) showed that intravenous hydrocortisone alone was ineffective in preventing acute adverse reactions to antivenom, but demonstrated a trend towards hydrocortisone reducing reactions when given with intravenous chlorphenamine [12]. However, all of the reactions were mild or moderate, and the trial was not designed to study the efficacy of chlorphenamine alone, making it difficult to interpret the results.

In contrast to these small studies, our trial enrolled just over 1,000 patients, and 752 patients experienced reactions. Our prespecified primary end point was the development of severe reactions to antivenom during the first 48 h after its administration. However, our data clearly showed that more than 80% of severe reactions occurred during the first hour after antivenom administration, and only a negligible number of severe reactions occurred more than 6 h after antivenom administration. Furthermore, about 40% of patients were given rescue medication (i.e., adrenaline, hydrocortisone, or promethazine as rescue medication irrespective of the randomization) in the first hour after antivenom administration. Such early administration of rescue medication may have diluted the effects of the randomization on reactions at the later time points, but should not have affected rates of reactions at 1 h, and we therefore chose to focus on severe reactions during the first hour. Previous studies have used a variety

Table 7. Heart rate, blood pressure, and number of patients with rise in blood pressure at 30 min and 60 min after pretreatment administered.

Time after Pretreatment	Measure	Adrenaline		Hydrocortisone		Promethazine	
		Yes (*n* = 502)	No (*n* = 505)	Yes (*n* = 505)	No (*n* = 502)	Yes (*n* = 505)	No (*n* = 502)
30 min	Heart rate	94.9 (0.91)	94.9 (0.93)	96.2 (0.95)	93.7 (0.89)	95.2 (0.94)	94.6 (0.90)
	Systolic BP	114.9 (0.97)	111.5 (1.01)	113.9 (0.98)	112.6 (1.00)	113.5 (1.02)	112.9 (0.95)
	Diastolic BP	70.3 (0.68)	68.9 (0.66)	70.3 (0.68)	69.0 (0.65)	69.4 (0.67)	69.9 (0.67)
	Number of patients with rise in BP[a]	63 (12.6)	52 (10.3)	63 (12.4)	52 (10.4)	66 (13.1)	49 (9.8)
60 min	Heart rate	93.4 (0.85)	93.0 (0.88)	94.2 (0.88)	92.3 (0.85)	93.4 (0.85)	93.1 (0.87)
	Systolic BP	117.1 (0.85)	114.2 (0.94)	115.6 (0.93)	115.6 (0.87)	116.5 (0.91)	114.7 (0.89)
	Diastolic BP	71.5 (0.61)	69.8 (0.64)	71.2 (0.65)	70.2 (0.61)	70.8 (0.63)	70.6 (0.63)
	Number of patients with rise in BP[a]	84 (16.7)	64 (12.7)	82 (16.1)	66 (13.3)	85 (16.8)	63 (12.6)

All values are mean (standard error).
[a]An increase in systolic BP of >30 mm Hg and/or diastolic BP of >20 mm Hg higher than baseline.
doi:10.1371/journal.pmed.1000435.t007

Table 8. Risk of death by treatment: main effects adjusted for clustering by trial site.

Treatment	Death			Logistic Regression Model, Main Effects		
	Yes	No	Total	OR	95% CI	*p*-Value
Adrenaline	0	123	123	0.85	0.39–1.85	0.681
Hydrocortisone	2	125	127	3.30	1.28–8.52	0.014
Promethazine	0	126	126	1.16	0.80–1.68	0.220
Adrenaline and hydrocortisone	3	123	126			
Adrenaline and promethazine	2	120	122			
Hydrocortisone and promethazine	4	122	126			
Adrenaline, hydrocortisone, and promethazine	1	130	131			
Triple placebo	1	125	126			
Total	13	994	1,007			

Data from five hospitals.
doi:10.1371/journal.pmed.1000435.t008

of different definitions for reactions, and we chose to use an established international grading [17] in an attempt to standardise this; rates of severity of reactions are therefore not directly comparable to previous studies. The factorial design enabled us to investigate both direct effects and interactions between the different medications in the most efficient manner.

We found that administration of adrenaline significantly and substantially reduced the risk of severe adverse reactions in the first hour and that this was still apparent at 48 h, but neither hydrocortisone nor promethazine had any clear effect. We have also unequivocally demonstrated that a dose of subcutaneous adrenaline of 250 micrograms is safe after snakebite, even where there is coagulopathy. While pretreatment with hydrocortisone or promethazine did not reduce severe reaction rates to antivenom significantly, hydrocortisone negated the beneficial effects of adrenaline when these treatments were given together. However, given the multiple comparisons and post-hoc nature of this finding, it should be interpreted cautiously. Hydrocortisone was also associated with an increased risk of death, but this finding was based on very small numbers. Given that hydrocortisone has no benefit and may even be harmful, we would discourage its current widespread empirical use as a pretreatment before antivenom administration.

The mechanism of reactions to antivenom is uncertain. Acute reactions may be due to type 1 (IgE-mediated) hypersensitivity, but antivenom reactions often occur in those with no previous exposure to equine proteins. Although some commercial antivenoms have anticomplement activity in vitro, complement activation has never been clearly demonstrated in patients with antivenom reactions [6,20,21]. Early reactions are most likely to be due to a combination of type 1 hypersensitivity, complement activation, and the effect of aggregates of immunoglobulin or immunoglobulin fragments, including Fc, which can be found in even highly refined antivenoms [22]. Although theoretically cleaving of the IgG molecule into smaller fragments should reduce the incidence of antivenom reactions, this has not been shown in clinical studies, and the major influence on reaction rates appears to be the manufacturing process [7]: there is emerging evidence that the use of caprylic acid, which results in a more pure IgG preparation, may reduce reaction rates [23,24]. Slow infusion of antivenom intravenously (rather than administration by bolus injection) has also been advocated as a way of reducing reaction rates, although the only small comparative study of methods of administration found no difference in the rates and severity of reactions between a 30-min infusion and intravenous injection over 10 min. Using a small test dose of antivenom to detect patients who may develop acute adverse reactions to the antivenom has no predictive value and can itself cause anaphylactic reactions [6,25].

