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Coagulation parameters in copperhead compared to other *Crotalinae* envenomation: secondary analysis of the F(ab’)2 versus Fab antivenom trial

Charles J. Gerardo, Joao R. Nickenig Vissoci, Michael W. J. Brown and Sean P. Bush

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**ABSTRACT**

**Context:** Coagulation derangements in copperhead envenomation are considered less severe than other crotaline envenomations, resulting in recommendations to limit both coagulation testing and antivenom treatment. A prospective, blinded, multicenter, randomized clinical trial comparing the effectiveness of F(ab’)2 versus Fab antivenom in crotaline envenomation patients was completed in 2011. We determined the difference between coagulation parameters in copperhead compared to other crotaline envenomations.

**Methods:** We performed a post hoc analysis comparing the coagulation parameters (platelets and fibrinogen) prospectively obtained in the aforementioned trial. All the patients received antivenin in one of three treatment arms [F(ab’)2 with maintenance, F(ab’)2 with placebo maintenance, or Fab with maintenance]. Coagulation parameters were measured at pretreatment baseline, during acute hospitalization, day 5, day 8, and day 15 post-envenomation. Mean platelet count and fibrinogen levels for the copperhead and other crotaline groups were compared. The platelet and fibrinogen point estimates with distribution are presented graphically over time.

**Results:** 122 patients were enrolled in the study. There were 22 patients with copperhead envenomation, 93 with other crotaline envenomations, and 7 that could not be definitively determined. The mean age was 42 (SD 20) years. There was a minor pretreatment difference in mean baseline platelet count between the copperhead group (246 × 10^9/L 95% CI 215, 277) compared to other crotaline envenomation patients (184 × 10^9/L 95% CI 167, 202). There was a modest pretreatment difference in mean fibrinogen level between copperhead patients (345 mg/dL 95% CI 277, 415) and other crotaline patients (261 mg/dL 95% CI 241, 281). Pretreatment coagulation parameter means were normal and converged post treatment.

**Conclusion:** On average, copperhead envenomations have less severe initial coagulation derangements. However, in mild envenomations, differences in laboratory values are minimal and there is substantial variation in individual patients regardless of species. Species alone should not be used to determine the need for laboratory testing or treatment in copperhead snakebite.

**Introduction**

*Crotalinae* (pit viper) snake venom is a complex mixture of proteins and peptides that cause clinical abnormalities in several organ systems, including hematologic venom effects.[1,2] The hematologic venom effects include both cellular and acellular components of coagulation. Venom-induced thrombocytopenia, hypofibrinogenemia, elevated prothrombin time (PT), and/or partial thromboplastin time (PTT) may lead to coagulopathy and subsequent bleeding.[3]

Rattlesnakes, pygmy rattlesnakes, cottonmouth and copperhead snakes are members of the *Crotalinae* subfamily of venomous snakes, and all can cause coagulopathy. Coagulation derangements from copperhead envenomation are considered less severe than other crotaline envenomation syndromes, resulting in recommendations for limiting both coagulation testing and antivenom treatment in these patients.[4–8] Although rare, there are reports of hemorrhage in copperhead envenomations.[9,10] Hemorrhage in other crotaline envenomation is also uncommon and it appears that the full spectrum of coagulopathy is present in both groups.[9–14] To date no study has evaluated coagulopathy in copperhead versus other crotaline envenomation in patient populations that were similar with the exception of snake species. It remains unclear if copperhead envenomation patients should be evaluated and treated differently based solely on snake species.

A prospective, blinded, multicenter, randomized clinical trial comparing the effectiveness of F(ab’)2 versus Fab antivenom enrolled patients between May 2008 and September 2011. This trial evaluated the coagulation profiles of crotaline envenomation at baseline and serially beyond two weeks post-envenomation. This trial included copperhead and other crotaline envenomation patients and presents an opportunity for comparison of these two groups.[15] Comparing the difference in coagulation parameters over time in these two groups is important to understand the risk of bleeding in...
copperhead versus other crotaline envenomation. This will inform our testing and treatment recommendations for copperhead envenomation.

The goal of this investigation is to determine the difference between coagulation parameters in copperhead and other crotaline envenomation.

