Continuous IV Crotalidae Polyvalent Immune Fab (Ovine) (FabAV) for selected North American Rattlesnake bite patients

Sean P. Bush a,*, Steven A. Seifert b, Jennifer Oakes c, Susan D. Smith a, Tammy H. Phan a, Sarah R. Pearl a, Ellen T. Reibling a

a Department of Emergency Medicine, Loma Linda University, School of Medicine, Loma Linda, CA, USA
b Department of Emergency Medicine, University of New Mexico, School of Medicine, New Mexico Poison and Drug Information Center, Albuquerque, NM, USA
c Department of Emergency Medicine, Royal Inland Hospital, Interior Health Authority, Kamloops, British Columbia, Canada

ARTICLE INFO

Article history:
Received 21 September 2012
Received in revised form 10 January 2013
Accepted 5 February 2013
Available online 6 March 2013

Keywords:
Antivenom
FabAV
Continuous IV infusion: recurrence
Hematologic effects
Crotalinae
Envenomation

ABSTRACT

Background: In patients bitten by North American rattlesnakes and treated with Crotalidae Polyvalent Immune Fab (Ovine) (FabAV), late hematologic abnormalities—persistent, recurrent, or late, new onset of hypofibrinogenemia, prolonged PT/INR, prolonged PTT, and/or thrombocytopenia beyond 48 h post-envenomation—are common, difficult to manage, and may result in morbidity and mortality. The optimal management of late hematologic abnormalities, particularly the use of further treatment with antivenom, has not been well defined. The current FabAV treatment regimen is to give antivenom as a bolus dose over a one-hour period. We describe our experience using a continuous intravenous infusion of FabAV for late hematologic effects and/or associated bleeding complications in rattlesnake envenomation.

Methods: This is a retrospective, observational case series of patients envenomated by North American rattlesnakes at three medical centers managed with a continuous intravenous infusion of FabAV for late hematologic abnormalities and/or associated bleeding complications. Indications, dilution and infusion protocols, and duration of therapy were individualized.

Results: Five cases were identified between July 2010 and September 2011. All patients had profound late hematologic abnormalities and/or were associated with bleeding complications. Several patients had received repeat bolus infusions of FabAV, with or without human blood products, with either inadequate or only transient beneficial response. All patients were then managed with a continuous intravenous infusion of FabAV and all appeared to respond to the continuous intravenous infusion of FabAV, titrated to effect, with cessation of progression and, in most cases, improvement in hematologic abnormalities. Rates of infusion varied from 2 to 4 vials per 24 h (mean = 3.1 ± 0.4 vials/day). The termination of FabAV infusion was between day 6 and day 14 from the time of envenomation (mean = 10 ± 3 days), after which hematologic values were normalized or were normalizing in all patients and continued to do so.

* Corresponding author. Tel.: +1 951 440 9600.
E-mail addresses: sbush@llu.edu (S.P. Bush), SSeifert@salud.unm.edu (S.A. Seifert).

Contents lists available at SciVerse ScienceDirect
Toxicon
journal homepage: www.elsevier.com/locate/toxicon

0041-0101/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxicon.2013.02.008
1. Introduction

In patients bitten by North American rattlesnakes and treated with Crotalidae Polyvalent Immune Fab (Ovine) (FabAV), late hematologic abnormalities—persistent, recurrent, or late, new onset of hypofibrinogenemia, prolonged PT/INR, prolonged PTT, and/or thrombocytopenia beyond 48 h post-envenomation—are common, difficult to manage, and may result in morbidity and mortality. The optimal management of late hematologic abnormalities, particularly the use of further treatment with antivenom, has not been well defined. The current FabAV administration method is to give antivenom as a bolus dose over a one-hour period, with sufficient doses to obtain initial control of venom effects, followed by maintenance dosing of 2 vials every six hours for three doses. The use of antivenom for late effects is not well established, with the package insert stating: “Optimal dosing following the 18 h scheduled dose of CroFab has not been determined. Additional 2 vial doses may be administered as deemed necessary by the treating physician, based on the patient’s clinical course” (CroFab™ Package Insert). We describe our experience using a novel approach of a continuous intravenous infusion of FabAV for late hematological effects in rattlesnake envenomation.

