Clinical Serum Therapy: Benefits, Cautions, and Potential Applications

Toru Hifumi, 1 Akihiko Yamamoto, 2 Manabu Ato, 3 Kyoko Sawabe, 4 Kazunori Morokuma, 5 Nobuya Morine, 6 Yutaka Kondo, 7 Eiichiro Noda, 8 Atsushi Sakai, 9 Jin Takahashi 10 and Kazuo Umezawa 11

1 Emergency Medical Center, Kagawa University Hospital, Kagawa, Japan
2 Department of Biosafety, National Institute of Infectious Disease, Tokyo, Japan
3 Department of Immunology, National Institute of Infectious Disease, Tokyo, Japan
4 Department of Medical Entomology, National Institute of Infectious Disease, Tokyo, Japan
5 The Chemo-Sero-Therapeutic Research Institute (KAKETSUKEN), Kumamoto, Japan
6 Okinawa Prefectural Institute of Health and Environment, Okinawa, Japan
7 Department of Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA
8 Department of Emergency Medicine, Fukuoka City Hospital, Fukuoka, Japan
9 The Japan Snake Institute, Gunma, Japan
10 Department of Emergency Medicine, Tokyo Bay Urayasu/Ichikawa Medical Center, Chiba, Japan
11 Department of Emergency and Critical Care Medicine, Tokai University School of Medicine, Kanagawa, Japan

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Blood serum from immunized humans or animals (e.g., horses) contains relevant antibodies and has been used as serum therapy to treat many diseases or envenomation events. The effectiveness of blood serum was initially discovered in 1890 when Kitasato and von Behring observed the effectiveness of this type of therapy against diphtheria and tetanus. Serum therapies played an important role in the advancement of modern medicine prior to the development of penicillin and steroids. At present, several types of serum therapy remain in clinical use. However, some physicians have a limited understanding of the nature and the benefits of serum therapy and the factors that require particular attention. In this review, we set out to clarify the benefits, cautions, and potential applications of serum therapy in the context of conditions such as gas gangrene, diphtheria, botulism, and tetanus and bites from three snake species (mamushi, habu, and yamakagashi) and the redback spider. It is hoped that this review will help clinicians to learn about clinical serum therapies and become familiar with their applications. (DOI: 10.2302/kjm.2016-0017-IR; Keio J Med 66 (4) : 57–64, December 2017)

Keywords: serum therapy, antivenom, critical care, envenomation, passive immunization

Background

Serum therapy involves the administration of purified blood serum from immunized humans or animals (e.g., horses), which contains relevant antibodies, as a form of passive immunization against many diseases and envenomation events. Serum therapy was first introduced in 1890, when Kitasato and von Behring discovered that serum derived from rabbits immunized against diphtheria or tetanus could protect mice exposed to the related pathogens. The principle of serum therapy, i.e., that sera from immune animals cured toxin-exposed animals, was
thereby established.\textsuperscript{3} von Behring won the 1901 Nobel Prize in Physiology or Medicine for his research on diphtheria. To date, the most effective reported treatment for Ebola virus infection is the transfusion of passive antibodies from a human survivor of a previous Ebola infection.\textsuperscript{4} In many other ways also, serum therapies played an important role in the advancement of modern medicine prior to the development of penicillin and steroids.\textsuperscript{5}

Today, the most common use of serum therapy in humans is as an antitoxin or antivenom to treat envenomation. Although several types of serum therapy remain in clinical use, many physicians lack knowledge about the nature and the benefits of serum therapy and also about the factors requiring particular clinical attention. This lack of knowledge has developed mainly because of the declining demands for serum therapy. Reasons why serum therapy has become rare in Japan are the extensively adhered to vaccination policies and high levels of environmental hygiene.\textsuperscript{6} Moreover, summaries of clinical serum therapies are not available in the literature. Consequently, clinicians have few opportunities to learn about and become familiar with serum therapies.

Our research group has investigated several types of serum therapy over many years.\textsuperscript{7,10} In this review, we aim to clarify the benefits, cautions, and potential applications of serum therapy in an attempt to facilitate understanding among clinicians.

