Inflammation and Coagulation
An Unholy Alliance

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Critical Care Research Group
Brisbane, Australia
ECMO cases and centres continue to grow
ECMO-Related Complications

- Systemic inflammatory response syndrome (SIRS)-like reaction
- Infections
- Sepsis
- Delayed recovery
- Acute & Long-term morbidities
- Death
- Bleeding & thrombotic events

- Delayed recovery
It all started to get tricky when ......
The Problem of ECLS
Bleeding and Clotting and what to do next?
How big a problem?

<table>
<thead>
<tr>
<th>Complication</th>
<th>Reported (%)</th>
<th>Survived (%)</th>
<th>Reported (%)</th>
<th>Survived (%)</th>
<th>Reported (%)</th>
<th>Survived (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clots: oxygenator</td>
<td>7.9</td>
<td>42</td>
<td>7.4</td>
<td>50</td>
<td>9.6</td>
<td>41</td>
</tr>
<tr>
<td>Clots: bridge</td>
<td>3.1</td>
<td>38</td>
<td>2.5</td>
<td>51</td>
<td>0.9</td>
<td>56</td>
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<tr>
<td>Clots: bladder</td>
<td>4.1</td>
<td>40</td>
<td>2.1</td>
<td>49</td>
<td>0.2</td>
<td>45</td>
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<tr>
<td>Clots: other</td>
<td>9.9</td>
<td>43</td>
<td>10</td>
<td>55</td>
<td>6.5</td>
<td>38</td>
</tr>
<tr>
<td>CNS: infarction</td>
<td>4.4</td>
<td>33</td>
<td>4.4</td>
<td>39</td>
<td>3.8</td>
<td>22</td>
</tr>
<tr>
<td>CNS: hemorrhage</td>
<td>6.0</td>
<td>30</td>
<td>4</td>
<td>20</td>
<td>2.2</td>
<td>8</td>
</tr>
<tr>
<td>GI hemorrhage</td>
<td>1.9</td>
<td>14</td>
<td>2.7</td>
<td>30</td>
<td>4</td>
<td>24</td>
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<tr>
<td>Cannula site bleeding</td>
<td>12.3</td>
<td>40</td>
<td>18.4</td>
<td>54</td>
<td>19.8</td>
<td>41</td>
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<tr>
<td>Surgical site bleeding</td>
<td>32.6</td>
<td>40</td>
<td>28.4</td>
<td>48</td>
<td>23</td>
<td>34</td>
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<tr>
<td>Hemolysis (P/He &gt;0.5 g/L)</td>
<td>9.8</td>
<td>33</td>
<td>8.4</td>
<td>44</td>
<td>7.1</td>
<td>30</td>
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<tr>
<td>DIC</td>
<td>3.3</td>
<td>24</td>
<td>3.9</td>
<td>35</td>
<td>4.1</td>
<td>24</td>
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<tr>
<td>Cardiac tamponade</td>
<td>5.2</td>
<td>36</td>
<td>5.2</td>
<td>49</td>
<td>5.4</td>
<td>31</td>
</tr>
<tr>
<td>Pulmonary hemorrhage</td>
<td>5.1</td>
<td>24</td>
<td>6</td>
<td>38</td>
<td>3.1</td>
<td>29</td>
</tr>
</tbody>
</table>

D.A. Murphy et al. / Transfusion Medicine Reviews 29 (2015) 90–101
The artificial endothelium, Annich GM, Organogenesis 2011 7:1, 42-49;
Where do they bleed from?

<table>
<thead>
<tr>
<th>Localization of bleeding</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral cannulation site</td>
<td>12</td>
</tr>
<tr>
<td>Thoracic</td>
<td>9</td>
</tr>
<tr>
<td>Epistaxis</td>
<td>3</td>
</tr>
<tr>
<td>Diffuse</td>
<td>2</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>1</td>
</tr>
<tr>
<td>Following puncture of gall bladder</td>
<td>1</td>
</tr>
<tr>
<td>Following fasciotomy</td>
<td>1</td>
</tr>
<tr>
<td>More than one bleeding site</td>
<td>7</td>
</tr>
</tbody>
</table>
ECLS Balancing Act

