Animal Model Benefits and Limitations for Traumatic Hemorrhagic Shock: Non-Human Primates

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Disclaimer

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NHP Primer:
Old World vs. New World

New World:
- Nostrils flare out
- Prehensile tail ~ 5th arm
- Arboreal
- Lack butt pads
- Monogamous
- Smaller

Old World:
- Nostrils flare down
- Different dental pattern
- Terrestrial
- Butt pads ~ attracts mates
- Polygynous
- Larger
Primate Phylogeny

- **Primates**
  - **Haplorhines**
    - **Platyrrhines / New World Monkeys**
      - Marmosets, Capuchins, Howler Monkeys
    - **Catarrhines**
      - **Old World Monkeys**
    - **Apes**
      - Macaques, Baboons, Langurs
      - Chimpanzees, Gorillas, Orangutans, Humans
      - Lemurs, Aye-Ayes, Bushbabies
## Interspecies Protein Homology

### Amino Acid Sequence Identity to Human Hemoglobin

<table>
<thead>
<tr>
<th>Species</th>
<th>Hemoglobin Alpha chain (141 AA)</th>
<th>Hemoglobin Beta chain (146 AA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gorilla</td>
<td>99.3%</td>
<td>99.3%</td>
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<tr>
<td>Rhesus</td>
<td>96.5%</td>
<td>94.5%</td>
</tr>
<tr>
<td>Pig</td>
<td>85.8%</td>
<td>80.8%</td>
</tr>
<tr>
<td>Mouse</td>
<td>86.5%</td>
<td>78.7%</td>
</tr>
<tr>
<td>Sheep</td>
<td>81.5%</td>
<td>77.4%</td>
</tr>
<tr>
<td>Rabbit</td>
<td>80.1%</td>
<td>89.0%</td>
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</tbody>
</table>

### Amino Acid Sequence Identity to Human Factor

<table>
<thead>
<tr>
<th>Species</th>
<th>Factor X</th>
<th>Factor VIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus</td>
<td>93.7%</td>
<td>97.0%</td>
</tr>
<tr>
<td>Chimpanzee</td>
<td>100%</td>
<td>99.5%</td>
</tr>
<tr>
<td>Pig</td>
<td>73.1%</td>
<td>84.3%</td>
</tr>
<tr>
<td>Bovine</td>
<td>72.7%</td>
<td>86.7%</td>
</tr>
<tr>
<td>Rat</td>
<td>77.7%</td>
<td>84.3%</td>
</tr>
</tbody>
</table>
# Interspecies Hematologic, Chemistry and Coagulation Comparison