**Basic Information about Antitoxin/Antivenom**

In Japan, antitoxins and antivenoms are generally manufactured in a freeze-dried state to ensure that quality is maintained to government standards. Currently, three types of antitoxin—gas gangrene equine antitoxin (Fig. 1A), diphtheria equine antitoxin (Fig. 1B), and botulism equine antitoxin (Fig. 1C)—are supplied by the government (i.e., they are available under the Kokuyu vaccine supply system), whereas tetanus antitoxin, *Gloydius blohmoffi* (mamushi) equine antivenom, and *Protobothrops flavoviridis* (habu) equine antivenom (Fig. 1D) are supplied commercially.\textsuperscript{5} *Rhabdophis tigrinus* (yamakagashi) equine antivenom (Fig. 1E) and *Latrodectus hasselti* (redback spider) equine antivenom (Fig. 1F) are supplied by research groups in Japan\textsuperscript{11–13} (Table 1).

Generally, antitoxin/antivenom should be administered as early as possible to ensure the most extensive neutralization of unbound toxin/venom. We note, however, that equine antitoxin/antivenom is associated with side effects such as anaphylaxis and serum sickness. A recent Japanese survey of mamushi bites reported that the incidence of anaphylaxis to antivenom was 2.4–9.0%.\textsuperscript{13} Miyagi reported that habu antivenom induced anaphylaxis in approximately 11% and serum sickness in approximately 24.2% of patients.\textsuperscript{13} Previously, a meta-analysis and Cochrane review concluded that premedication with epinephrine, but not with other agents, significantly reduced the incidence of early adverse reactions.\textsuperscript{14} Additionally, epinephrine can effectively be administered shortly after an anaphylactic reaction to antivenom administration.\textsuperscript{15}

**Specific Serum Therapies**

**Gas gangrene**

Currently, clostridial gas gangrene, a life-threatening muscle infection, is rarely reported,\textsuperscript{16} in contrast to the relatively frequently encountered non-clostridial gas gangrene. This fact has been attributed to the aging of society.\textsuperscript{17,18} The main etiologic agent in the former condition, α-toxin secreted by *Clostridium perfringens*, causes intravascular hemolysis followed by severe anemia, disseminated intravascular coagulopathy (DIC),\textsuperscript{19,20} and multiple organ failure (Table 2).

Gas gangrene antitoxin comprises equine serum immunoglobulins raised against three specific gas gangrene toxins (*C. perfringens* type A, *C. septicum*, and *C. oedematiens*), and has been approved for commercial distribution.\textsuperscript{6} However, this product is no longer available in the United States because of poor efficacy and severe allergic reactions.\textsuperscript{21}

Despite the decrease in reported cases of clostridial gas gangrene, the incidence of *C. perfringens* septicemia without gas gangrene has been increasing. Van Bunderen et al. retrospectively reviewed cases of *C. perfringens* septicemia that rapidly progressed to intravascular hemolysis, metabolic acidosis, and death within a few hours after patient admission.\textsuperscript{22} In their review, the mortality rate was >80% despite treatment with high-dose antibiotics and surgical debridement. Therefore, we must redefine conventional standard treatments by re-examining the pathophysiology of *Clostridium* infections. To this end, we recommend that administration of antitoxin along with antibiotics should be considered as early as possible to neutralize the toxin.\textsuperscript{21}

**Diphtheria**

Diphtheria is associated with an infection of the gram-positive bacillus *Corynebacterium diphtheriae*. Specifically, diphtheria toxin acts intracellularly to cause cell death, particularly in the diaphragm and myocardium. *Corynebacterium diphtheriae* infection may lead to respiratory disease, cutaneous disease, or an asymptomatic carrier state (Table 2). In developed countries, the estimated mortality associated with diphtheria range from 5% to 10%.\textsuperscript{23}

*Corynebacterium ulcerans*, which shares a similar, but not identical, toxin antigen, induces a rare diphtheria-like infection, and isolated cases of this illness have been identified not only in the United States\textsuperscript{24} but also in Japan, where a related fatality was reported.\textsuperscript{23,24} Similar to...
Corynebacterium diphtheria, Corynebacterium ulcerans can produce diphtheria toxin, leading to a life-threatening disease that requires urgent treatment with diphtheria antitoxin and macrolide antibiotics.25,26