2. Materials and methods

This is a retrospective, observational case series of patients envenomated by North American rattlesnakes and presenting to three medical centers with clinically challenging snake envenomation. A continuous intravenous infusion of FabAV was administered at some point during the course of medical management for patient benefit to control and reverse late hematologic abnormalities and/or associated bleeding complications. Indications, dilution and infusion protocols, and duration of therapy were individualized as detailed below.

Discussion: The use of FabAV as a continuous intravenous infusion, particularly after the acute phase of envenomation has passed, provides a continuous source of circulating antibodies to neutralize venom components reaching circulation from tissue stores and allows natural replenishment of hematologic factors such as platelets and/or fibrinogen. This method is an efficient use of FabAV, avoiding the wasteful excess of a bolus dose, may be more effective, eliminating the potential for destruction of hematologic factors when protective antivenom levels are lost between bolus FabAV doses, and appears to be safe. Further assessments of the stability and sterility of the product during infusion are needed. The need to continue hospitalization is the major drawback, but continued observation and inpatient care may be needed for other indications (e.g. bleeding) in this subset of patients.

Conclusions: A continuous intravenous infusion of FabAV between 2 and 4 vials per day, titrated to effect, and continued for 6–14 days post-envenomation appeared to be associated with reversal of late hematologic effects of rattlesnake envenomation and, when combined with indicated human blood products, control of significant bleeding. Continuous intravenous infusion of FabAV may be safer, more efficacious, and more cost-effective than observation without FabAV treatment or as-needed bolus dosing in selected patients with late hematologic abnormalities.

3. Results

3.1. General analysis

Five cases were identified between July 2010 and September 2011. All patients had profound hematologic abnormalities that persisted, recurred and/or were associated with serious bleeding. Several patients received repeat bolus infusions of FabAV, with or without human blood products, with either inadequate or only transient beneficial response. All patients were then managed with a continuous intravenous infusion of FabAV and all appeared to respond to the continuous intravenous infusion of FabAV, titrated to effect, with cessation of progression and, in most cases, improvement in hematologic abnormalities. Rates of infusion varied from 2 to 4 vials per 24 h (mean = 3.1 ± 0.4 vials/day) for control or reversal of hematologic abnormalities. The termination of FabAV infusion was between day 6 and day 14 from the time of envenomation (mean = 10 ± 3 days) with a duration of FabAV infusion of 4–14 days (mean = 6 ± 2 days), after which hematologic values were normalized or were normalizing and continued to do so.

3.2. Case 1

A 16-year-old healthy male presented 2 h after a rattlesnake (Crotalus sp.) envenomation to his right lower extremity in Southern California. Tenderness extended from the bite site into his distal thigh. Diagnostic studies revealed with thrombocytopenia (platelet count 2 bil/L) and coagulopathy (Prothrombin Time non-clotting, INR 9.4, fibrinogen not done). Shortly after arrival, patient developed hematemesis, bloody diarrhea, and bruising to his snake bitten leg. Hemoglobin decreased 7 g/dL in the 2-h interval between draws. So the patient was given 12 vials of FabAV, 4 units of frozen plasma, and 2 platelet packs.

Repeat labs showed INR 1.7 and platelets of 79 bil/L. Reassessment of the patient post infusion showed no
further advancements of local effects, improvement of systemic effects and a trending of abnormal coagulation parameters/platelet counts toward normal (Fig. 1) indicating initial control. Another dose of FabAV 2 vials were given at 6 and 12 h after initial control. The patient’s clinical status and labs were followed closely. However, the patient’s platelet counts and fibrinogen began to trend downward again. A continuous intravenous infusion of antivenom was proposed. FabAV 2 vials in 500 mL normal saline at a rate of 21 mL/h was initiated (2 vials per 24 h). However, platelet counts continued to trend downward, so the rate was increased to 4 vials per 24 h. After the increased continuous intravenous infusion rate was started, a gradual improvement in fibrinogen and stabilization and subsequent improvement of the platelet count was observed and there were no further platelet counts below 100 bil/L or fibrinogen levels less than 100 mg/mL. Lab values continued to improve after cessation of the continuous intravenous infusion on hospital day (HD) 6, ultimately normalizing and remaining normal through HD 15.