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Unit</th>
<th>Pig</th>
<th>Cyno</th>
<th>Rhesus</th>
<th>Human</th>
<th>Rhesus</th>
<th>Range</th>
<th>NAMRU-SA</th>
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</thead>
<tbody>
<tr>
<td><strong>Hematology</strong></td>
<td>WBC</td>
<td>/mm³</td>
<td>18.6</td>
<td>11.9</td>
<td>8</td>
<td>3.8-10.6</td>
<td>6</td>
<td>2.3-15.9</td>
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<tr>
<td></td>
<td>Hemoglobin</td>
<td>g/dL</td>
<td>10</td>
<td>13</td>
<td>13.2</td>
<td>11.6-14.6</td>
<td>11.4</td>
<td>9.7-12.9</td>
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<tr>
<td></td>
<td>Hematocrit</td>
<td>%</td>
<td>37.9</td>
<td>43.1</td>
<td>40.9</td>
<td>34.1-43.3</td>
<td>32.5</td>
<td>27.3-37.3</td>
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<tr>
<td></td>
<td>Platelets</td>
<td>x10^3/mm³</td>
<td>506.6</td>
<td>431.7</td>
<td>359</td>
<td>156-369</td>
<td>278.8</td>
<td>193.0-414.0</td>
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<tr>
<td></td>
<td>Lymphocyte</td>
<td>%</td>
<td>45.5</td>
<td>58.4</td>
<td>38.2</td>
<td>13-44</td>
<td>39.1</td>
<td>15.2-69.3</td>
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<tr>
<td></td>
<td>Monocyte</td>
<td>%</td>
<td>4.7</td>
<td>4.4</td>
<td>3.5</td>
<td>4-13*</td>
<td>8</td>
<td>4.8-18.7</td>
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<tr>
<td><strong>Renal Function and Electrolytes</strong></td>
<td>Sodium</td>
<td>mmol/L</td>
<td>142.9</td>
<td>158.4</td>
<td>147.3</td>
<td>136-146</td>
<td>142.5</td>
<td>136.0-136.0</td>
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<tr>
<td></td>
<td>Potassium</td>
<td>mmol/L</td>
<td>5.3</td>
<td>5.8</td>
<td>4</td>
<td>3.5-5.0</td>
<td>3.4</td>
<td>2.7-5.1</td>
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<tr>
<td></td>
<td>Chloride</td>
<td>mmol/L</td>
<td>104.8</td>
<td>113.4</td>
<td>112.6</td>
<td>95-110</td>
<td>109.4</td>
<td>100.0-116.0</td>
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<td></td>
<td>Calcium</td>
<td>mg/dL</td>
<td>11</td>
<td>11.1</td>
<td>9.4</td>
<td>8.4-10.2</td>
<td>7.3</td>
<td>6.2-8.5</td>
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<tr>
<td></td>
<td>Phosphorus</td>
<td>mg/dL</td>
<td>8.6</td>
<td>5.6</td>
<td>4.1</td>
<td>2.5-4.5</td>
<td>4.7</td>
<td>3.1-6.0</td>
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<td></td>
<td>CO2</td>
<td>mmol/L</td>
<td>29</td>
<td>n.a.</td>
<td>13.9</td>
<td>21-32</td>
<td>44.2</td>
<td>37.0-72.9</td>
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<tr>
<td></td>
<td>Urea</td>
<td>mg/dL</td>
<td>12.4</td>
<td>23.1</td>
<td>17.3</td>
<td>5.0-20.0</td>
<td>17.9</td>
<td>11.1-40.3</td>
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<td>Creatinine</td>
<td>mg/dL</td>
<td>1</td>
<td>0.8</td>
<td>1</td>
<td>0.6-1.1</td>
<td>0.9</td>
<td>0.5-1.4</td>
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<tr>
<td><strong>Liver Function</strong></td>
<td>AST</td>
<td>IU/L</td>
<td>40.1</td>
<td>33.1</td>
<td>33.6</td>
<td>&lt;40</td>
<td>20.9</td>
<td>14.0-36.0</td>
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<tr>
<td></td>
<td>ALT</td>
<td>IU/L</td>
<td>58.5</td>
<td>47.1</td>
<td>44.6</td>
<td>&lt;40</td>
<td>30.4</td>
<td>11.0-72.0</td>
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<tr>
<td></td>
<td>ALP</td>
<td>IU/L</td>
<td>263.1</td>
<td>1138.6</td>
<td>183.7</td>
<td>38-126</td>
<td>176.1</td>
<td>19.9-1309.0</td>
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<td>GGT</td>
<td>IU/L</td>
<td>43</td>
<td>124.1</td>
<td>61</td>
<td>&lt;40</td>
<td>51.5</td>
<td>35.0-76.0</td>
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<td>Tot Bilirubin</td>
<td>mg/dL</td>
<td>0.5</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3-1.5</td>
<td>0.1</td>
<td>0.1-0.2</td>
<td></td>
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<tr>
<td></td>
<td>Tot Protein</td>
<td>g/dL</td>
<td>5.2</td>
<td>7.9</td>
<td>7.1</td>
<td>6.3-7.7</td>
<td>5.8</td>
<td>4.8-6.8</td>
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<td></td>
<td>Albumin</td>
<td>g/dL</td>
<td>2.6</td>
<td>4.3</td>
<td>4</td>
<td>3.4-5.0</td>
<td>3.3</td>
<td>2.4-4.1</td>
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<td></td>
<td>Cholesterol</td>
<td>mg/dL</td>
<td>100.6</td>
<td>131.5</td>
<td>141.7</td>
<td>&lt;200</td>
<td>94.5</td>
<td>52.0-163.0</td>
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<td></td>
<td>Glucose</td>
<td>mg/dL</td>
<td>92.1</td>
<td>81</td>
<td>67.1</td>
<td>70-99</td>
<td>72.4</td>
<td>55.0-107.0</td>
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<tr>
<td><strong>Coagulation Profile</strong></td>
<td>PT</td>
<td>sec</td>
<td>11.7</td>
<td>9.4</td>
<td>14.2</td>
<td>11.3-14.5</td>
<td>14.6</td>
<td>12.1-18.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PTT</td>
<td>sec</td>
<td>15.5</td>
<td>21.6</td>
<td>43</td>
<td>22.7-35.6</td>
<td>29.5</td>
<td>20.8-233.2</td>
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<tr>
<td></td>
<td>D-Dimer</td>
<td>μg/mL</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>&lt;0.45</td>
<td>0.4</td>
<td>0.03-1.45</td>
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<tr>
<td></td>
<td>Fibrinogen</td>
<td>mg/dL</td>
<td>235.8</td>
<td>n.a.</td>
<td>n.a.</td>
<td>200-400</td>
<td>191.9</td>
<td>130.0-308.0</td>
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</tr>
</tbody>
</table>