The high rate of adverse reactions to antivenom observed in our study is common to large areas of South Asia, and is an example of how poor manufacturing and quality control by antivenom producers causes substantial problems for patients and their doctors. This highlights the importance of addressing issues of poor quality and potentially unsafe antivenom. Even well-manufactured antivenom may be associated with severe adverse reaction rates of up to 5% [15]. We therefore welcome the recent World Health Organization guidelines on production, control, and regulation of antivenom [26]. The need for concerted action by local health and regulatory authorities, the World Health Organization, and other stakeholders, including technology transfer programmes between antivenom manufacturers, to improve the quality of antivenom can not be overemphasized. Ultimately, the prevention of antivenom reactions will depend on improving the quality of antivenom. The increasing recognition of the considerable burden of snakebite and its treatment will hopefully lead to such improvements. Until these overdue improvements come about, we have shown that pretreatment with low-dose adrenaline is an effective and safe therapy to prevent acute reactions to antivenom. This finding may be of particular relevance in areas where adverse reactions to antivenom are common. Meanwhile, we continue to reiterate that the need for careful observation of patients receiving antivenom and prompt treatment of acute reactions when they occur remains undiminished.

Supporting Information

Table S1 Risk of severe reaction during the first 6 h by treatment. Main effects and two-way interactions adjusted for clustering by trial site.
Found at: doi:10.1371/journal.pmed.1000435.s001 (0.04 MB DOC)

Table S2 Risk of severe reaction during first 24 h by treatment. Main effects and two-way interactions adjusted for clustering by trial site.
Found at: doi:10.1371/journal.pmed.1000435.s002 (0.04 MB DOC)

Text S1 Study protocol.
Found at: doi:10.1371/journal.pmed.1000435.s003 (0.10 MB DOC)

Text S2 CONSORT checklist.
Found at: doi:10.1371/journal.pmed.1000435.s004 (0.22 MB DOC)

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Author Contributions

ICMJE criteria for authorship read and met: HAdS AP CDR SJ SBS AH RK GAR WU JKA JMA DGL HJdS. Agree with the manuscript's results and conclusions: HAdS AP CDR SJ SBS AH RK GAR WU JKA JMA DGL HJdS. Designed the experiments/the study: HAdS CDR AH JKA JMA DGL HJdS. Analyzed the data: HAdS AP JKA JMA DGL. Collected data/did experiments for the study: HAdS SJ SBS AH RK GAR WU. Enrolled patients: HAdS SJ SBS AH RK GAR WU. Wrote the first draft of the paper: HAdS CDR HJdS. Contributed to the writing of the paper: HAdS AP CDR SJ SBS JKA DGL HJdS. Provided comments on the manuscript: JMA. Participated in the design; supervised the study: HJdS.

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Editors' Summary

Background. Of the 3,000 or so snake species in the world, about 600 are venomous. Venomous snakes, which are particularly common in equatorial and tropical regions, immobilize their prey by injecting modified saliva (venom) into their prey's tissues through their fangs—specialized hollow teeth. Snakes also use their venoms for self-defense and will bite people who threaten, startle, or provoke them. A bite from a highly venomous snake such as a pit viper or cobra can cause widespread bleeding, muscle paralysis, irreversible kidney damage, and tissue destruction (necrosis) around the bite site. All these effects of snakebite are potentially fatal; necrosis can also result in amputation and permanent disability. It is hard to get accurate estimates of the number of people affected by snakebite, but there may be about 2 million envenomings (injections of venom) and 100,000 deaths every year, many of them in rural areas of South Asia, Southeast Asia, and sub-Saharan Africa.

Why Was This Study Done? The best treatment for snakebite is to give antivenom (a mixture of antibodies that neutralize the venom) as soon as possible. Unfortunately, in countries where snakebites are common (for example, Sri Lanka), antivenoms are often of dubious quality, and acute allergic reactions to them frequently occur. Although some of these reactions are mild (for example, rashes), in up to 40% of cases, anaphylaxis—a potentially fatal, whole-body allergic reaction—develops. The major symptoms of anaphylaxis—a sudden drop in blood pressure and breathing difficulties caused by swelling of the airways—can be treated with adrenaline. Injections of antihistamines (for example, promethazine) and hydrocortisone can also help. In an effort to prevent anaphylaxis, these drugs are also widely given before antivenom, but there is little evidence that such "prophylactic" treatment is effective or safe. In this randomized double-blind controlled trial (RCT), the researchers test whether low-dose adrenaline, promethazine, and/or hydrocortisone can prevent acute adverse reactions to antivenom. In an RCT, the effects of various interventions are compared to a placebo (dummy) in groups of randomly chosen patients; neither the patients nor the people caring for them know who is receiving which treatment until the trial is completed.

What Did the Researchers Do and Find? The researchers randomized 1,007 patients who had been admitted to secondary referral hospitals in Sri Lanka after snakebite to receive low-dose adrenaline, promethazine, hydrocortisone, or placebo alone and in all possible combinations immediately before treatment with antivenom. The patients were monitored for at least 96 hours for adverse reactions to the antivenom; patients who reacted badly were given adrenaline, promethazine, and hydrocortisone as "rescue medication." Three-quarters of the patients had acute reactions—mostly moderate or severe—to the antivenom. Most of the acute reactions occurred within an hour of receiving the antivenom, and nearly half of all the patients were given rescue medication during the first hour. Compared with placebo, pretreatment with adrenaline reduced severe reactions to the antivenom by 43% at one hour and by 38% over 48 hours. By contrast, neither hydrocortisone nor promethazine given alone reduced the rate of adverse reactions to the antivenom. Moreover, adding hydrocortisone negated the beneficial effect of adrenaline.

What Do These Findings Mean? These findings show that pretreatment with low-dose adrenaline is safe and reduces the risk of acute severe reactions to snake antivenom, particularly during the first hour after infusion. They do not provide support for pretreatment with promethazine or hydrocortisone, however. Indeed, the findings suggest that the addition of hydrocortisone could negate the benefits of adrenaline, although this finding needs to be treated with caution because of the design of the trial, as does the observed increased risk of death associated with pretreatment with hydrocortisone. More generally, the high rate of acute adverse reactions to antivenom in this trial highlights the importance of improving the quality of antivenoms available in Sri Lanka and other parts of South Asia. The researchers note that the recent World Health Organization guidelines on production, control, and regulation of antivenom should help in this regard but stress that, for now, it is imperative that physicians carefully monitor patients who have been given antivenom and provide prompt treatment of acute reactions when they occur.

Additional Information. Please access these Web sites via the online version of this summary at http://dx.doi.org/10.1371/journal.pmed.1000435.

