Venom Induced Consumption Coagulopathy
Pathophysiology and implications for antivenom therapy

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Newcastle, Australia

Snake bite...

- 22 year old male bitten by a brown snake
- Holding snake and attempting to kill himself
  - Initial collapse at the scene with spontaneous recovery
- Brought in by police and ambulance
- Blood alcohol = 0.24

What is Snake Bite Coagulopathy?

- Venoms contain a procoagulant toxin:
  - Prothrombin activator
  - Causes a clot in vitro
- Venom-induced consumption coagulopathy (VICC)
  - Clotting factor consumption in vivo
    - Specific factor deficiencies:
      - Fibrinogen
      - Factors V and VIII
    - Most Australasian elapids:
      - Brown snake, tiger snake group and taipan

VICC ... factor deficiencies
Australasian elapids

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Mild</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>INR</td>
<td>0.9 – 1.3</td>
<td>1.7 (1.2 – 10)</td>
<td>&gt;12 ‡ (1.3 – &gt;12)</td>
</tr>
<tr>
<td>aPTT (seconds)</td>
<td>26 – 38</td>
<td>44 (30 – 140)</td>
<td>&gt;180 ‡ (30 – &gt;180)</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>1.5 – 4.0</td>
<td>0.6 (0.3 – 1.2)</td>
<td>&lt;0.2* (&lt;0.2 – 2.5)</td>
</tr>
<tr>
<td>Factor II (%)</td>
<td>50 - 150</td>
<td>76 (28 – 95)</td>
<td>58 (0.1 – 97)</td>
</tr>
<tr>
<td>Factor V (%)</td>
<td>50 - 150</td>
<td>53 (14 – 91)</td>
<td>7.1 (0 – 119)</td>
</tr>
<tr>
<td>Factor VIII (%)</td>
<td>50 - 150</td>
<td>194 (3 – 82)</td>
<td>10.1 (0.4 – 118)</td>
</tr>
<tr>
<td>d-Dimer (mg/L)</td>
<td>&lt; 0.25</td>
<td>140 (12 – 746)</td>
<td>826 (1.7 – 906)</td>
</tr>
</tbody>
</table>

Isbister et al J Thromb Haem 2010

severe haemophilia + afibrinogenaemia + severe factor V deficiency
Recovery of Factors

Recovery to INR<2 = median 14.4h (IQR 11.5-17.5 h)

Model of snakebite coagulopathy

Procoagulant toxins

Sri Lankan Russell’s Viper ... factor deficiencies
Venom contains factor X and factor V activators

Other Snakes ... factor deficiencies

Table 1. Pretreatment levels of clotting factors and FDP in a group of 18 patients bitten by E. carinatus
Warrell et al QJM 1977

Table 5. Pretreatment levels of clotting factors and FDP in a group of 18 patients bitten by E. carinatus
Warrell et al QJM 1977
Antivenom and coagulopathy: ...what do we really know?

1. Does it WORK?
   a) Efficacy:
      i. *In vitro*: Does it bind toxins? Does it neutralise activity?
      ii. *In vivo*: Does it bind free venom? Does it increase elimination of toxins?
   b) Clinical effectiveness: Does it improve patient outcomes?

2. Dosing?
   a) Initial dose: How do we determine this?
   b) Re-dosing: Do we need to?

Efficacy vs. Effectiveness

Antivenom efficacy:
- Central Compartment (e.g., coagulant toxin)
- Peripheral Compartment (e.g., neurotoxin, myotoxin)

Antivenom prevents OR reverses clinical effects

Antivenom Pharmacokinetics

Australia ... “Previous / Current” practice

- Increasing doses of antivenom being used
  - Commence with a larger dose of antivenom
- Antivenom needs to be titrated:
  - Repeat coags 1 to 3 hours post-AV, fibrinogen best
  - If abnormal repeat dose antivenom
  - Regular coagulation testing
  - Continue antivenom until coagulation normal
- Does NORMAL = measurable fibrinogen, normal INR and aPTT, or even normal D-dimer?

Increasing Dose of Antivenom

- Animal work suggesting insufficient:
  - Tibballs 1990: up to 25 x dose needed
  - 341 studies
  - Spriulis 1996: unable to neutralise 20 x
  - Massive amounts of venom (100 x clinical)
  - Insufficient antivenom being tested
- Madaras 2005
- Clinical studies:
  - Yeung et al 2004:
    - Variable fibrinogen assays (derived, LOQ)
    - Did not consider time for clotting recovery

Australia ... Increasing Antivenom Dose

- CSL initially recommended 500 U
  - Now equivalent to ½ vial
- CSL antivenom handbook:
  - 3 to 4 vials
- Toxicologists in 2006:
  - 5 vials (Eastern States)
  - 10 vials (WA) = 20 x initial CSL dose

Antivenom dosing in 35 patients with severe brown snake (Pseudonaja) envenoming in Western Australia over 10 years

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>Patients</th>
<th>Antivenom Dose</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeung et al</td>
<td>2004</td>
<td>35 patients</td>
<td>20 x initial dose</td>
<td>Improved fibrinogen levels, reduced D-dimer levels</td>
</tr>
</tbody>
</table>

**Main outcome measure:**
The dose of antivenom required to neutralise venom, deferred prophylactically and in patients with significant antivenom in titre.
In Australia...