Xenotransfusion Considerations

Exaggerated aggregation of human platelets infused into swine with porcine von Willebrand factor implicated.

Thrombotic complications reported in one study in which human platelet derived hemostatic agents (PDHA) infused into swine hemorrhage model.

Porcine platelet glycoproteins have been shown to be recognized by human natural “anti-gal” antibodies.

- Anti-Gal is the most abundant natural antibody in humans, constituting ~1% of immunoglobulins.
- Anti-Gal is naturally produced in Apes and Old World monkeys.
- Gal sugars are found in Non-Primate Mammals, Strepsirrhines, and New World Monkeys.
Galili. Immunology. 2013;140:1-11

Xenogeneic and immunologic compatibility between Rhesus monkeys and human-derived blood products have also been reported
Xenotransfusion Considerations

Ex Vivo Work at NAMRU-SA

Rhesus Macaques
- Human Plasma and Human Platelets infusions are compatible into Rhesus Macaques
- Currently undertaking Ex Vivo studies to determine Human pRBC compatibility into Rhesus Macaques
The ‘Lung in Shock’ as a Result of Hypovolemic-Traumatic Shock in Baboons

INCREASED CIRCULATING D-LACTATE LEVELS PREDICT RISK OF MORTALITY AFTER HEMORRHAGE AND SURGICAL TRAUMA IN BABOONS

LYMPH FROM A PRIMATE BABOON TRAUMA HEMORRHAGIC SHOCK MODEL ACTIVATES HUMAN NEUTROPHILS

Similarities in Thromboelastometric (ROTEM®) Findings between Humans and Baboons

LARGE ANIMAL MODELS: BABOONS FOR TRAUMA, SHOCK, AND SEPSIS STUDIES

DEVELOPMENT OF A NONHUMAN PRIMATE (RHEUSUS MACAQUE) MODEL OF UNCONTROLLED TRAUMATIC LIVER HEMORRHAGE
Rhesus Macaques

- Why Rhesus Macaques:
  - Availability
  - 8 – 14 Kg
  - Widespread familiarity
  - Husbandry considerations
  - Reagent availability
  - Old World Primate
  - Cost-return on investment
Rhesus Trauma Models

- Uncontrolled Liver Hemorrhage
- PtCHS, 30 minute shock duration
- PtCHS, 60 minute shock duration
- PtCHS, 60 minute shock duration + soft tissue injury
- PtCHS, 60 minute shock duration + soft tissue injury + musculoskeletal injury
- PtCHS, physiologic decompensation + soft tissue injury + musculoskeletal injury
Uncontrolled Hemorrhage