- The MedlinePlus Encyclopedia has pages on snakebite and on anaphylaxis (in English and Spanish)
- The UK National Health Service Choices website also has pages on snakebite and on anaphylaxis
- The World Health Organization has information on snakebite and on snake antivenoms (in several languages); its Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins are also available
- The Global Snakebite Initiative has information on snakebite
- A *PLoS Medicine* Research Article by Anuradhani Kasturiratne and colleagues provides data on the global burden of snakebite
- A *PLoS Medicine* Neglected Diseases Article by José María Gutiérrez and colleagues discusses the neglected problem of snakebite envenoming

Posttraumatic Mucormycosis

A Nationwide Study in France and Review of the Literature

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Abstract: Data on clinical, mycologic characteristics, and outcome of posttraumatic mucormycosis are scarce and often limited to case reports. From the French nationwide ''RetroZygo'' study, we compared posttraumatic mucormycosis cases with other forms of mucormycosis. We also reviewed reports of posttraumatic mucormycosis in the English-language literature from 1993 to 2013. We included all proven or probable cases for which underlying condition, route of infection, surgical and antifungal treatments, and outcome were detailed. From our cohort, posttraumatic mucormycosis (n = 16) differed significantly from other forms (n = 85) by rarity of underlying disease (31.2% vs 81%, $p < 0.0001$), frequency of cutaneous localization (87% vs 7%, $p < 0.0001$), short time before diagnosis (4.5 vs 21 d, $p = 0.0002$), species involved (*Apophysomyces elegans* complex and *Saksenaea vasiformis*), surgical requirement (93.7% vs 47%, $p = 0.0006$) and better survival (87.5% vs 47.6% at day 90, $p = 0.03$). We studied 122 cases of posttraumatic mucormycosis through our literature review. Most frequently reported traumas were traffic (37%), domestic accidents (15.1%), or natural disasters (13.4%). Mucormycosis occurred after extensive soft-tissue damage in 47.5% cases, with symptoms occurring a median of 9.5 days after trauma with necrosis being reported in 76.2% cases. Dissemination was found in 9% of patients, and bacterial coinfection in 41%. Nineteen percent of cases occurred in the Middle East or in India where *Apophysomyces elegans* complex was the predominant species recovered. Awareness of mucormycosis as a cause of posttrauma soft-tissue infection is warranted, especially in cases of soil-contaminated wounds. Survival is higher than in other forms of mucormycosis, but morbidity remains high.

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Abbreviations: ITS = internal transcribed spacer, L-AmB = liposomal amphotericin B, PTM = posttraumatic mucormycosis.

INTRODUCTION

Mucormycosis is a rare emerging angioinvasive infection caused by ubiquitous filamentous fungi. During the past decade, several countries reported an increase in mucormycosis incidence.[4,28] *Mucorales* are present in soil and plant debris and responsible for rhinocerebral and pulmonary infections following inhalation in immunocompromised hosts such as those with diabetes mellitus or hematologic malignancy.[17,32] By contrast, acquisition through disrupted cutaneous barriers, occurring after traumatic injury, is the major route of infection in immunocompetent hosts. To our knowledge, there has been no definitive, comprehensive review of the literature to date on posttraumatic mucormycosis (PTM) to guide our understanding of the epidemiology and outcome of this entity. Therefore, we took advantage of the French nationwide retrospective ''RetroZygo'' study to identify the clinical and epidemiologic features of PTM.[17] We also reviewed the English-language literature for all cases of PTM, from 1993 to 2013 to describe their predisposing conditions, route of infection, modalities of treatment, and outcome.

PATIENTS AND METHODS

The French nationwide retrospective RetroZygo study[17] aimed to collect all proven or probable mucormycosis cases diagnosed in France between 1 January 2005 and 31 December 2007. Cases were identified through 2 independent sources of recording: the French hospital information system and the National Reference Center for Mycoses and Antifungals (NCRMA). They were defined according to the 2008 European Organisation for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria, only modified by the inclusion of diabetes mellitus and trauma in case of temporal relationship with mucormycosis.[17] From these cases, we selected those related to a traumatic injury and compared them with other forms of mucormycosis. A case of PTM was defined as a compatible clinical presentation and histologically and/or culture documented infection occurring after a skin or mucosal injury. Clinical features, diagnostic methods, treatment and outcome of posttraumatic cases were described and compared to other mucormycosis cases of the RetroZygo cohort. The

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*Members are listed in the Appendix.

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identification of strains was assessed by morphology and confirmed by molecular analysis of the ITS1-5.8S-ITS2 region.[10,11] The study was approved by the Institut Pasteur Internal Review Board (2009-34/IRB) and by the Commission Nationale de l'Informatique et des Libertés according to French law.

Literature Review

Search Methods

We retrospectively reviewed the English- and French-language literature from May 1993 to May 2013 to identify all reported cases of PTM. A computer-based search of PubMed (National Library of Medicine, Bethesda, MD) was conducted, using the following keywords: ''zygomycosis,'' ''mucormycosis,'' ''*Rhizopus*,'' ''*Mucor*,'' ''*Rhizomucor,*'' ''*Cunninghamella,*'' ''*Mycocladus*,'' and ''*Absidia*'' (now classified as ''*Lichtheimia*''), ''*Apophysomyces*,'' ''*Saksenaea*,'' ''wound,'' ''trauma,'' ''injury,'' ''cornea,'' ''cutaneous,'' and ''immunocompetent.'' The individual references listed in each publication were also reviewed to identify additional case reports.

Selection Criteria

We restricted our analysis to cases diagnosed either by histology and/or tissue cultures and for which the trauma was identified as the only source of infection. Underlying condition, mechanism of injury, clinical features, and details of antifungal and surgical treatments were required for each reported case. Mortality was assessed as all-cause mortality at 3 months from diagnosis. Because healthcare-related mucormycosis have already been reported,[24] we excluded the latter infections. We also excluded burn-related mucormycosis without associated trauma because distinction between colonization and infection requires specific criteria that were not detailed enough in most reports.

Statistical Analysis

Results are expressed as median and interquartile range for continuous variables and as percentage with 95% confidence interval for categorical variables. Comparisons of 2 medians were performed using Wilcoxon rank test. Associations between categorical data were analyzed using the chi square or Fisher exact test, when appropriate. Survival analyses were realized using Kaplan-Meier method for overall survival estimation and the log rank test for survival rates comparison. All analyses were performed using SAS v. 9.1. All tests were bilateral with $p < 5\%$.