Why had previous in vitro studies suggested larger doses?
- Assumed much higher venom concentrations i.e. 50,000 ng/mL

VICC... antivenom dose and efficacy
- Dose with binding efficacy:
  - 1 vial binds all venom in vitro
  - 1 vial binds all venom in vivo
- In vitro efficacy:
  - 1 vial neutralises procoagulant activity in vitro
- Clinical Effectiveness:
  - Antivenom does not prevent coagulopathy occurring:
    - need to give <30 minutes
  - Antivenom does not speed recovery

Venom assay: Enzyme Immunoassay

Sandwich ELISA

Biotinylated Horseradish Peroxidase conjugate

Antigen

Antibody

Anti-snake venom IgG

Streptavidin Horseradish Peroxidase conjugate

Serum

Microplate reader – 405nm

Development of a sensitive enzyme immunoassay for measuring taipan venom in serum

Toxicon 2010
Kulawickrama et al

In vivo binding...after antivenom
- Brown snake (27 patients)
  - No venom detected in any sample; 9 given only 1 vial
  - Rough-scale snake (20 patients)
    - No venom detected except 1 patient given small amount of polyvalent; 5 given only 1 vial
  - Tiger snake (46 patients)
    - No venom detected after antivenom; 7 given only 1 vial
  - Taipan (17 patients)
    - No venom detected after antivenom; 7 given only 1 vial

Our patient...

<table>
<thead>
<tr>
<th>Time</th>
<th>Venom (ng/mL)</th>
<th>Antivenom (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1hr</td>
<td>121</td>
<td>0</td>
</tr>
<tr>
<td>2hr</td>
<td>0</td>
<td>1044</td>
</tr>
<tr>
<td>6hr</td>
<td>0</td>
<td>685</td>
</tr>
<tr>
<td>60hr</td>
<td>0</td>
<td>137</td>
</tr>
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2 vials of antivenom
Antivenom efficacy...neutralisation

- In vitro clotting studies
  - Venom added to plasma
  - Time to cloudiness (clotting) recorded
  - Venom induces clot formation in vitro
  - Venom + antivenom mixtures added to plasma
  - Increasing time to cloudiness
  - Antivenom prevents clot formation

Antivenom Dose

<table>
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<tr>
<th>Venom Type</th>
<th>Dose</th>
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<tr>
<td>Brown Snake</td>
<td>4 to 10 vials</td>
</tr>
<tr>
<td>Tiger Snake/Rough Scale</td>
<td>4 vials</td>
</tr>
<tr>
<td>Black Snakes: Mulga Snake</td>
<td>1 vial</td>
</tr>
<tr>
<td>Red-bellied Black Snake</td>
<td>1 vial tiger/black</td>
</tr>
<tr>
<td>Taipan</td>
<td>3 vials</td>
</tr>
<tr>
<td>Death Adder</td>
<td>1 vial</td>
</tr>
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</table>

- Can we trust the science OR do we just give more because it makes us feel better
  - False attribution of FAILURE to not enough antivenom
  - and SUCCESS to giving the antivenom

Australia: Do we need to re-dose?

- Once all venom (toxins) bound no further doses are required:
  - i.e. no venom detectable in serum
- Assumes:
  - No ongoing absorption of venom
  - Can only be determined with venom concentrations:
    - Not possible in real time
    - Establish dose in large prospective studies (ASP)

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<th>Antivenom (U/L)</th>
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<tr>
<td>1hr</td>
<td>15:25</td>
<td>0</td>
</tr>
<tr>
<td>2hr</td>
<td>16:20</td>
<td>1044</td>
</tr>
<tr>
<td>6hr</td>
<td>&gt;180</td>
<td>685</td>
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PKPD delay between venom removal and recovery of coagulopathy

Recovery of coagulopathy

- Delay between venom neutralisation and coagulopathy resolving:
  - Dependent on resynthesis of clotting factors
  - Will antivenom speed this process? No
- INCORRECT ASSUMPTION:
  - Ongoing coagulopathy = insufficient antivenom
  - BASED ON:
    - Antivenom will treat the coagulopathy
  - SO, no reason to re-dose

Other snakes: Dose and re-dose?

Russells viper: Venom Concentrations
Issues:
• Venom concentrations:
  o Venom not all bound
  o Decreased venom without re-dose

Questions:
Can we assume that all venom is absorbed???
1. Is antivenom binding and immediately neutralising venom
   OR
2. Is antivenom assisting in elimination of venom

Does venom have to be undetectable in serum for antivenom to be EFFECTIVE?