**Thromboembolic Events**
- Platelet Activation
- Pro-Thrombotic Milieu

**Hemorrhagic Events**
- Anti-Coagulation Therapy
- Impaired Plt Function
- vWF Multimer Loss
Extra Corporeal Circulation

Blood – Surface Interaction

Coagulation  Fibrinolysis  Kinins

Platelets  Neutrophils

Shear Stress

Transfusion  Drugs

Bleeding and Clotting
Whole Circuit
- foreign surface → adhesion & activation
- haemodliution on commencement

Oxygenator
- consumption of platelets & plasma proteins

Pump
- altered shear
- VWF dysfunction
- palatelet activation
- reduced ADAMTS13

Low Flow Zones and Connectors
(e.g. cannula to circuit connectors, back perfusion cannula ------)
- turbulence & increased shear
- platelet activation

Patient

Underlying Disease
- intercardiac due to stasis
- infection/sepsis
- post resuscitation
- trauma

Drugs
- heparin → bleeding/HIT
- antiplatelet agents

Systemic Inflammatory Response
- microparticle release
- leucocyte activation and NETs
- DIC
- platelet/endothelial activation

Patholgy
- negative pressure
- haemolysis → reduced NO
- cavitation

Liver
- synthetic function –procoagulant and natural anticoagulants
- fibrinolytic pathways
The inflammatory response to extracorporeal membrane oxygenation (ECMO): a review of the pathophysiology

Jonathan E. Millar, Jonathon P. Fanning, Charles I. McDonald, Daniel F. McAuley, and John F. Fraser
Balancing on the tight rope

• Already inflamed pt becomes more hypercoaguable state, with extracorporeal circuits and components at risk of thrombosis.

• Minimal anticoag to reduce clot formation in the ECLS circuit whilst avoiding bleeding
The Ying and the Yang

- 75% of Adult ECMO patients who underwent autopsy had systemic thromboembolic complications (Brain / Bowel / other organs)

- ECMO during H1N1: Hemorrhagic complications occurred in 54%,
  - ECMO cannulation sites in 22%,
  - gastrointestinal tract in 10%
  - respiratory tract in 10%
  - vaginal bleeding in 9%
  - intracranial hemorrhage in 9%

Davies et al 2009 JAMA
Ecmo Induced Coagulopathy

- Fibrinolysis
  - Tissue plasminogen to plasmin
  - Antithrombin

- Inflammatory system
  - Complement
  - Cytokines
  - Inflammatory cells

Contact activation on the ECMO circuit

- PreKallkrein and Factor XII to Kallikrein and Factor XIIa

- Activated platelets
- Endothelium, fibrinogen & vWF
  - GPIIe-IIIb & GP Ib
- Thrombin mediated:
  - PAR-1 & PAR-4
- Amplification of coagulation

- Fibrinogen-fibrin-insoluble fibrin
- Xa-Ca++, Va,PL to Prothrombin-thrombin

Tissue factor mediated
- Damaged endothelium and tissue factor bearing cells - VII to Vil

Management of Anticoagulation and Hemostasis for Pediatric Extracorporeal Membrane Oxygenation

Saini & Spinella
The Endothelial Glycocalyx

- Chondroitin sulfate
- Heparan sulfate
- Glypican
- Hyaluronan
- Syndecan
- Soluble molecules
- Glycoproteins

Endothelium

TM
The “endothelium” in ECMO

Reynolds MM. The artificial endothelium. Organogenesis 2011; 7: 42-9
**Ex vivo ECMO Model**

Human whole blood (<24 hours old)

Flow rates assessed:
- 4 L/min - high flow (n=5)
- 1.5 L/min - low flow (n=5)

Blood collected at different time points

Immune Response

Coagulation
High Flow or Low Flow - which causes more problems?