RESULTS

Results From the RetroZygo Study

General Results

The RetroZygo study[17] included 101 cases of proven ($n = 60$) and probable ($n = 41$) mucormycosis. The mean age of the patients was 50.7 years (range, 9–87 yr), with men representing the majority (58%). The 2 most common underlying diseases were hematologic malignancies (50%) and diabetes mellitus (23%). Sites of infection were lungs (28%; 79% in hematology patients), rhinocerebral (25%; 64% in diabetic patients), skin (20%), and disseminated (18%). Ninety-day survival was 56%; it was reduced in cases of dissemination compared with rhinocerebral (hazard ratio [HR], 5.38 [2.0–14.1], $p < 0.001$), pulmonary (HR, 2.2 [1.0–4.7], $p = .04$), or skin localization (HR, 5.73 [1.9–17.5], $p = .002$). Mucormycosis localization remained the only independent factor associated with survival.

Recent trauma were present in 18 (18%) patients. Two cases were subsequently excluded because of absence of identified skin damage in 1 case and because infection occurred at distance from the initial traumatic injury in the other case. Therefore, we considered 16 cases of PTM (15.8%) that actually occurred at the site of injury (Tables 1 and 2). One of these cases is here described in detail.

Illustrative Case From the RetroZygo Study

A 14-year-old girl with no significant medical history was run over by a car while she was walking in a rural setting. She sustained multiple severe traumatic injuries including right lower extremity crush injury with skin degloving from the top of the thigh to the ankle, open fracture of the tibia with loss of bone fragment, deep lacerations of the anterior and posterior tibial muscles with massive contamination of the wounds by soil and plant debris. At the referring hospital, initial surgery consisted of reimplantation of the tibial fragment that was found at the scene of the accident, osteosynthesis with external fixation, and debridement of nonviable tissues. The patient initially received intravenous amoxicillin-clavulanate and massive blood transfusion. Seven days after admission, wounds of the right lower limb developed a necrotic appearance requiring an extensive debridement of all skin, subcutaneous tissue, and muscles of the posterior thigh and the anterior part of the leg (Figure 1). Direct mycologic examination and histology of the debrided tissues showed fungal hyphae within the dermis and deep subcutaneous tissue, confirming a clinically significant mould infection. Several cultures grew with a Mucorale. *Rhizopus oryzae* was identified on the basis of its phenotype and this identification was confirmed by internal transcribed spacer (ITS) sequencing. Because of positive culture from bone specimen, 12 days later tibial fragment was removed and a cement spacer was placed. At this time, no necrosis was seen and cultures from surgical specimens remained negative. Liposomal amphotericin B (350 mg/d) was started on the day after the first surgical debridement and continued for 21 days. Given the exposition of the tibial diaphysis, a negative pressure dressing (V.A.C therapy) was used for 10 days and reconstruction of the leg was then carried out by a latissimus dorsi flap plus several skin grafts. The patient was discharged after extensive physical therapy at a rehabilitation facility. She remained disease-free at 2 years and was able to ambulate successfully despite an arthrodesis of the right ankle with a 50° equinus deformity.

Circumstances of Trauma and Mechanism of Injury

Trauma occurred outside Europe in 3 cases: 2 terrorist blasts in Lebanon and Egypt, and 1 traffic accident in Tunisia. In France, traumas were mostly due to traffic accident ($n = 5$) or farm working accident ($n = 3$). With the exception of 1 case, all infections consecutive to a minor injury ($n = 4$) occurred in context of soil-contaminated wounds: 2 patients had plant injury while gardening and 1 patient had minor abrasion of the foot after a stone fall (see Table 2). The remaining case was considered healthcare-associated mucormycosis and occurred 2 weeks after setting up a plaster in a young girl suffering from osteosarcoma.[27] Finally, 1 patient had deep burn related community-acquired mucormycosis secondary to house fire. Compared with patients who had other forms of mucormycosis, the

TABLE 1. Comparison of Characteristcs of Patients With Posttraumatic Mucormycosis (PTM) and Other Forms of Mucormycosis (Other Patients) Recorded in the RetroZygo Study

	Patients with PTM (n = 16)	Other patients (n = 85)	p
Median (IQC) age (yr)	42.9 (19.1–68.5)	55.5 (40.5–65.3)	0.42
Male sex	11 (68.7)	48 (56.5)	0.36
Underlying disease	5 (31.2)	81 (95.3)	< 0.0001
Cutaneous localization	14 (87.5)	6 (7.1)	< 0.0001
Diagnosis			
Positive histology or microscopy	8 (50)	70 (82.3)	0.005
Positive culture	16 (100)	52 (61.2)	0.002
Positive histology	5 (31.2)	52 (61.2)	0.03
Median time between symptoms and diagnosis, days (range)	4.5 (0–317)	21 (0–210)	0.0002
Treatment			
Surgical treatment	15 (93.7)	39 (47)	0.0006
Antifungal therapy	13 (81.2)	74 (87.1)	0.53
Death	6 (37.5)	56 (65.9)	0.03
Death rate at day 90	2 (12.5)	43 (52.4)	0.03
Fungal species	n = 16	n = 46	
Apophysomyces elegans complex	1 (7.7)	0	
Cunninghamella bertholletiae	0	4 (8.7)	
Lichtheimia species	4 (25)	13 (28.3)	
Lichtheimia corymbifera	1 (7.7)	13 (28.3)	
Lichtheimia ramosa complex	3 (23)	0	
Mucor species	3 (23)		
Mucor circinelloides	2 (15.4)	0	
Mucor racemosus	1 (7.7)		
Rhizomucor pusillus	0	4 (8.7)	
Rhizopus species	6 (37.5)	25 (54.3)	
Rhizopus microsporus	1 (7.7)	9 (19.6)	
Rhizopus arrhizus	3 (23.1)	16 (34.8)	
Saksenaea vasiformis	2 (15.4)	0	

presence of an underlying disease was less frequently reported in patients who developed PTM (31.2% vs 81%, $p < 0.0001$).

Clinical Presentation

First symptoms occurred a median of 8 days (1 d to 7 yr) after the trauma. For 13 of the 16 patients, the lesion developed on the limbs (see Table 2). Except 2 cases (1 tenosynovitis and 1 keratitis), all infections involved cutaneous or subcutaneous tissues (87.5%). Only 1 patient had a secondary pulmonary dissemination of the infection. Necrosis was observed in 10 patients (62.5%), while a moldy appearance wound was noted in only 1 case. In PTM, cutaneous location was more frequent than for nontrauma-related mucormycosis (87% vs 7%, $p < 0.0001$).