**Methods**

**Study design**

We performed a post hoc analysis of the coagulation parameters prospectively obtained in the aforementioned clinical trial. The Institutional Review Board at each site approved the study protocol. The study was registered at ClinicalTrials.gov, NCT00636116. The details of this trial protocol are previously described elsewhere, and are summarized in brief below.[15]

**Setting and selection of participants**

The study enrolled crotaline envenomation patients aged 2–80 years presenting to emergency departments from 18 clinical sites across the USA. Signs of envenomation included predefined local signs, systemic signs and symptoms, and decreased platelets or fibrinogen. The study excluded pregnant or breastfeeding patients, those without signs of envenomation, or those with underlying medical conditions or medications that significantly effect platelets or fibrinogen. All patients received antivenom in one of three treatment arms [F(ab')2, with maintenance, F(ab')2, with placebo maintenance, or Fab with maintenance].

**Measurements and outcomes**

Study personnel obtained the subfamily, genus and species of the offending snake when possible. The site investigator made the identification by live snake or carcass, or photo of the offending snake. The primary investigator cross-checked the identification against locally existing indigenous snakes at each site to ensure accuracy. Study personnel obtained the pretreatment baseline platelets and fibrinogen. Laboratory studies were repeated serially during the acute post-treatment phase at the following time points: 2 h after initial dose of antivenom, 2 h after the start of each additional antivenom dose required to obtain initial control and 2 h after the third maintenance dose. They were also obtained during follow-up on days 5 (±1), 8 (±1), and 15 (±1). As multiple measurements occurred during the acute hospitalization phase, both the baseline and acute post-treatment nadir was chosen for comparison. Additionally, we report the baseline snakebite severity score (SSS) to ensure similarity between treatment groups.

**Analysis**

We report the baseline characteristics for copperhead and other crotaline envenomation patients. We also report the proportion of patients in each group with any severe thrombocytopenia, defined as platelet count <50 x 10^9/L and severe hypofibrinogenemia, defined as fibrinogen less than 50 mg/dL. Continuous data is reported as mean and SD or median and interquartile range as appropriate. We compare baseline characteristics between the groups using chi-square and t test for independent samples.

We report the platelet count and fibrinogen point estimates with 95% confidence intervals for each group. We present the platelet count and fibrinogen level individual values and median graphically over time. We compare the coagulation outcomes per time of recovery with bivariate comparisons adjusting for multiple comparisons.

We created linear mixed effects models to assess the relationship between time of recovery, coagulation parameters, and type of snake. We modeled each coagulation parameter separately. Fixed effects were time of recovery (baseline, acute phase nadir, 5, 8, and 15 d) and type of snake (copperhead or other crotaline), with an interaction term used in the model. Random effects were intercepts for subjects, as well as by-subject and by-time of recovery random slopes for the effect of type of snake and time of recovery. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. We obtained p values by likelihood ratio tests of the full model with the effect in question against the model without the effect and without the interaction term. We used R Language for Statistical Consulting (R Core Team, 2015) through the lme4 package (Bates, Maechler & Bolker, 2012) to perform all analysis and figures.

Power for the analysis was calculated to ensure power to detect moderate effect size for change over time. Moderate effect size was based on standards defined by Cohen’s D effect size for distribution comparisons. Our sample size for the copperhead group would give a power of 80 and 5% significance, in a paired mean comparison test. Specifically for the mixed models, considering a moderate change over 5 time points of follow up, 5% significance and 80% power would be reached with 22 patients by group when the outcome modeled was platelets.[16,17]

**Results**

**Characteristics of study subjects**

One hundred and twenty-two patients were analyzed in the initial clinical trial. In seven patients, they could not definitively identify the offending snake, leaving 115 patients for our analysis. This included 22 patients with copperhead envenomation and 93 involving other crotaline species (92 rattlesnakes and 1 cottonmouth). The patients were predominantly male (74.8%) and white (68.7%), with a mean age of 41.6 (SD 20.1) years. There were no statistical differences in baseline demographic data between groups except race. The other crotaline patient population had more Hispanic patients (23%), while the copperhead group had relatively more African-Americans (14%). When applying a Bonferroni correction for multiple comparisons, race is no longer statistically significant. Median SSS was 3 (IQR 2, 5),
Table 1. Demographics and severity baseline data.