A

IL-1β (pg/mL)

B

TNF-α (pg/mL)

C

IL-6 (pg/mL)

D

IL-8 (pg/mL)

ECMO duration (h)

High flow

Low flow
Higher Flow (4L/min) led to higher neutrophil activation

**Neutrophil Elastase**

**Myeloperoxidase**

- High flow
- Low flow

**ECMO duration (h)**

<table>
<thead>
<tr>
<th>ECMO duration</th>
<th>NE (ng/mL)</th>
<th>MPO (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>2</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>6</td>
<td>500</td>
<td>500</td>
</tr>
</tbody>
</table>

- *P < 0.05
- **P < 0.01
- ***P < 0.001
- ****P < 0.0001
Coagulation

• Full blood count
• Platelet function (Multiplate)
  • ADP, TRAP-6, Ristocetin
• ROTEM (HEPTEM, EXTEM)
  • Clot formation time (CFT)
  • Maximum clot firmness (MCF)
• Coagulation factors (FXIII, FXII and ATIII)
Multiplate & ROTEM results

- Platelet counts were unchanged
- Coagulation Factors (FXII, FXIII, ATIII) unchanged
Lower flow has higher haemolysis and vWF degradation
### Summary

<table>
<thead>
<tr>
<th></th>
<th>Low flow</th>
<th>High flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets</td>
<td>Unchanged</td>
<td>Unchanged</td>
</tr>
<tr>
<td>Multiplate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADP</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>TRAP-6</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Ristocetin</td>
<td>Decreased</td>
<td>Unchanged</td>
</tr>
<tr>
<td>ROTEM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEPTEM-CFT</td>
<td>Prolonged</td>
<td>Unchanged</td>
</tr>
<tr>
<td>HEPTEM-MCF</td>
<td>Decreased</td>
<td>Unchanged</td>
</tr>
<tr>
<td>EXTEM-MCF</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Coagulation factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FXII, FXIII, ATIII</td>
<td>Unchanged</td>
<td>Unchanged</td>
</tr>
</tbody>
</table>
vWF (blue dye) acts like a glue between platelets (green and red dye)

Shear-induced conformational changes in vWF
What effect of pulsatility on refolding vWF to prevent cleavage from ADAMTS13

Coiled, inactive vWF

High shear stress generated in mechanical assist device induces unfolding of vWF

Continuous-flow

Pulsatile-flow induces refolding of vWF

Stretched, active vWF

ADAMTS13

vWF fragment

Coiled, inactive vWF
What about pulse on endothelial cells and release more vWF

Continuous Flow and Low Pulsatility
- Coiled, inactive vWF
- Mechanical assist device

Lack of Vasoconstriction

Pulsatile Flow and High Pulsatility
- Stretched, active vWF
- ADAMTS13 Cleavage
- vWF fragment

Vasoconstriction
- vWF-rich stored in endothelial cells
- HMW vWF Secretion

Blood flow

HMW Multimer
Study the effect of CF and PF on total blood damage using Blood-Shearing Device
The effect of shear stress on total blood damage – blood shearing device

**Haemolysis**
- Exposure time:
  - 0 min
  - 5 min
  - 15 min

**Platelet Activation**
- Exposure time:
  - 0 min
  - 5 min
  - 15 min
Continuous flow can do harm to RBC and Pts

Haemolysis

- Exposure time
  - Control_0min
  - CF_5min
  - CF_15min
  - PF_5min
  - PF_15min

Platelet Activation

- Exposure time
  - Control_0min
  - CF_5min
  - CF_15min
  - PF_5min
  - PF_15min

Shear Stress (Pa)

<table>
<thead>
<tr>
<th>Shear Stress (Pa)</th>
<th>14</th>
<th>43</th>
<th>162</th>
</tr>
</thead>
<tbody>
<tr>
<td>pfHb (mg/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control_0min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF_5min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF_15min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF_5min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF_15min</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CD42b (%)

- Exposure time
  - Control_0min
  - CF_5min
  - CF_15min
  - PF_5min
  - PF_15min
Study the effect of shear stress on platelet aggregation

- Un-sheared vWF bind more platelets tightly than post-sheared vWF
Study the mechanobiology of vWF under continuous and pulsatile flow using Synchronised Pulsatile Device and Speed Modulation Controller.

Pulsing assist device

Pulsing in HVAD using Speed modulation controller
vWF multimeric profile in a swine model associated with sequential changes of pressure pulse (PP) using different speeds of HVAD.