3.3. Case 2

A 61-year-old female presented after a rattlesnake envenomation in Southern California with unmeasurable fibrinogen, platelets of 39 bil/L, and gross hematuria. She was hypotensive with angioedema and was orotracheally incubated. She had total body myokymia and later developed rhabdomyolysis. She was pharmacologically paralyzed to prevent myokymia from worsening her rhabdomyolysis. Her neurological status was difficult to follow because of the sedation. Meanwhile, her platelet counts began to trend downward. Because of concerns for a profound recurrence of thrombocytopenia and the difficulty of clinically detecting a significant bleed, a continuous intravenous infusion of FabAV at 2 vials/24 h was initiated and increased to 4 vials/24 h when the decline continued and her platelet count dropped below 100 bil/L. On this infusion rate, her platelet counts leveled off and returned to the normal range by the second week as illustrated in Fig. 2.

3.4. Case 3

A 73-year-old patient presented with thrombocytopenia of 106 bil/L and an INR of 6.4 following an envenomation to her ankle by a speckled rattlesnake (*Crotalus m. mitchellii*) in Southern California. Assessment of the role of the envenomation in the prolongation of the INR and its optimal management was complicated by the patient being on warfarin therapy for her mechanical prosthetic valve. Her venom-induced thrombocytopenia responded to antivenom therapy but recurred, trending downward reaching a nadir of 93 on HD 4. Fibrinogen was normal during the entire hospital course. Her warfarin was held and her INR dropped to sub-therapeutic. Her cardiologist recommended enoxaparin bridging therapy until her INR was therapeutic (between 2.5 and 3.5). To prevent potentially multiple episodes of thrombocytopenia if treated with repetitive boluses, it was judged to be safer and more efficient to start a continuous intravenous infusion of FabAV after a single bolus of 4 vials. Her platelet count promptly returned to normal and remained so after the continuous intravenous infusion was started (Fig. 3).

![Fig. 1. Case 1 – trend in platelet count and fibrinogen level during continuous intravenous infusion of FabAV.](image-url)
3.5. Case 4

The patient was a 40 year-old female who was bitten on her left foot by a rattlesnake while walking in her garden in New Mexico. She experienced immediate pain and swelling and presented for care within an hour of the bite. Her swelling had progressed to just below her knee, with good neurovascular function distally. Her initial hematologic laboratories, obtained two hours post bite were: Platelets 203 bil/; Fibrinogen 288 mg/dL; d-dimer 4.45; PT 15.6 s;

![Fig. 2. Case 2 – trend in platelet count during continuous intravenous infusion of FabAV.](image)

![Fig. 3. Case 3 – trend in platelet count during continuous intravenous infusion of FabAV.](image)
INR 1.07; PTT 34 s. She was treated with six vials of antivenom (CroFab<sup>®</sup>; FabAV), with initial control of both local and systemic effects. Platelets increased to 270 bil/L within two hours. Repeat fibrinogen was 315 mg/dL; and PT/INR 14.7 s/1.15. She received three maintenance doses (2 vials q 6 h) of FabAV and was discharged at 36 h. Discharge labs were platelets 161 (nadir 154) bil/L, fibrinogen 312 mg/dL, PT/INR 14.2 s/1.11.

The patient returned two days later and had no new symptoms or bleeding, but platelets were 87 bil/L; fibrinogen 327 mg/dL, PT/INR 13.3 s/1.02. She was instructed to return the next day for follow up labs but did not present again until two days later, when she was bleeding excessively from her menses. Platelets were 19 bil/L, with continued normal values for fibrinogen and PT/INR, which remained normal for the remainder of her care. The patient was given 2 vials of FabAV and readmitted to the hospital. Repeat platelets 2 h later were 15 bil/L and another 2 vials of FabAV were given, after which the platelet count had increased to 22 bil/L. Because of continued menstrual bleeding and a declining hematocrit from 37% to 32%, the patient was given an additional two vials of FabAV, and one unit of a platelet transfusion, after which the platelet count was 55,000 bil/L and her bleeding decreased to a normal menses flow. The next day, the platelet count had decreased to 35,000 bil/L and the patient received another 2 vials of FabAV. The platelet count increased slightly to 37,000 bil/L, the Hct remained stable at 32% and menstrual bleeding was unchanged. Her platelet count declined to 21,000 bil/L and she was given another 2 vials of FabAV as well as 2 units of platelets, with an increase to 62,000 bil/L. Because the patient was requiring frequent boluses of FabAV, a decision was made to start a continuous intravenous infusion, initially at a rate of 2 vials per 24 h. When the platelet count decreased to 49,000 bil/L 12 h later, the FabAV infusion rate was increased to 3 vials per 24 h. The platelets declined to a nadir of 46,000 bil/L shortly thereafter and then increased to 60,000 12 h later. The infusion was continued for another 12 h and then discontinued. Platelets remained greater than 60,000 for the next 24 h without additional antivenom or platelet transfusions. Bleeding had stopped and the patient was discharged home. The next day, platelets were 135,000, there was no further bleeding and the patient had an otherwise uneventful recovery. See Fig. 4.