<table>
<thead>
<tr>
<th></th>
<th>Total (N = 115)</th>
<th>Other Crotalinae (N = 93)</th>
<th>Copperhead (N = 22)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, mean (SD)</td>
<td>41.6 (20.1)</td>
<td>39.8 (19.9)</td>
<td>44.1 (20.9)</td>
<td>0.38</td>
</tr>
<tr>
<td>Sex, male, N (%)</td>
<td>86 (75)</td>
<td>68 (73)</td>
<td>18 (82)</td>
<td>0.57</td>
</tr>
<tr>
<td>Ethnicity, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>79 (69)</td>
<td>62 (67)</td>
<td>17 (77)</td>
<td>0.03</td>
</tr>
<tr>
<td>African-American</td>
<td>5 (4)</td>
<td>2 (2)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hispanic</td>
<td>23 (20)</td>
<td>21 (23)</td>
<td>2 (9)</td>
<td>–</td>
</tr>
<tr>
<td>Other*</td>
<td>8 (7)</td>
<td>8 (9)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sex, male, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>23 (20)</td>
<td>21 (23)</td>
<td>2 (9)</td>
<td>–</td>
</tr>
<tr>
<td>Hispanic</td>
<td>23 (20)</td>
<td>21 (23)</td>
<td>2 (9)</td>
<td>–</td>
</tr>
<tr>
<td>Other*</td>
<td>8 (7)</td>
<td>8 (9)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ethnicity, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
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<td>5 (4)</td>
<td>2 (2)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hispanic</td>
<td>23 (20)</td>
<td>21 (23)</td>
<td>2 (9)</td>
<td>–</td>
</tr>
<tr>
<td>Other*</td>
<td>8 (7)</td>
<td>8 (9)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Race Misclassified</td>
<td>37 (32)</td>
<td>28 (30)</td>
<td>9 (41)</td>
<td>0.45</td>
</tr>
<tr>
<td>Other*</td>
<td>17 (15)</td>
<td>14 (15)</td>
<td>3 (14)</td>
<td>0.87</td>
</tr>
<tr>
<td>Severity score, median (Q1:Q3)</td>
<td>3 (IQR 2, 5)</td>
<td>3 (2, 5)</td>
<td>2.5 (2, 3.8)</td>
<td>0.19</td>
</tr>
<tr>
<td>Required additional antivenom, N (%)</td>
<td>0.03 for all groups</td>
<td>0.03 for all groups</td>
<td>0.03 for all groups</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Includes Asian, American Indian, Native Hawaiian.

Table 2. Modeled coagulation measure point estimate, 95% confidence interval and standard error.

<table>
<thead>
<tr>
<th></th>
<th>Other Crotalinae</th>
<th>Copperhead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets (10^9/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Point estimate &amp; distribution Mean (SE)</td>
<td>Precision of point estimate CI 95%</td>
<td>Predicted difference CI 95%</td>
</tr>
<tr>
<td>Baseline</td>
<td>263 (10.0)</td>
<td>243, 282</td>
</tr>
<tr>
<td>Acute nadir</td>
<td>217 (10.0)</td>
<td>197, 237</td>
</tr>
<tr>
<td>5 d</td>
<td>377 (10.1)</td>
<td>356, 396</td>
</tr>
<tr>
<td>8 d</td>
<td>354 (10.3)</td>
<td>334, 374</td>
</tr>
<tr>
<td>15 d</td>
<td>315 (10.3)</td>
<td>294, 335</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>263 (10.0)</td>
<td>243, 282</td>
</tr>
<tr>
<td>Acute nadir</td>
<td>217 (10.0)</td>
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<tr>
<td>15 d</td>
<td>315 (10.3)</td>
<td>294, 335</td>
</tr>
</tbody>
</table>

Modeled pretreatment baseline reported. Modeled acute nadir is during the treatment phase. Other time points are reported in post-treatment days.

without a statistical significant difference between groups (Table 1).

Main results

The mean pre-treatment baseline platelet count in both groups was within normal limits; the copperhead group patients had a higher mean platelet count (246 × 10^9/L 95% CI 215, 277; SD 91) compared to other crotaline patients (184 × 10^9/L 95% CI 167, 202; SD 74) (Table 2). No copperhead patients had marked thrombocytopenia <50 × 10^9/L, whereas 7 (9%) of other crotaline patients did. The platelet counts in each population rapidly increased and converged during the acute (post-treatment) phase and remained similar (Figure 1).