Stage 1
High pump speed
Low pulsatility

Stage 2
Low pump speed
Normal pulsatility

Stage 3
High pump speed
Low pulsatility

BL BP 0 5 15 30 40 55 70 85 100 110 115 120 125 140 (in minutes)

High to low molecular weight vWF multimers

LMW vWF (1-5)

IMW vWF (6-9)

HMW vWF (>9)
NO as novel anticoagulant in ECMO

- Flow rate
- ACT
- Platelet count
- Platelet aggregation (TRAPtest)
- Cytokine (IL8, TNFa)
### Improved ECMO performance over 8 hr

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>240mins</th>
<th>480mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.01 (0.01)</td>
<td>2.25 (0.77)</td>
<td>1.63 (0.73)</td>
</tr>
<tr>
<td>Heparin</td>
<td>3.99 (0.06)</td>
<td>4.21 (0.01)*</td>
<td>2.15 (0.65)</td>
</tr>
<tr>
<td>NO = 30ppm</td>
<td>3.96 (0.03)</td>
<td>4.12 (0.17)*</td>
<td>4.13 (0.13)**</td>
</tr>
<tr>
<td>NO = 50ppm</td>
<td>4.05 (0.02)</td>
<td>4.2 (0.06)*</td>
<td>4.1 (0.11)**</td>
</tr>
<tr>
<td>NO = 80ppm</td>
<td>4.04 (0.01)</td>
<td>4.03 (0.02)*</td>
<td>4.02 (0.02)**</td>
</tr>
</tbody>
</table>

Values are means (SEM)

*p < 0.0001 vs control and **p < 0.0001 vs heparin.

### Prevented platelet loss

- **Control**
- **Heparin**
- NO = 30ppm + Heparin
- NO = 50ppm + Heparin
- NO = 80ppm + Heparin

### Reduced platelet aggregation

- No Anticoagulation
- NO = 30 ppm + Heparin
- NO = 50ppm + Heparin
- NO = 80ppm + Heparin

### Higher ACT

- No Anticoagulation
- NO = 30ppm + Heparin
- Heparin
- NO = 50ppm + Heparin
- NO = 80ppm + Heparin
NO also act as anti-inflammatory
MSCs and ECMO?
Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial

Jennifer G Wilson, Kathleen D Liu, Hanjing Zhuo, Lizette Caballero, Melanie McMillan, Xiaohui Fang, Katherine Cosgrove, Rosemary Vojnik, Carolyn S Calfee, Jae-Woo Lee, Angela J Rogers, Joseph Levitt, Jeanine Wiener-Kronish, Ednan K Bajwa, Andrew Leavitt, David McKenna, B Taylor Thompson, Michael A Matthay

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>6 h</th>
<th>Day 1</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Interleukin 6 (pg/mL)</strong></td>
<td>762 (419–1198)</td>
<td>557 (91–734)</td>
<td>317 (150–736)</td>
<td>62 (20–140)</td>
</tr>
<tr>
<td><strong>Interleukin 8 (pg/mL)</strong></td>
<td>35 (18–49)</td>
<td>26 (16–39)</td>
<td>29 (19–55)</td>
<td>16 (8–47)</td>
</tr>
<tr>
<td><strong>ANGPT2 (pg/mL)</strong></td>
<td>7507 (3977–14950)</td>
<td>8168 (4415–13000)</td>
<td>10900 (4593–18200)</td>
<td>6922 (4783–18700)</td>
</tr>
<tr>
<td><strong>AGER (pg/mL)</strong></td>
<td>2749 (894–5060)</td>
<td>2841 (1055–4333)</td>
<td>1790 (882–5080)</td>
<td>1308 (1268–2437)</td>
</tr>
</tbody>
</table>

Data are median (IQR). ANGPT2=angiopoietin-2. AGER=receptor for advanced glycation endproducts.
Mesenchymal stromal cells and the acute respiratory distress syndrome (ARDS): challenges for clinical application