Sri Lanka: Russell’s viper

How do we determine dose?
• Australia:
  – Snakes with small fangs delivering small doses
  – Small range of doses 1 to 200ng/mL
  – Most likely rapidly and completely absorbed
  – Highly potent venoms
  – Free venom levels go to ZERO
  – DOSE can be determined in a cohort study
• Sri Lanka:
  – Snakes with very large fangs (vipers) deliver large doses
  – Large range of doses 2 to 5000ng/mL
  – Absorbed over 24+ hours
  – Less potent venoms
  – Free venom remains DETECTABLE
  – More difficult to establish dose

Antivenom: proposing a mechanism of action?
• Binding of all venom
  – No detectable venom on ELISA
  – Probable immediate neutralisation (coagulant toxins)
  – Requires excess antivenom ie. AV:venom > 5
• Increasing venom elimination
  – Free venom still detectable by ELISA
  – Delay in neutralisation (coagulant toxins)
  – Requires at least 1:1 ratio of AV:venom

Neutralisation

Toxin in central compartment e.g. coagulant toxin

NOT detectable with ELISA for free venom
Appears to be the case in Australia

DETECTABLE with ELISA for free venom
Might be the case in Sri Lanka
Are ‘free’ venom assays enough?

- Free venom assay:
  - “0” means there is no free venom
    = neutralisation
  - “non-zero” does NOT mean that antivenom is not bound to venom
  - Venom can probably still be detected when there is a 1:1 binding of venom-antivenom
- Can we measure the venom-antivenom complex?

[venom – AV] assay: Enzyme Immunoassay

![VAV Assay](image)

Measuring the Venom-Antivenom complex

Venom-Antivenom Assay

![Venom-Antivenom Assay](image)

Antivenom:venom ratio?

**Slope = 0.075 mU AV / ng venom**

This implies that 0.075 mU of AV is required for each nanogram of venom (at VAV\(_{\text{max}}\))
- ie 0.075 U for each microgram of venom
- ie 0.75 U for 10 micrograms of venom

This is very close to CSL definition of 1 U required for 10 micrograms of venom
Antivenom: venom ratio
Can we use this for other antivenoms?

11E1Feb23 Plate2: Measurement of VAV complex (solid lines) and of free RV (dotted lines) in mixtures of RV and RVAV.

**EFFECTIVENESS?**

- Antivenom – toxin interactions
  - Effectiveness per se?
  - Effective dose
- Toxin target:
  - Peripheral
  - Central
- Toxin reversibility

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**Failure of antivenom to improve recovery in Australian snakebite coagulopathy**
G.K. Irister, S.B. Duffull, and S.G.A. Brown, for the ASP investigators

- Question does antivenom shorten the course of the coagulopathy?
  - Not possible to do a placebo controlled trial of AV
- Objective: to investigate predictors of VICC recovery
  - Antivenom:
    - timing of antivenom
    - dose of antivenom
  - Factor replacement
  - Other covariates: snake, age, sex
- Main outcome measure:
  - Time until INR < 2 = ‘VICC recovery’

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**Interpretation**

- Recovery of VICC
  - Administration of FFP within 4 hours only important determinant
  - EARLIER administration of antivenom or INCREASED dose of antivenom had NO effect
    - Suggests antivenom has no effect
  - ONLY for Australian elapids

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**Recovery of VICC**

Antivenom dose – no effect
Antivenom time – no effect
FFP use: large highly probable positive effect

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**VICC ... Echis spp.**

- Prothrombin activator toxin
  - Snake venom metalloproteinase (not like human factor Xa)
  - Also a haemorrhagin → vascular injury
- Bleeding/Coagulopathy:
  - Spontaneous bleeding
  - Prolonged coagulopathy: reported to continue for up to 10 days untreated

Warrell QJM 1977

• 12 year period
• 71 envenomed viper bites
  – 62 got antivenom
    • 39 got early antivenom < 24 hours post-bite
    • 23 got delayed antivenom > 24 hours post-bite
  – 9 did not get antivenom

Echis ... recovery of VICC

• No relationship between time of AV and recovery of VICC?
  – Authors: delayed antivenom is still beneficial
    • Difficult for this to be biologically plausible –
    • i.e. if antivenom is given late then VICC must recover even faster in these patients?
    • Assume antivenom works – supported by 9 patients not getting antivenom
  – Cynic: Does antivenom speed the recovery at all?
    • Not dissimilar to Australian elapids
    • Are the 9 untreated patients convincing (small no. etc)

HOW DO WE EXPLAIN THIS?

Echis ... recovery of VICC

Faster recovery of VICC with antivenom

HOWEVER ... They all did recover

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http://star.ferntree.com

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