The mean pre-treatment fibrinogen level in both the copperhead group (345 mg/dL 95% CI 277, 415; SD 140) and the other crotaline group (261 mg/dL 95% CI 241, 281; SD 105) were within normal limits. This constituted a modest difference of 84 (95% CI 2, 119) mg/dL in the pre-treatment mean fibrinogen level. Two (2.6%) patients with envenomation by other crotaline snakes and no copperhead patients had severe hypofibrinogenemia. The mean acute post-treatment nadir fibrinogen level was higher in copperhead patients (314 mg/dL 95% CI 267, 414) compared to other crotaline envenomations (214 mg/dL 95% CI 195, 234). These mean differences resolved during the recovery phase as the other crotaline and copperhead fibrinogen curves converged at 5, 8, and 15 d (Figure 2).

The models with interaction terms between time of recovery and type of snake, for both platelets and fibrinogen, showed lower values for AIC, BIC, and Log Likelihood measures. Model comparison showed statistical significance in relation to the model without interaction terms, showing type of snake influences the recovery time during first two initial laboratory collection times (Table 2). Comparing platelets levels for both groups show that there is a difference in predicted values at baseline, but the curves collapse in the sequential follow-up times without differences in predicted means (Table 2). A similar pattern is observed for fibrinogen, but with significant differences for baseline and the nadir; while at 5, 8, and 15 d the predicted means reach similar sizes.

Discussion

Copperhead envenomations are widely considered to have less severe hematologic venom effects compared to other crotaline envenomations.[4,5,8] In 2011, Walker et al. reported a retrospective series of 88 copperhead bites over 15 years from their facility in east Texas. In this series, only 11% had elevated PTs, none had bleeding, and none received antivenom. Gale et al. found similar results in a retrospective chart review in 2016. In their series of 318 copperhead envenomated patients, 23.6% had coagulation abnormalities, and only 13.8% received antivenom. Both studies made recommendations to withhold antivenom in copperhead envenomation. Unfortunately, these studies were
retrospective chart reviews that did not adequately adhere to existing recommendations for the performance of this study design.\[18,19\] Data abstractors were not blinded to the hypothesis, nor were intra- or inter-rater reliability of data abstraction evaluated. Additionally, no systematic follow-up or serial coagulation testing was performed. Verification bias may have systematically underestimated coagulation abnormalities due to the lack of a comprehensive search.\[20\]

In 2015, Ali et al. published a retrospective series of 106 copperhead envenomation patients with more robust methods. They found a low rate of coagulation derangements and no bleeding.\[4\] However, this study did not compare copperhead with rattlesnakes or other crotaline envenomations, and the rate of significant coagulopathy and bleeding in these populations are also low.\[12–14\] As the difference is understudied it remains unclear if the clinician should approach the evaluation and treatment of copperhead envenomation any differently than other crotaline envenomations.

Based on the statistically significant differences in baseline coagulation parameters and the finding that only severe derangements occurred in the other crotaline group, our study supports the adage that the average copperhead envenomation has less severe coagulopathy than do other crotaline envenomations. Yet it also demonstrates that a more nuanced picture exists regarding this comparison. Most importantly, the mean platelet count and mean fibrinogen level prior to treatment are normal in both groups. There was a statistically significant difference in the pre-treatment baseline platelet count between the copperhead and other crotaline groups of $64 \times 10^9$, but this is unlikely to be a clinically important difference. The curves measuring platelet count over time for copperhead and
other crotaline patients rapidly converge after treatment and maintained their similarity throughout the measured recovery phase. There does not appear to be either a clinically important difference in platelet count after treatment based solely on snake type.

Although the average crotaline envenomation patient has a platelet count within normal range, there is a wide distribution of values around the mean. Consequently, there is a substantial area of overlap between the copperhead and other crotaline patients, and both groups had patients with thrombocytopenia. Individual patient presentation appears to be a more important indicator of venom-induced platelet effects than does the specific species of crotaline snake.