3.6. Case 5

A 29 year-old female had been walking at late dusk in Canada when she felt a very sharp stabbing sensation to her left foot. Within minutes her foot began to become painful and swollen. No snake was seen or heard, but both the Western Rattlesnake (Crotalus viridis oreganus) and the Northern Pacific Rattlesnake (Crotalus oreganus oreganus) are native to the region. She presented to an emergency department approximately 45 min later. Upon arrival, edema and erythema extended to her left knee, with local ecchymosis over foot. Two puncture wounds were noted to the proximal dorsal L foot approximately 2 cm apart. The patient described paresthesias to the left leg as well as perioral numbness. Vital signs were HR 140s, BP 103/58, T 36.6°C, RR 24, O2sat 98%RA. She complained of feeling ‘shaky’ but no tremors or fasciculations were noted.

Initial laboratory investigations revealed platelet count of 20 bil/L, hemoglobin 148 g/L, WBC count 12.2 K/L, INR...
1.2, fibrinogen 240 mg/dL. ECG showed sinus tachycardia but was otherwise unremarkable.

The patient received 6 vials of FabAV approximately 1 h after presentation. She continued to have progressive edema, ecchymosis and pain to left leg. She received an additional 2 vials of FabAV 7 1/2 h after envenomation, and another 4 vials at 13 h due to progressive swelling. She received maintenance doses of 2 vials of FabAV 8 h later, followed by 2 vials 6 h thereafter. The patient had persistent edema and ecchymosis extending from foot to hip of left lower extremity and was unable to weight bear secondary to pain and edema of the limb. She had other areas over her arms and torso of spontaneous ecchymosis and was noted to have mild hematuria. No other bleeding was manifest.

On HD 4, the patient was noted to have severe, recurrent thrombocytopenia (Fig. 5). She was administered 4 vials FabAV by bolus and then 2 vials every 6 h × 6 additional doses. On HD 6 FabAV was changed to a continuous intravenous infusion of 1 vial infused over 8 h (=3 vials per 24 h), and increased to 1 vial over 6 h (=4 vials per 24 h) on HD 8 and continued at this rate for an additional 24 h. On HD 9 the patient had improved considerably in terms of pain, edema and ecchymosis of the limb. She was able to weight bear without significant pain, and had no new signs of bleeding. The FabAV infusion was stopped and her platelets remained stable for the following 48 h 48 h after discharge and platelets were stable at 72 bil/L and hemoglobin was within normal range. She had no further hematologic manifestations, but has persistent pain and lymphedema of the envenomated limb.

4. Discussion

We describe a series of patients who received FabAV as a continuous intravenous infusion at various points of time during their envenomations for control of late hematologic effects and/or associated bleeding complications. To our knowledge, this is the first such use and report of this novel dosing method.

In the period following initial control, maintenance dosing (2 vials every 6 h for 3 doses) is recommended in prescribing information for continued control of local venom effects. Although an early post-marketing study found maintenance dosing superior to as needed dosing for this indication (Bogdan et al., 1997), late hematologic abnormalities were not prevented by either regimen (Bush et al., 2002; Offerman et al., 2002).