J E Millar,¹ J F Fraser,² D F McAuley³

Thorax July 2015 Vol 70 No 7
Take home message: Don’t put IV MSCs in ECMO

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Conditions during ex-vivo ECMO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4x10⁶ IPSC-MSCs (n=4)</td>
</tr>
<tr>
<td>Time to 25% decrease in blood flow (min±SE)</td>
<td>68±32</td>
</tr>
<tr>
<td>Time to 50% decrease in blood flow (min±SE)</td>
<td>85±39</td>
</tr>
<tr>
<td>Blood flow at 2000 RPM (L/min±SE)</td>
<td></td>
</tr>
<tr>
<td>30 s</td>
<td>4.05±0.02</td>
</tr>
<tr>
<td>15 min</td>
<td>3.79±0.35</td>
</tr>
<tr>
<td>30 min</td>
<td>2.83±0.98</td>
</tr>
<tr>
<td>60 min</td>
<td>1.94±1.14</td>
</tr>
<tr>
<td>120 min</td>
<td>1.39±0.92</td>
</tr>
<tr>
<td>240 min</td>
<td>0.60±0.35</td>
</tr>
<tr>
<td>Transoxygenator pressure gradient (mm Hg±SE)</td>
<td></td>
</tr>
<tr>
<td>30 s</td>
<td>20±6</td>
</tr>
<tr>
<td>15 min</td>
<td>27±12</td>
</tr>
<tr>
<td>30 min</td>
<td>51±24</td>
</tr>
<tr>
<td>60 min</td>
<td>555±31</td>
</tr>
<tr>
<td>120 min</td>
<td>50±18</td>
</tr>
<tr>
<td>240 min</td>
<td>101±9</td>
</tr>
<tr>
<td>MSCs detectable in blood (cells/μl±SE)</td>
<td></td>
</tr>
<tr>
<td>30 s</td>
<td>21.8±1.9</td>
</tr>
<tr>
<td>30 min</td>
<td>9.4±4.2</td>
</tr>
<tr>
<td>60 min</td>
<td>6.9±3.4</td>
</tr>
<tr>
<td>120 min</td>
<td>4.1±3.8</td>
</tr>
<tr>
<td>240 min</td>
<td>0.3±0.2</td>
</tr>
</tbody>
</table>

* Circuits terminated after a 25% reduction in blood flow (3L/min), performed to optimise conditions for microscopy.
– Did not occur; ECMO, extracorporeal membrane oxygenation; IPSC, induced pluripotent stem cell; MSCs, mesenchymal stem cells; RPM, revolutions per minute.
Thorax 2018;0:1–3. doi:10.1136/thoraxjnl-2017-211439
Administration of mesenchymal stem cells during ECMO results in a rapid decline in oxygenator performance

Jonathan Edward Millar, Viktor von Bahr, Maximillian V Malferttheiner, Katrina K Ki, Meredith A Redd, Nicole Bartnikowski, Jacky Y Suen, Danny Francis McAuley and John F Fraser

This is the first study to directly address the feasibility of MSC therapy during ECMO. Our data suggest that intravascular administration of MSCs during ECMO may have important consequences for oxygenator function, as well as for their efficacy as a therapy for severe ARDS in this setting. This may have occurred due to the characteristic plastic adhesiveness of MSCs.

Thorax 2018;0:1–3. doi:10.1136/thoraxjnl-2017-211439
Animal Study - ARDS + ECMO + MSCs

ARDS Sheep (n=7 per group) supported by ECMO ± MSCs

• T0 – 2-hit ARDS ovine model (Oleic Acid + LPS)
• T1 – ECMO cannulation
• T2 – MSCs administration
• T23 – ECMO stops
• T24 – Completion of experiment
It ain’t all glamour in the sheep lab!
MSCs reduced inflammation but increased coagulation

Lung histology showed significant level of thrombi!
Upcoming study: Oxygen is a drug!

- Oxygen is the most commonly prescribed drug around the globe.
- The “right dose” and the risks associated with giving the “wrong dose” are unclear.

Munshi et al., Crit Care Med, 2017.
Upcoming study: Oxygen is a drug!

Clinical consequences
- Neurological damage (e.g. confusion, delirium)
- Organ injuries (lung collapse, alveolar leak, chest pains)
- Increased risk of death

Hyperoxemia
$\text{PaO}_2$ 101 - >300 mmHg

High-dose oxygen management in ECMO
80 - 100% $\text{FiO}_2$

Ex vivo ECMO study
A better understanding of the immunological mechanisms involved will help clinicians titrate the optimal level of oxygen.

Cellular consequences - Homeostatic imbalance:

Hayes et al, Perfusion, 2013
Summary

Inflammation and Coagulation – An Unholy Alliance

• Two sides of the same coin
• Inter-connected and Inter-dependent
• ECMO impacts on both inflammation and coagulation
• Both needs to be considered simultaneously
• If we do this, we will achieve better outcomes
Acknowledgements