Operative Ports
Ports Removed
Liver Laceration
Hospital Arrival
Euthanasia
Pre-Injury
Hemorrhage
Resuscitation
Hospital Care
2 hour pre-hospital phase with invasive monitoring

Mean Arterial Pressure (mmHg)
Heart Rate (bpm)
Hematocrit (%)
Lactate
pH
IL-6
MCP-1

Sheppard F, et al. Shock 2015;44(S1):114-122
NAMRU-SA Pressure Targeted Controlled Hemorrhagic Shock ± Traumatic Injury

Developed in response to sub-clinical physiologic, metabolic, inflammatory and coagulation responses in the uncontrolled hemorrhage model.

Even with 60% removal of the left lobe of the liver (~Grade 4), hemorrhage appeared to stop once the blood pressure hit a MAP of 20mmHg.

Moved to target a hemorrhage MAP based on experience in uncontrolled hemorrhage in Rhesus, while minimizing variability inherent in uncontrolled models and control severity of overall response by duration of shock and addition of additional injuries.
NAMRU-SA Pressure Targeted Controlled Hemorrhagic Shock ± Traumatic Injury

Starting MAP ~ 70mmHg → Target MAP ~ 20mmHg

Duration of Shock
- 30 Minutes
- 60 Minutes
- >60 Minutes, when compensation lost

Injuries
- None
- Soft Tissue: 15cm laparotomy
- Soft Tissue + Femur Fracture: 15cm laparotomy + mid-shaft femur fracture.

Survival: To 24 hours after initiation of shock

Table/list of readings:
- CBC
- Serum Chemistries
- Blood Gases
- ROTEM®
- Multiplate®
- STAGO®
- FLOW Cytometry
- Lumines (Cytokine/Chemokine)
- ELISA
- EEG
- TCD
- TEE
- StO₂
- Continuous Vitals Monitoring
- Necropsy/Histopathology
Isolated PtCHS: 30 vs 60 minutes

- Catheter Placement
- Hemorrhage
- Shock Period
- Resuscitation
- Observation (Under Anesthesia)
- Survival Period + Observation

**Crystallloid**
- 30 minutes
- 2X Shed Blood Volume

**Whole Blood**
- 30 minutes
- ½X Shed Blood Volume

**Crystallloid**
- 60 minutes
- 1X Shed Blood Volume

**Graphs:***
- Time to T=0 (min)
- % Blood Loss
- Base Deficit (mM)
- Lactate (mM)

**Graphs:**
- MAP (mmHg)
- Heart Rate (bpm)
- Shock Index

# - indicates difference from baseline in 30 min PtCHS
$ - indicates difference from baseline in 60 min PtCHS
* - indicates difference between groups
60 min PtCHS + Injuries

- Soft Tissue Injury
- Musculoskeletal Injury

Timeline:
- Catheter Placement
- Hemorrhage
- Shock Period
- Resuscitation
- Observation (Under Anesthesia)
- Survival Period + Observation

- Repair Laparotomy
- Femur Fixation/Repair

Blood Loss:
- 30 minutes: 2X Shed Blood Volume
- 30 minutes: 1/3X Shed Blood Volume
- 60 minutes: 1X Shed Blood Volume

- Time to T=0 (min)
- % Blood Loss
- Lactate (mmol/L)
- MAP (mmHg)
- HR (bpm)
- SI

Graphs showing:
- Time to T=0 (min) for different groups:
  - PTHS-60
  - PTHS-60+ST
  - PTHS-60+ST+FF

- % Blood Loss for different groups:
  - PTHS-60
  - PTHS-60+ST
  - PTHS-60+ST+FF

- Lactate (mmol/L) over time points:
  - BSLN
  - EOS
  - EOR
  - 360

- MAP (mmHg) over time points:
  - BSLN
  - T0
  - EOS
  - EOR
  - T360

- HR (bpm) over time points:
  - BSLN
  - T0
  - EOS
  - EOR
  - T360

- SI over time points:
  - BSLN
  - T0
  - EOS
  - EOR
  - T360

* indicates difference between PthCHS60 vs. PthCHS60+ST+FF
† indicates difference between PthCHS60 vs. PthCHS60+ST
Worst case scenario from PtCHS models:
- 60 min PTHS (MAP 20-25mmHg) + soft tissue + femur fracture...
- Outcome: 88% survival and moderate physiologic, metabolic, coagulation and inflammatory derangements.