In our study, the difference in mean fibrinogen level in copperhead versus other crotaline patients is more striking. Similar to the platelet counts, there is also a wide range of individual patient’s fibrinogen levels. The mean pretreatment baseline level is different between the two groups and becomes even greater when assessing the mean acute post-treatment fibrinogen nadir. The fibrinogen level decreased from the pre-treatment baseline level to the acute treatment phase nadir likely due to the ongoing hematologic venom effects even with treatment. Whereas the platelet count in the other crotaline group increased during the acute phase nadir. It is possible that platelet counts respond more quickly to antivenom than does fibrinogen level. Fibrinogen level is likely a greater discriminator than platelet count when discussing the difference in coagulation parameter severity in copperhead versus other crotaline envenomation. But other individual patient factors likely make contributions to the course of the patient’s venom-induced coagulopathy as well.[21–23]

The fact that on average, copperhead envenomation tends to have less severe hematologic venom effects than does other crotaline envenomation, has given rise to recommendations that copperhead patients do not require treatment, testing, and/or observation.[4,5,8] Our study shows that within a group of treated crotaline envenomation patients, there was little important difference in coagulation between copperhead and other crotaline envenomation patients that could justify a dramatically different approach to evaluation based solely on species. As we must care for all patients as individuals, not just the average patient, our approach to clinical care must include recommendations to treat the full spectrum of severity that presents. This is particularly important in this disease state that has such a wide variation in presenting severity. In all likelihood, snake species is only one of many variables, along with patient age, size, comorbidities, medications, venom load, etc., that should be considered when deciding to observe, test, and/or treat victims of crotaline envenomation.[12,24,25] Future research into the variation in severity of venom effects on other organ systems, such as tissue injury, cardiovascular affects, and renal function will help us better define appropriate therapeutic options in copperhead envenomation as compared to other crotaline envenomation. Research into the efficacy of antivenoms in treating these diverse venom effects in copperhead envenomation specifically will yield better answers regarding the need to treat or withhold antivenom in this patient population.

**Limitations**

This study is a post hoc analysis of two crotaline snakebite cohorts from an existing clinical trial, and the conclusions must be tempered by the limitations of its design. No a priori sample size calculation is possible on this existing data set. We performed a post hoc power analysis that demonstrated adequate power for paired comparisons, but our study may be underpowered for multiple comparisons. However, these results do give us a clearer starting point in determining the true difference between copperhead and other crotaline (primarily rattlesnake) venom-induced coagulopathy.

The primary outcome of this study is a surrogate marker of venom effects and is not the patient-oriented outcome of clinically important bleeding. Yet this outcome is valuable for two reasons. The first is that clinically important bleeding in crotaline envenomation is uncommon regardless of species and bleeding would not be an obtainable outcome measure.[10,12,26] Consequently, laboratory coagulation abnormalities are a widely used and accepted surrogate outcome measure.[15,26–29] Secondly, the laboratory values themselves drive clinical decisions. Current guidelines recommend antivenom therapy for low or down trending laboratory values as these patients are at increased risk of bleeding.[24] As gastrointestinal, intracranial, retroperitoneal, and other remote hemorrhage can be life-threatening, the clinician should not wait for actual hemorrhage in order to institute antivenom therapy.[13]

All patients in this study received one of two antivenoms and the results of this study can only be generalized to a population of treated crotaline envenomation patients. As the acute phase measurements were made 2 h after antivenom infusion this limitation may impact those measurements in particular. It is unknown if the difference in coagulation parameters between copperhead and other crotaline envenomation patients would diverge, converge, or remain the same in an untreated populations. Lastly, there is some risk of recruitment bias in the two groups, as not all patients that presented enrolled in the clinical trial. Specifically, enrollment may have systematically excluded mild copperhead envenomation patients as the medical providers may have felt that no antivenom was necessary. Or conversely, more severe rattlesnake envenomation patients may have been excluded as the trial included experimental antivenom. In either scenario, the actual difference in mean coagulation parameters would be larger than the measured difference. This limitation is minimized by having no exclusion criteria based on severity.

**Conclusion**

On average, copperhead envenomations have less severe initial coagulation derangements than do other crotaline
envenomations. However, individual patients have substantial variation regardless of snake species. Species alone should not be used to determine the need for laboratory testing in crotaline snakebite and the need for treatment should depend on the summation of all the available clinical data for the individual patient.

Disclosure statement
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References