Botulism

Botulism, a potentially life-threatening neuro-paralytic syndrome caused by a neurotoxin produced by C. botulinum, is generally characterized by the acute onset of bilateral cranial neuropathies and symmetrical descending weakness. Botulism cases can be classified as foodborne, infant, wound, adult enteric infectious, inhalational, and iatrogenic, based on the mode of acquisition.27 Notably, infant and adult enteric infectious botulisms occur in Japan at a rate of approximately one case per year. The currently available heptavalent botulism antitoxin is administered only to adults because its efficacy and safety have not been determined for infants28,29; to date, all efficacy studies have been conducted in animals because it is not feasible or ethical to conduct such studies in human populations. In an attempt to evaluate human efficacy, Tacket et al. retrospectively evaluated data of 132 patients with type A foodborne botulism and found that patients treated with antitoxin had decreased mortality rates compared with untreated patients.29 However, details such as the severity of foodborne botulism and comorbidities were not compared.

In Japan, antitoxins to the A, B, E, and F botulism toxins are manufactured, stored in a national stockpile, and provided for the treatment of primary severe adult dietary food poisoning. However, with regard to the manufacturing of botulinum equine antitoxin preparations worldwide, the ranch maintenance required for equine management and securing the human resources for manufacturing plants remain as obstacles in many countries. Additional difficulties associated with the market economy and technology inheritance have led to the cessation of antitoxin production in many countries. Accordingly, antitoxin stockpiles held by countries, such as Japan, have been used during outbreaks for the humanitarian rescue of many patients in countries that do not stockpile antitoxins30,31. In addition, the potential nature of botulinum toxin as a weapon of terrorism has led to the need for national antitoxin stockpiles as a counter-terrorism measure.31
Tetanus

Tetanus is caused by a toxin produced by *C. tetani*, which is found in soil, human feces, and objects lying on the ground. This tetanus toxin blocks neurotransmission by cleaving membrane proteins, leading to muscle spasms of the jaw, chest, neck, back, abdominal muscles, buttocks, and back muscles, a condition referred to as opisthotonos. Other symptoms of tetanus include drooling, excessive sweating, fever, hand or foot spasms, irritability, dysphagia, and urinary or rectal incontinence (Table 2). The annual incidence of tetanus in Japan has remained consistent at approximately 100 cases, with a 10% mortality rate. Tetanus immunoglobulin (TIG) is derived from human serum and has been approved for commercial distribution. TIG neutralizes only the unbound endotoxin produced by *C. tetani*; this agent possesses neither antibacterial activity nor the capacity to prevent bacterial growth, and cannot restore organ function. The tetanus toxin initially binds to peripheral nerve terminals to cause the clinical symptoms. Therefore, theoretically, TIG does not resolve clinical symptoms, although such assertions are likely to be incorrect. However, Vollman et al. reported an interesting case of tetanus in which the patient’s symptoms were resolved after human TIG administration. Thirteen hours after TIG administration, the patient had an increased range-of-motion in the lower jaw and decreased neck pain. Approximately 27 h after TIG administration, the patient recovered the full range-of-motion in his neck and was able to move his jaw more completely. We note that no additional pharmacologic treatment was given in that case.

Under the Preventive Vaccination Law, enacted in Japan in 1968, four doses of diphtheria, tetanus, and pertussis vaccine are administered during infancy, and a vaccine against diphtheria and tetanus is administered at the age of 11–12 years. However, the immunity induced by vaccination wanes after approximately 10 years. In 2008, Takahashi et al. reported that individuals older than 45 years exhibited only 10% of the protective level of antibody titer for tetanus; in other words, tetanus preventive measures are insufficient. In contrast, in the United

### Table 1 Basic information about antitoxins and antivenoms

<table>
<thead>
<tr>
<th>Antitoxin/antivenom</th>
<th>Kokuyu vaccine</th>
<th>Property</th>
<th>Approved drug</th>
<th>Blood</th>
<th>Dose</th>
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<td>Yes</td>
<td>Equine</td>
<td>A,B,E: 10,000 U F: 4000 U</td>
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<td>No</td>
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<td>Yes</td>
<td>Human</td>
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<tr>
<td>Mamushi (<em>Gloydius blomhoffii</em>)</td>
<td>No</td>
<td>Freeze-dried</td>
<td>Yes</td>
<td>Equine</td>
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<tr>
<td>Habu (<em>Protobothrops flaviviridis</em>)</td>
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<tr>
<td>Yamakagashi (<em>Rhabdophis tigrinus</em>)</td>
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### Table 2 Basic information about toxins and venoms