Diagnosis

Diagnostic findings are represented in Figure 2. All cases had a mucorale growing in culture. Mucormycosis was proven for 8 patients; diagnosis was assessed by positive histology: muscle (n = 3), skin (n = 1), and bone biopsy (n = 1) or by the presence of *Mucorales* species in culture from a sterile site: bone biopsy (n = 1) and muscle biopsy (n = 2). Mucormycosis was probable for the remaining 8 patients. Diagnosis was based on a positive culture from a nonsterile site either alone—cutaneous biopsy (n = 3), cornea (n = 1), wound swab (n = 1)—or associated with positive direct examination (n = 3). Of note, bacteria grew from the wound in half of the patients (at the same time of PTM diagnosis in 5 cases and before diagnosis in 2 cases). Species involved were *Acinetobacter baumannii* (n = 1), *Aeromonas* species (n = 1), *Bacillus cereus* (n = 1), *Enterobacter cloacae* (n = 2), *Enterococcus* species (n = 2), *Escherichia coli* (n = 2), *Morganella morganii* (n = 1), *Pseudomonas aeruginosa* (n = 5), and *Staphylococcus* species (n = 2). *Aspergillus niger* was also recovered from the wound in 1 patient after a bomb explosion in Egypt. The median time between first symptoms and diagnosis was significantly shorter in patients with PTM than in those with other forms (4.5 vs 21 d, $p = 0.0002$).

Mucorales Species Involved

Molecular identification to the species level was obtained from culture for 13 (81%) patients (see Table 1). *Rhizopus* species were predominant (n = 6) followed by *Lichtheimia* (n = 4), *Mucor* (n = 3), *Saksenaea vasiformis* (n = 2), and *Apophysomyces elegans* complex (n = 1). Remarkably, no *Mucor, Saksenaea*, or *Apophysomyces* species were found in patients with other forms of mucormycosis. However, *Rhizopus* and *Lichtheimia* species were found in the same proportion in PTM and non-PTM patients.

Treatment

In most cases, initial management consisted of a combination of medical and surgical treatments (n = 12) (Table 3). No systemic antifungal treatment was recorded for 3 patients due to

TABLE 2. Characteristics of Posttraumatic Mucormycosis in the RetroZygo Study and in the Literature

Characteristics	RetroZygo (n = 16), n (%)	Literature (n = 122), n (%)
Age, yr median (range)	42.9 (9–87)	38 (2–85)
Male sex	11 (68.7)	82 (67.2)
Underlying disease		
None (immunocompetent)	11 (68.8)	92 (75.4)
Cancer	1 (6.2)	2 (1.8)
Corticosteroids	1 (6.2)	3 (2.4)
Diabetes mellitus	2 (12.5)	20 (16.4)
Hematological malignancy	1 (6.2)	5 (4.1)
Trauma		
Farm accident	3 (18.7)	15 (12.6)
Explosion	2 (12.5)	5 (4.2)
Traffic accident	6 (37.5)	44 (37.0)
Domestic accident	1 (6.2)	18 (15.1)
Plant injury	2 (12.5)	6 (5.0)
Insect or animal bite	–	10 (8.4)
Natural disaster	–	16 (13.4)
Other	2 (12.5)	5 (4.2)
Mechanism of injury		
Abrasion	3 (18.7)	31 (25.4)
Deep burn	2 (12.5)	8 (6.5)
Minor penetrating wound	1 (6.2)	25 (20.5)
Extensive soft-tissue damage without fracture	3 (18.7)	16 (13.1)
Extensive soft-tissue damage with fracture	7 (43.7)	42 (34.4)
Primary site of infection		
Upper limb	3 (18.7)	10 (8.2)
Lower limb	10 (62.5)	47 (38.5)
Abdominal wall	1 (6.2)	8 (6.6)
Thorax	–	8 (6.6)
Cephalic extremity	1 (6.2)	28 (22.9)
Cornea	1 (6.2)	3 (2.5)
Solid organ	–	8 (6.6)
Disseminated	–	10 (8.2)
Symptoms		
Necrosis	10 (62.5)	93 (76.2)
Mouldy appareance	1 (6.2)	27 (22.1)
Redness	4 (25)	53 (47.7)
Swelling	4 (25)	48 (43.2)
Purulent discharge	2 (12.5)	26 (23.2)
Ulceration	–	21 (18.9)
Keratitis	1 (6.2)	3 (2.5)
Extension		
Localized cutaneous disease	2 (12.5)	10 (8.2)
Deep extension	10 (62.5)	103 (84.4)
Bone	2 (12.5)	14 (12.6)
Secondary dissemination	1 (6.2)	10 (9.0)
Tenosynovitis	1 (6.2)	–
Median time between, days (range)		
Trauma and symptoms	8 (1–2555)	9.5 (1–63)
Trauma and diagnosis	15.5 (3–2879)	14 (3–3650)
First symptoms and diagnosis	4.5 (0–317)	2 (−4–3620)
Microbiologic assessment of wound		
Bacteria recovered	8 (50)	50 (41.0)
Other fungi recovered	1 (6.2)	19 (15.6)

ocular location, immediate premortem diagnosis, or favorable response following surgery alone. Multiple debridements were performed in 6 cases (37.5%) and the number of surgical procedures ranged from 1 to 16 (median, n = 1). Two patients needed an amputation. Only 1 patient, with favorable outcome, did not undergo surgical intervention, but liposomal amphotericin B monotherapy. Antifungal therapy was prescribed for a median duration of 23 days (range, 5–216 d). The most commonly used drug was liposomal amphotericin B (L-AmB), administered in 11 patients at a median daily dose

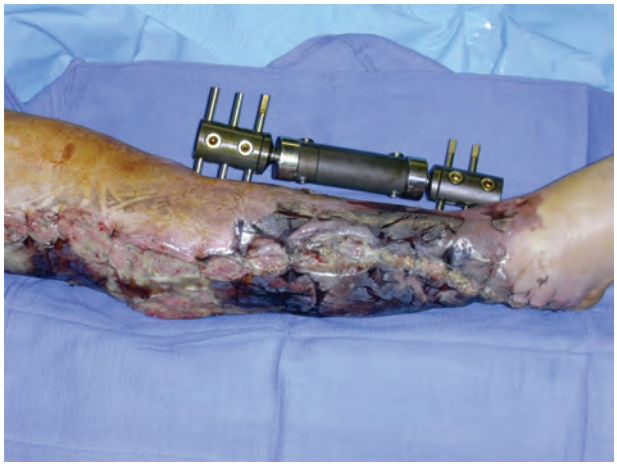

FIGURE 1. Right leg wound 7 days after a traffic accident in a young girl. The presence of subcutaneous necrosis suggested a Mucorale infection, thereafter confirmed by mycologic culture.