An initial presentation with venom-induced thrombocytopenia and/or coagulopathy is highly predictive for persistent or recurrent hematological abnormalities of a similar nature and severity (Boyer et al., 1999). The phenomenon of late hematologic abnormalities is likely secondary to the short half-life of FabAV and its rapid clearance from circulation. This allows unneutralized venom, reaching circulation from tissue sites, to again produce its hematologic effects (Boyer et al., 1999; Seifert et al., 2011). Venom antigens have been detected in blood and urine for days to weeks after envenomation (Nielsen et al., 1978; Gillissen et al., 1994; Ownby et al., 1996) and late hematologic abnormalities may persist, reoccur or newly appear during this time (Boyer et al., 1999; Seifert et al., 2011). While routine monitoring of hematologic
parameters in all snakebite patients post-discharge has been advocated to detect this phenomenon (Ruha et al., 2011; Seifert et al., 2011), the optimal management of late hematologic venom effects in the days and weeks following FabAV-treated rattlesnake envenomation has not been determined. The administration of additional antivenom for recurrent hypofibrinogenemia or thrombocytopenia post-discharge has been reported (Seifert et al., 1997; Boyer et al., 1999) but resulted in minimal and transient improvement. A “conservative” approach to management of delayed, multi-component coagulopathy following rattlesnake envenomation, consisting of observation alone, has been described (Camilleria et al., 2005). However, poor outcomes, including death, following severe late hematologic abnormalities have since been reported (Kitchens and Eskin, 2008; O’Brien et al., 2009; Stanford et al., 2006a,b).

If there is a clinical benefit to the use FabAV in the management of late hematologic abnormalities, it is most likely to be seen in the context of severe envenomations, the criteria for which were met in each of the cases in this series (Yin et al., 2011). All of five of our cases had persistent, recurrent or late, new onset thrombocytopenia and one patient also had recurrent hypofibrinogenemia. All of these patients also had either associated bleeding complications or complicated medical conditions in which hemorrhage or anticoagulation was problematic.

In each of our cases, considered individually, a continuous intravenous infusion of FabAV was clinically judged to provide advantages to conventional repeat bolus dosing. In all cases, once a sufficient rate of antivenom infusion was obtained, platelet counts appeared to stabilize and then to increase. The one patient who also had recurrent hypofibrinogenemia also had and maintained improvement in fibrinogen concentrations. Despite the disparate nature of the specific case scenarios and management protocols, the use of FabAV as a continuous intravenous infusion, particularly after the acute phase of envenomation had passed, appeared to provide continuous control of ongoing venom effects. By providing a continuous source of circulating antibodies to neutralize venom components reaching circulation from tissue stores and allowing natural replenishment of hematologic factors such as platelets or fibrinogen, the lowest required dose of antivenom was used and episodes of new recurrent effects were eliminated. Although isolated hypofibrinogenemia may be associated with a lower risk of spontaneous hemorrhage than thrombocytopenia (Boyer et al., 1999), profound hypofibrinogenemia may be associated with increased risk of bleeding in trauma and may also increase the risk of bleeding with multi-component hematologic abnormalities (Boyer et al., 2001).

We feel that this method is an efficient use of FabAV, avoiding the wasteful excess of a bolus dose, much of which will not find venom antigens in circulation to neutralize and which will then be excreted. This approach may also provide a continuous neutralization of venom coming into circulation and both prevent additional recurrences between bolus doses and also allow for re-accumulation of hematologic clotting components. The initial rate of FabAV infusion of 2 vials per 24 h was based on prior, published theoretical work (Seifert, 1998). Titration of the infusion rate and the duration of need for therapy were based on empiric response to treatment. All patients in this series appeared to require at least 3 vials per 24 h for control of venom effects. Although thrombocytopenia and/or hypofibrinogenemia may persist for more than 3 weeks, in our series, no patient required additional FabAV beyond 14 days after envenomation. It is possible that at some point, the endogenous rate of replenishment of platelets, fibrinogen or clotting factors will be sufficient to maintain stable to increasing serum concentrations despite some rate of ongoing venom-induced destruction.

The optimal manner of FabAV titration and cessation is not clear. Since this was not a systematic study and each clinician used his or her own judgment to decide when to initiate, maintain, increase, or stop the infusion, no evidence-based recommendations can be made. For the most part, “titration” starts at a rate that may be effective and adjustments are upward until stabilization or improvement in the target parameter (e.g. platelets or fibrinogen) is seen. Termination of the infusion is done when the clinician feels that the natural rate of replenishment of fibrinogen or platelets will exceed any venom-related destruction. In the absence of a commercial venom assay or other assessment tools, this is largely a matter of trial and error, informed by what is known about the persistence of venom levels and the experience of prior reports, such as this.