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<tr>
<th>Toxin/venom</th>
<th>Typical symptoms</th>
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<tr>
<td>Gas gangrene</td>
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<td>Diphtheria toxin</td>
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<tr>
<td>Botulism</td>
<td><em>Clostridium botulinum</em> toxin (A,B,C,D,E,F,G,H,I)</td>
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<tr>
<td>Tetanus</td>
<td>Tetanus toxin</td>
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<td>Mamushi</td>
<td>Tetanus toxin</td>
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<td>Habu</td>
<td>Habu venom</td>
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<tr>
<td>Yamakagashi</td>
<td>Prothrombin activator</td>
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<td>Redback spider</td>
<td>α-Latrotoxin</td>
</tr>
</tbody>
</table>

**Tetanus**

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States, the reported level of seroprotection against tetanus in adults older than 65 years is approximately 80%. In Japan, adult antibody titers remain remarkably low when compared to those in other regions, and twice as many tetanus patients are reported in Japan than in the United States, with an accompanying high mortality rate (approximately 10%). Physicians should bear in mind that proper passive immunization with TIG for tetanus should be considered in patients older than 50 years, given the existing low immunity levels.

Mamushi (Gloydius blomhoffii) bites

Mamushi is a pit viper with fangs about 5 mm long with very thin tips (Fig. 2A). Approximately 2000–3000 mamushi bites occur annually in Japan, and mamushi venom is generally associated with local pain and swelling (Table 2). However, no previous randomized controlled trials (RCTs) or meta-analyses have examined the effects of antivenom. Hifumi et al. conducted a large, multi-center, population-based study of 234 mamushi bite cases and found that among severe cases (redness and swelling beyond the elbow or knee joint), patients who received mamushi antivenom had significantly shorter hospital stays compared to those who were administered cepharanthine ($P = 0.03$). In contrast, no significant difference in the duration of hospital stay was observed between the two groups for mild cases (redness and swelling limited to the wrist or foot) ($P = 0.77$). In other words, mamushi antivenom effectively reduces the hospital stay for patients with severe mamushi bites. Until 1990, antivenom was usually administered subcutaneously or intramuscularly to avoid adverse reactions, which were of great concern. Theoretically, intravenous administration of antivenom leads to earlier venom neutralization compared with subcutaneous or intramuscular administration. We recommend antivenom administration in patients with a grade >III mamushi bite, based on our previously reported data. Other types of snake antivenom, such as yamakagashi or habu antivenom, cannot neutralize mamushi venom.

Habu (Protobothrops flavoviridis) bites

Habu is the most dangerous of the three snakes considered here because it is large, reaching up to 2 m in length, and is the most aggressive. Habu fangs are tubular and 1.5–2 cm in length (Fig. 2B). Habu venom causes extremely severe local swelling, necrosis, and bleeding at the bite site (Table 2). To date, five types of pit vipers are known to inhabit Okinawa and Amami islands, and habu bites are limited to areas outside of the Japanese main island. However, the shifts promoted by climate change and increasingly intensive interactions between people have led to concerns regarding the potential for increased frequencies of habu bites on the main island.

Although habu antivenom is considered effective when administered after a bite, no RCTs or meta-analyses have examined the effects of this antivenom. Okinawa prefecture is known to house a large population of habu, and, accordingly, it has a higher rate of habu antivenom use compared with that of the Amami islands. No deaths from habu bites have been reported in the past 12 years in Okinawa (2004–2015, no deaths in 603 cases).
er, between 1965 and 1969, approximately 24 deaths were reported among 1770 cases in Okinawa because of the unavailability of habu antivenom. Recently, Nishimura et al. reported 86 cases of habu bites from 2003 to 2014 in Amami Ohshima, a known habu habitat in Kagoshima prefecture. Good clinical outcomes were reported in this area, with 97.7% (84/86 patients) receiving antivenom therapy and a mortality rate of only 1.1% (1/86 cases). In contrast, the mortality rate reported in the same area in 1978 was 10%. In summary, habu antivenom therapy appears to decrease mortality and should be considered when treating habu bites.