of 5 mg/kg (range, 3–10 mg/kg). When prescribed, local antifungal therapy was always associated with systemic therapy excepted for keratitis. It consisted of antifungal drops for ocular

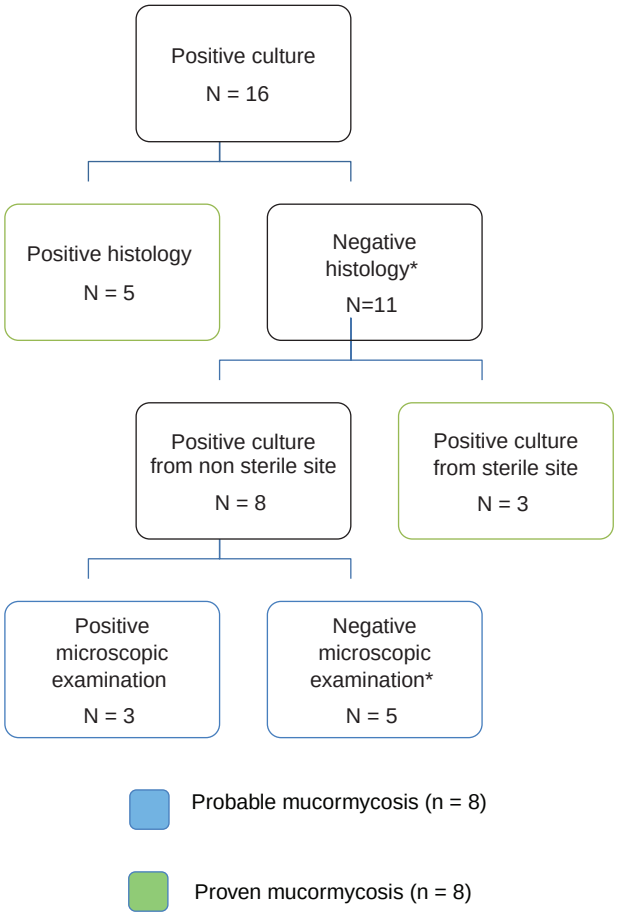

FIGURE 2. Histologic and mycologic results obtained in 16 proven or probable cases of posttraumatic mucormycosis from the RetroZygo study. *Negative or not done.

localized infection, local application of amphotericin and amphotericin-impregnated beads in 1 case each. Most of patients (n = 9, 60%) were admitted to intensive care unit and all except the 1 with keratitis received concomitant antibacterial therapy. Compared to patients with other forms of mucormycosis, surgery was more frequently performed in patients with PTM (93.7% vs 47%, p = 0.0006).

Outcome

The global survival rate was 62.5% (median duration of follow-up, 36 months; range 0–67), which was significantly higher than patients with other form of infection (34.1%, p = 0.03). Similarly, survival rate at day 90 was significantly higher than patients with other forms of infection (87.5% vs 47.6%, p = 0.03) (Figure 3).

Review of 122 Other Cases of PTM From the Literature

A total of 122 individual cases of PTM were identified from our literature review between 1993 and 2013, of which 85 (69.7%) were recorded after 2002 (Figure 4). Of these cases, 99 were defined as proven and 23 as probable mucormycosis. The majority of cases were from Europe (n = 34) or North America (n = 40), but a significant number occurred in Middle East or in India (n = 23) (Figure 5). There was a larger proportion of male (67.2%) with a median age of 38 years (see Table 2). An underlying disease was reported for 30 patients (24.6%). Most common traumatisms were traffic accident (37%), domestic accidents (15.1%), natural disaster (14%), farm working accident (12.6%), and animal or insect bite (8.4%) (including 2 scorpion stings, 1 spider bite, 1 magpie peck, and 1 dog scratch).[2,3,15,18,23,29,34,35,37,39] Natural disasters were Joplin tornado (13 cases),[22] collapse of building (1 case),[2] and Asian tsunami (2 cases)[1,33] (Figure 6). Half of wounds were described as extensive soft-tissue damage, 8 (6.5%) deep burns that occurred in a soil contamination context, 25 (20.5%) as penetrating wound and 31 (25.4%) as superficial lesions. The latter 2 included a great proportion of insect or animal bites,[2,3,15,18,23,29,34,35,37,39] penetrating injury with plant material (for example, a thorn), but also surprising traumas as crab trap[16] or wooden matchstick injuries.[13] More than one-third of the wounds were observed on the lower limbs (n = 47), followed by the cephalic extremity (n = 28) (see Table 2). Three patients had keratitis and solid organ was the primary site of infection in 8 cases: kidney (n = 1), liver (n = 1), stomach (n = 1), lungs (n = 2) and peritoneum (n = 2). Of the 98 patients with available data, patients developed symptoms of mucormycosis after a median of 9.5 days (range, 1–63 d) after trauma. Necrosis was reported in 93 (76.2%) patients while a mould appearance was noted in 27 (22.1%) patients. Subsequent dissemination occurred in 10 cases, including 2 cases in a context of immunosuppression. Organs involved were lungs (n = 4), kidney (n = 1), abdominal cavity (n = 2), stomach and brain (n = 1); the remaining 2 patients had multivisceral dissemination, which was diagnosed at autopsy.

Culture was positive in 111 cases leading to the identification of *Absidia* and *Apophysomyces* species as the most frequent pathogens (21.1% and 31.7%, respectively), followed by *Rhizopus* (14.4%), *Mucor* species (15.4%), and *Saksenaea* species (9%) (see Figure 5). The median interval between symptoms onset and diagnosis was 2 days (range, −4–3620 d]. Bacteria and other fungi were recovered from incident wounds in 50 (41%) and 19 (15.6%) patients, respectively.