In our series, no adverse effects of administering FabAV as a continuous intravenous infusion were seen. Under current prescribing guidelines, FabAV is presumed to be sterile and stable for at least 5 h after mixing (administration of the drug to be started no longer than 4 h after mixing, with the usual bolus infusion time of 1 h) (CroFab™ Package Insert). Although some of the patients in this series received a single bag of FabAV over a 24 h period without adverse effect, it would seem prudent to limit the duration of infusion of any given bag of FabAV. Since all of our patients in our series required at least 3 vials per 24 h, initiating the infusion at that rate would limit the duration of infusion of a single bag containing 1 vial, to 8 h. Further assessment of the safety of this drug administration protocol over various infusion periods is needed.

A continuous IV infusion generally requires an inpatient setting. However, this management protocol is only likely to be needed for the most severely envenomed patients and continued hospitalization of these patients may be needed for a variety of indications. If the stability and sterility of a continuous intravenous infusion is determined to be safe over a 24-h or longer period, outpatient administration by a portable infusion pump may be possible.

Because, compared with a Fab antivenom, free antivenom remains in circulation far longer with a F(ab′)2 antivenom (Gutiérrez et al., 2003), there may be a lower likelihood of recurrent hematologic venom effects and, if a recurrence should occur, is likely to occur later and be milder than that seen with an Fab antivenom. A clinical trial of a F(ab′)2 antivenom, compared directly against the existing Fab antivenom, recently concluded in the U.S. and may provide information on the relative rates of hematologic recurrence. However, at present and for the immediate future, the only available FDA-approved antivenom is the existing Fab product.
5. Limitations

This is a small, retrospective case series of patients treated with a continuous intravenous infusion of IV FabAV with different clinical scenarios, indications, and evaluation and treatment regimens and no controls for comparison. Post-hoc analysis of platelet and fibrinogen trends is, to some extent, subjective, and in some cases did not result in immediate or dramatic improvements, although stabilization is another potential interpretation of those findings. And it is possible that apparent improvements in platelets or fibrinogen following initiation of continuous IV FabAV was spontaneous and coincidental to antivenom administration. Thus, an uncontrolled case series such as this is not able to determine causation and whether this treatment methodology is effective or safe. The inability to measure serum venom and antivenom concentrations is a further limitation, on the extrapolation of the experiences of these cases, the ability of clinicians to monitor and treat hematologic recurrences and increases the difficulty of studying this phenomenon prospectively.

6. Conclusions

A continuous intravenous infusion of FabAV between 2 and 4 vials per day, titrated to effect and continued for 6–14 days post-envenomation appeared to be associated with reversal of persistent and/or recurrent hematologic effects of rattlesnake envenomation and, combined with human blood products, control of significant bleeding. Continuous intravenous infusion of FabAV may be more efficient and more cost-effective than either observation without FabAV treatment or as-needed bolus dosing in high-risk patients with severe persistent, recurrent or late, new-onset hematologic abnormalities and/or in patients with associated bleeding complications.

Ethical statement

The patients who are included in this report were treated by their providers in what was felt to be in their immediate best interests. The cases were selected retrospectively for this report because of the similarity of the management approach employed. The report was granted an exemption by the institutional review board of the University of New Mexico.

Acknowledgments

An abstract of this work was presented at the IST/Venom Week meeting in Honolulu, HI, July 2012 and published as part of the proceedings of the meeting (Bush et al., 2012).

Appendix A

Laboratory normal values
Platelets 140 bil/L–375 bil/L
Fibrinogen > 170 mg/dL–450 mg/dL
D-dimer < 0.5 mg/dL

Conflict of interest statement

SPB and/or this author’s affiliated organization has/have received funding and/or remuneration for educational presentations related to medical management of venomous bites from BTG International Inc., and for clinical trials investigations of antivenom(s)-in-development from Rare Disease Therapeutics, Inc./Instituto Bioclon.

SAS and/or this author’s affiliated organization has/have received funding and/or remuneration for clinical trials investigations of antivenom(s)-in-development from Protherics (CroFab™) and Rare Disease Therapeutics, Inc./Instituto Bioclon.

The other authors have no disclosures to report.

There were no sources of funding for this research, and the aforementioned sponsor(s) had no involvement in the design of this study; in the collection, analysis, or interpretation of this data; in the writing of this report; in the decision to submit this paper for publication.

References