Loss of compensated shock = 25% reduction in the avg MAP (T0-60 min) without a compensatory increase for 30 seconds. If compensation occurs prior to 30 seconds, resuscitation begins on the 2nd occurrence of a 25% reduction in MAP.
- Example: If average MAP from T0 – T60min = 23mmHg, a 25% reduction would = MAP of 18mmHg as trigger point.
Plasma Coagulation: 60 min PtCHS + Injuries vs. Decomp + Injuries

- Indicates difference between groups
- Indicates difference from baseline in Decomp group
- Indicates difference from baseline in PTHS-60+ST+FF group

**Graphs:**
- **PT (sec)**
- **PTT (sec)**
- **Fibrinogen (mg/dL)**
- **ATIII (%)**
- **vWF:Ag (%)**
- **D-dimer (µg/mL)**

*Study Periods: BSLN, EOS, EOR, T360*
Thromboelastometry: 60 min PtCHS + Injuries vs. Decomp + Injuries

* indicates difference between groups
# indicates difference from baseline in Decomp group
@ indicates difference from baseline in PTHS-60+ST+FF group
Platelet Aggregation: 60 min PtCHS + Injuries vs. Decomp + Injuries

- **ADP**
  - Time Points: BSLN, EOS, EOR, T360
  - Graphs show aggregation levels with indications for differences.

- **AA**
  - Time Points: BSLN, EOS, EOR, T360
  - Graphs show aggregation levels with indications for differences.

- **COL**
  - Time Points: BSLN, EOS, EOR, T360
  - Graphs show aggregation levels with indications for differences.

- **ADP** (on a log scale)
  - Time Points: BSLN, EOS, EOR, T360
  - Graphs show aggregation levels on a log scale with indications for differences.

- **AA** (on a log scale)
  - Time Points: BSLN, EOS, EOR, T360
  - Graphs show aggregation levels on a log scale with indications for differences.

- **COL** (on a log scale)
  - Time Points: BSLN, EOS, EOR, T360
  - Graphs show aggregation levels on a log scale with indications for differences.

**Legend**:
- * indicates difference between groups.
- # indicates difference from baseline in Decomp group.
- @ indicates difference from baseline in PTHS-60+ST+FF group.
**NHP Considerations**

**Phylogeny:** Other than an Ape, no closer to man than OWM.

**Extremely High Protein Homology:** Multiple proteins, to include coagulation factors.

**Xenocompatibility:** Human blood product into OWMs works.

**Physiology:** Close to human:
- Can breath spontaneously in supine position.
- Lack of splanchnic congestion during shock.
- Lack of pulmonary lung pooling in response to shock.
- Compliment activation comparable to humans.

**Coagulation:** Response to poly-traumatic hemorrhagic shock very similar to that observed in humans.

**Immune response:** Initial reports suggest that the immune response in our NHP poly-traumatic model is similar to the human response.
NHP Considerations

**Facility:** Capable of handling NHPs, willing to handle NHPs, cost, per diem cost.

**Occupational health:** TB monitoring, Herpes B, measles titer, additional PPE (always double gloved, face shield, bunny suit, shoe covers) +/- respiratory protection. Exposure is a huge deal. Implications for collaborations.

**Personnel:** Always 2 people with NHP when being manipulated. Animal NEVER left alone while out of cage. Veterinarian, experienced personnel.

**Animals (Rhesus Macaque):** Cost ~ $6,500 - $9,000, + shipping Mandatory quarantine.

**IACUC and Institution:** Scrutiny, optics, oversight, administrative aspects, safety.

Able to use human assay kits/reagents & pediatric probes.

The NHP study cost is similar to a true clinical trial study (~ $25,000 - $40,000 per animal).
THANK YOU!