TABLE 3. Treatment and Outcome of Patients With Posttraumatic Mucormycosis in the RetroZygo Study and in the Literature

	RetroZygo (n = 16), n (%)	Literature (n = 122), n (%)
Treatment		
Only medical	1 (6.2)	6 (4.9)
Only surgical	3 (18.7)	9 (6.4)
Medical and surgical	12 (80)	104 (85.2)
None		3 (2.5)
First-line antifungal therapy		
L-AmB	8 (50)	45 (36.9)
Amphotericin B deoxycholate	1 (6.2)	54 (44.3)
Amphotericin B lipid complex	–	5 (4.1)
Posaconazole	2 (12.5)	1 (0.8)
L-AmB + posaconazole	2 (12.5)	3 (2.4)
L-AmB + caspofungin	–	2 (1.6)
Median duration antifungal therapy (days) (range)	23 (5–216)	26.5 (3–530)
Surgery	15 (93.7)	113 (92.6)
Multiple debridments	6 (37.5)	85 (69.7)
No. of debridements per patient, median (range)		2 (0–19)
Amputation	2 (12.5)	18 (16.5)
Enucleation		9 (8.1)
Adjuvant mesures		
Hyperbaric oxygen	–	9 (8.3)
Local treatment	3 (18.7)	16 (14.7)
V.A.C	3 (18.7)	5 (4.6)
Outcome		
Cure	12 (80)	90 (74.4)
Death at day 90	2 (12.5)	28 (22.9)

Abreviations: L-AMB = Liposomal Amphotericin B; V.A.C = Vaccuum Assisted Closure.

The majority of patients underwent surgery (91.6%). As the infection progressed, additional debridements were performed for 85 (69.7%) patients with a median of 2 surgical procedures per patient (range, 0 to 19). Amputation and enucleation were performed in 18 (16.5%) and 9 (8.1%) patients respectively. Antifungal treatment consisted of monotherapy of amphotericin B deoxycholate for 54 patients (44.3%) or L-AmB for 45 patients (36.9%). Finally, 90 (74.4%) patients had a favorable outcome.

DISCUSSION

To our knowledge, this is the largest series to date of trauma-related mucormycosis on the basis of a nationwide retrospective study. Based on 2 independent data sources, these cases are estimated to be representative of 77% of all PTM cases occurring in the country over a 3-year period.[5] Although hematologic malignancy is the predominant risk factor of mucormycosis, PTM are a major concern as they represent the third cause of mucormycosis in the RetroZygo study (15.8%)[17] and the second cause (17%, equal with diabetes mellitus) in a recent European study.[32] Moreover, concordant with the increased description of mucormycosis in Europe, United States, and India,[8,21,32] the number of published cases increased significantly over time.

It is noteworthy that epidemiologic, clinical, mycologic and outcome characteristics of PTM differ from those observed

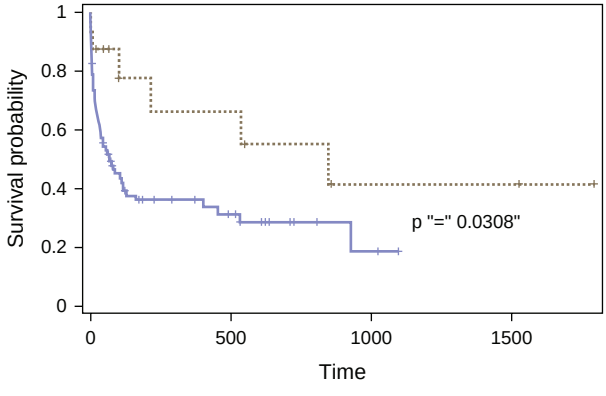

FIGURE 3. Survival of posttraumatic (dotted line) versus other forms of mucormycosis (blue line) in the RetroZygo study.

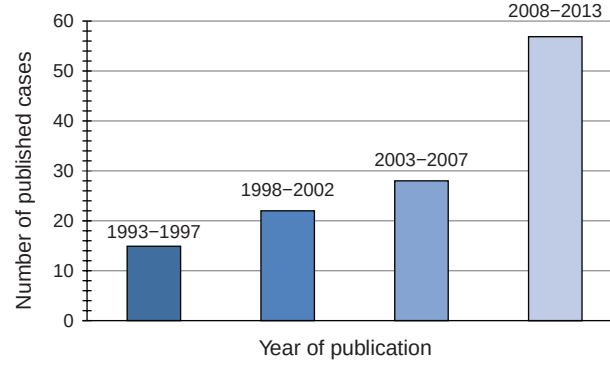

FIGURE 4. Distribution of 122 published cases of posttraumatic mucormycosis, 1993–2013. Each bar represents a 5-year period except for the last, which represents 6 years.

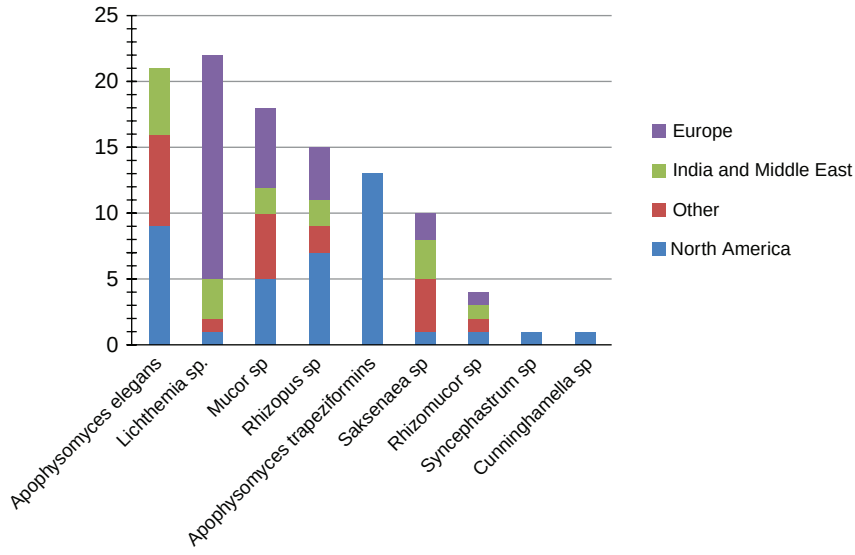

FIGURE 5. Distribution of *Mucorales* species according to the geographical origin in the literature review.

during other forms of mucormycosis. Indeed, most patients with PTM both in the RetroZygo cohort and in the present literature survey did not present any underlying condition, in accordance with results of a European study, that reported 35/39 PTM patients to be immunocompetent.[32] In contrast with other forms of mucormycosis, PTM occurs as the result of direct inoculation of spores into damaged skin with contaminated material during trauma. In addition to huge number of spores present in soil contaminated wounds, acidosis due to large soft tissue damage and lack of tissue viability associated with local immunodepression could explain the pathogenicity of *Mucorales* after trauma. Indeed, a major trauma by itself has been shown to cause a systemic immunocompromised state.[38]

In both RetroZygo and literature studies, PTM occurred mainly after extensive tissue damage as degloving, amputation and tear, where a massive soil contamination is observed. However, the initial trauma can also be minimal since 18% to 25% cases in our study were related to superficial abrasion. Cutaneous inoculation of *Mucorales* may have underestimated consequences since infection can rapidly invade deeper tissue, and eventually disseminate, that occurred in 84.4% and 9% cases in our review, respectively. A necrotic lesion in an injured patient should raise suspicion of mucormycosis. However, this sign may be absent in the first stages of the disease, and symptoms may be non-specific as those commonly observed during bacterial infections.