Yamakagashi (Rhabdophis tigrinus) bites

Yamakagashi grow to about 1 m in the plains and 1.5 m in the hills and mountains of Japan (excluding Hokkaido). The fangs of yamakagashi are not tubular, and the venom gland duct opens at the base of the fang (Fig. 2C). Yamakagashi venom, which acts as a prothrombin activator, exhibits strong blood coagulation activity and a weak thrombin-like effect, leading to DIC with a fibrinolytic phenotype (Table 2). Yamakagashi antivenom is derived from an equine source and is used as an off-label drug in Japan. Therefore, clinicians are required to use it in the context of clinical research. Over a period of 40 years, Hifumi et al. conducted a retrospective survey of 34 patients, 19 of whom were treated with antivenom. The authors found no significant differences in baseline characteristics and laboratory data between those treated and not treated with yamakagashi antivenom. However, hospital mortality was significantly reduced among patients treated with yamakagashi antivenom (0% vs. 26.7% without antivenom; \(P = 0.03\)). Moreover, the number of patients with renal failure who required hemodialysis was significantly lower among those treated with yamakagashi antivenom (5.3% vs. 40.0% without antivenom; \(P = 0.03\)). Therefore, antivenom is considered to be an effective treatment for yamakagashi bites. Fibrinogen levels <100 mg/dL are considered to be an appropriate indicator for antivenom administration in clinical practice.

Yamakagashi antivenom was manufactured via the immunization of rabbits in 1986 and of goats in 1987. However, low supply levels recently led to the immunization of horses, a practice that was supported by Health Science Grants (1998–1999) from the Ministry of Health, Labor, and Welfare in 2000. The fact that more than 16 years have passed since the most recent round of antivenom production is of great concern. Although our research group has examined the potency and quality of this antivenom, we cannot guarantee its quality in the future.

Redback spider (Latrodectus hasselti) bites

Since the first case reported in Osaka in 1997, redback spider bites have been a clinical and administrative issue in Japan. The adult female redback is characterized by a spherical black body with a prominent red stripe on the upper side of the abdomen and a red streak on the underside (Fig. 3). Alpha-latrotoxin, the main toxin produced by the redback spider, causes synaptic vesicle exocytosis from the presynaptic terminal via a calcium-dependent mechanism, leading to the release of catecholamines and acetylcholine.

Currently, two types of redback spider antivenom exist; these are produced by the Commonwealth Serum Laboratories in Australia and by our research group in Japan. Because these antivenoms are used as off-label drugs in Japan, clinicians are required to join a clinical research group to use either of the antivenoms in clinical practice.

To date, fatalities related to redback antivenom have not been reported in Australia since the development of antivenom. In 2014, Isbister et al. conducted a RCT to evaluate the effect of redback antivenom, and concluded that the addition of antivenom to standardized analgesia in patients with latrodectism did not significantly improve pain or systemic effects. However, that study has raised some concerns. In Australian emergency departments, some patients with redback spider bites, including children, pregnant women, and the elderly, have increased risks of developing severe or systemic symptoms and are more likely to receive antivenom. However, children, pregnant women, and the elderly were not included in the abovementioned target study population.

In 2013, the Ministry of Health, Labor, and Welfare of Japan established a research group to evaluate the safety
and efficacy of antivenom and to organize and maintain information about redback spider bites. Our research group conducted a 5-year retrospective study to elucidate the clinical characteristics of these bites and clarify the therapeutic efficacy of antivenom. Our survey evaluated the efficacy of antivenom in a small number of patients, including children and the elderly. In addition, we carefully discussed the domestic production of redback spider antivenom, which thereafter was completed in December 2015. According to this study, the routine use of antivenom for redback spider antivenom is not recommended; however, the efficacy of antivenom for the treatment of patients in the above-mentioned high-risk group should be evaluated further. We further note that redback spider antivenom protects against toxicity from widow spider venom, and therefore could be useful as a preventive measure in the event of a pandemic of widow spiders.55,56

Conclusions

It is hoped that this review will provide clinicians with an opportunity to learn about and become familiar with clinical serum therapies.

Acknowledgments

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Conflicts of Interest

The authors have no conflicts of interest to report.

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