Most patients developed symptoms in the first 10 days after injury, but the disease can progress slowly, especially in cases of superficial abrasions. Recently, Lu et al reported 5 cases of PTM from China caused by *Mucor irregularis* (formerly *Rhizomucor variabilis*) that all shared unusual clinical features.[19,20] Following minor trauma, infection was confined to superficial tissues and progressed very slowly, in the range of 7 months to 18 years. As a consequence, *Mucorales* should always be suspected as potential agents of soft-tissue infections in injured patients even in case of minor injury, indolent development or aspecific symptoms. This is of major concern because early diagnosis improves outcome.[7] To prove

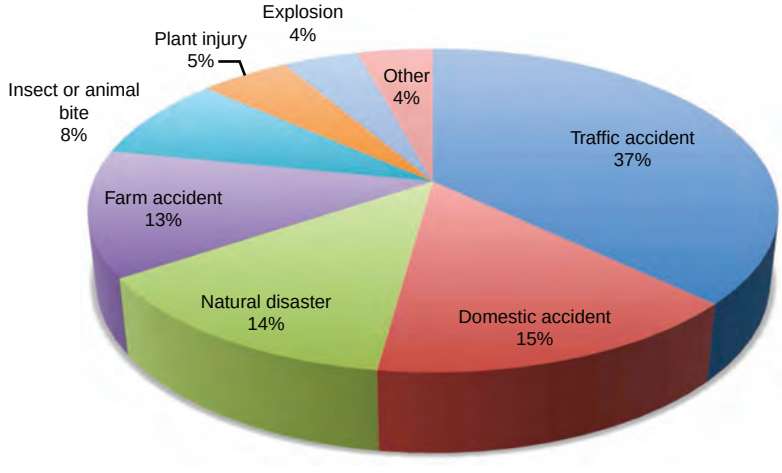

FIGURE 6. Traumas responsible for 122 mucormycosis in the literature.

fungal tissue infection, biopsy sample from infected lesions should be performed systematically for histologic and mycologic investigations. We evidence that bacterial coinfection can frequently be recovered from wounds either before or together with *Mucorales*, which may finally lead to misdiagnose isolated bacterial infection. Recovering bacteria should rather make clinicians even more suspicious since it reflects generally a massive soiled contamination. Furthermore, it was recently reported that bacterial infections of wounds increased the risk of subsequent mucormycosis after tornado injury.[22]

Of major importance, distribution of *Mucorales* species differed between PTM patients compared to others, especially for 2 species. Indeed, *S. vasiformis* and *A. elegans* complex were respectively responsible for 2 and 1 cases in RetroZygo study (including 2 cases for whom trauma occurred in Liban [Lebanon] and in Egypt) and for 9% and 18.9% cases of PTM in the literature, respectively. In contrast, these species were not recovered in other patients from RetroZygo study and recovered in only 5% and 6% cases in the review of 929 cases of mucormycosis by Roden et al.[26] *S. vasiformis* and *A. elegans* complex, are different from the typical opportunistic *Mucorales* as they are mainly involved in cutaneous infections from immunocompetent patients, especially from southern countries.[12] Warkentien et al have recently reported a series of 37 invasive mold infections following combat-related injuries in United States military personnel.[36] Of the 16 *Mucorales* infections reported, 6 were related to *A. elegans* and 2 to *S. vasiformis*. Most cases occurred in Afghanistan. To our knowledge, *A. elegans* was first isolated in 1979 from soil samples in a mango orchard in Northern India. Since that time, this species has been reported with increasing frequency as a cause of cutaneous PTM, especially on the Indian subcontinent, where it is the second most common isolate (19%) following *Rhizopus arrhizus (oryzae)*.[6] Other forms of mucormycosis due to *A. elegans* are rare and seem to occur mostly in apparently immunocompetent patients.[12] Two other species of the genus *Apophysomyces* were recently described: *Apophysomyces variabilis* was identified as the infecting pathogen in 5 Indian patients with primary cutaneous mucormycosis[14] and *Apophysomyces trapeziformis* was responsible for 13 cases of PTM following the tornado that occurred in Missouri in 2011.[22] Similarly, *S. vasiformis* has increasingly been reported as a cause of cutaneous infections in immunocompetent patients, but only rarely as a cause of rhino-orbito-cerebral, disseminated, and pulmonary infections.[25]

Most patients with PTM underwent surgical treatment including multiple debridements or amputation. Surgical resection is of primary importance in PTM as infection spreads rapidly, leading to a large area of necrosis with poor penetration of antifungal agents. Surgery was demonstrated as predictive of better outcome in previous studies.[26,30,32] Both recent third European Conference of Infections in Leukemia (ECIL-3) and European Society of Clinical Microbiology and Infectious Diseases (ESCMID) recommendations emphasize a timely debridement of all devitalized tissues to treat PTM. The wound must be closely monitored and surgery must be repeated when new clinical features strongly suggest disease progression.[9,31]

Prognosis of PTM is better than that observed during other forms of mucormycosis (90-day mortality of 12.5% vs 52.4%). This is in accordance with a previous major report in which patients with PTM had an 86% smaller probability of death compared with patients with hematological malignancies.[32] Such differences may have several explanations. First, fewer patients had an underlying disease in the PTM group; secondly, median time between first symptoms and diagnosis was significantly shorter in patients with PTM. Finally, we can raise the question of the role of the greater proportion of patients with PTM who underwent surgery may contribute to a better outcome.

In conclusion, this study provides new insights into PTM by comparison with other forms of mucormycosis. Fungal species involved vary with geographical location and are frequently associated with bacterial coinfection. Increased attention to environmental fungi as a possible cause of soft-tissue infection in trauma patients is thus warranted and early surgical treatment and adapted antifungal therapy are required to avoid local extension and fungal dissemination, even in apparently nonimmunocompromised patients.

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