Immunosuppression in Early Sepsis

Aleksandra Leligdowicz, MDCM DPhil FRCPC
Canadian Critical Care Forum 2019
12.November.2019
Sepsis in a nutshell

1. **TRIGGER → Infection**

2. **Systemic immune response**

3. **Endothelial & epithelial injury, coagulopathy**

4. **End-organ dysfunction**

5. ±Death

**Heterogeneity** at a molecular, cellular, & organ-level

ARDS, AKI, Cardiomyopathy, Encephalopathy...
1. Source of infection

- 29 ICUs/7,974 patients with septic shock
- 20 infection sources

- **Obstructive uropathy–associated UTI** → lowest mortality (26%)
- **Ischemic bowel** → highest mortality (75%)
2. Immune exhaustion in late sepsis

![Graph showing decreased TNFα expression and function in sepsis compared to no sepsis.](image)

- **CD4 and CD8** cell counts indicate a decrease in quantity and function in sepsis.
- **TNFα after LPS stimulation** shows a significant drop in sepsis compared to no sepsis.

Boomer, JAMA 2011: 306(23): 2594-2605
3. Endothelial injury

- **Baseline function**
  - Barrier
  - Cell & nutrient movement
  - Inflammatory signaling
  - Vasomotor tone
  - Coagulation control
  - New blood vessel generation

- **Sepsis**: Loss of barrier
  - Edema/leak
  - ↑ leukocyte adhesion & diapedesis
  - Bacterial translocation
  - Vasodilation/vasoplegia
  - Procoagulant state
    - microthrombi, fibrin deposits
  - Endothelial cell apoptosis

Ince, et al., SHOCK 2016; 45(3): 259-270.
Immunosuppression theory

Early deaths due to overwhelming inflammation

Late deaths due to persistent immunosuppression and recurrent infections

Competing evidence

No evidence of nosocomial infection in most patients dying at late state of sepsis

Goldenberg. Critical Care 2014, 18(540).

Incidence, Risk Factors, and Attributable Mortality of Secondary Infections in the Intensive Care Unit After Admission for Sepsis

Lonneke A. van Vught, MD; Peter M. C. Klein Klouwenberg, MD, PharmD, PhD; Cristian Spitoni, PhD; Brendan P. Scicluna, PhD; Maryse A. Wiewel, MD; Janneke Horn, MD, PhD; Marcus J. Schultz, MD, PhD; Peter Nürnberg, PhD; Marc J. M. Bonten, MD, PhD; Olaf L. Cremer, MD, PhD; Tom van der Poll, MD, PhD; for the MARS Consortium

Competing evidence

Attributable mortality fraction of ICU-acquired infections

Sepsis

Non-infectious
Heterogeneity in Sepsis

Risk factors
- Age
- Comorbidities
- Source of infection
- Causative organism
- Treatment

Biomarkers
- Metabolites
- Proteins & mRNA
- Cellular function & phenotype

Outcome
- End-organ injury ± Death

Leligdowicz, CCM 2019
Aims

1. Investigate *ex vivo* immune response heterogeneity in *early* sepsis
   - Leukocyte response to endotoxin
   - Endothelial cell injury

2. Relate *ex vivo* immune response to *in vivo* host inflammation
Methods

Whole blood

- RBC/WBCs
  - 4h stimulation (Endotoxin + Control)

  - Supernatant
    - Endothelial Permeability

  - Cell pellet
    - Endothelial ICAM-1
      - RNA Seq

- Plasma
  - Biomarkers

- Supernatant
  - Biomarkers

<24h of ER presentation
Leukocytes produce inflammatory cytokines after endotoxin stimulation

- Whole blood
- Plasma
- RBC/WBCs
- Supernatant
- Endothelial ICAM-1
- Endothelial Permeability

Biomarkers

- IL-1α
- IL-1β
- IL-6
- IL-8
- IL-10
- IL-12
- IL-13
- IL-17f
- RANTES
- Eotaxin
- MIP-1α
- MIP-1β
- MCP1
- EGH
- HGF
- VEGF
- IFNa
- IFNγ
- IP10
- TNFα
- GMCSF
- GCSF
- FGFbasic

Median fold change in supernatant biomarkers
#1. **Heterogeneity in leukocyte response to endotoxin**

Fold change in supernatant biomarkers after LPS stimulation
#2. *Ex vivo* endothelial permeability

Electric Cell-substrate Impedance Sensing (ECIS)

1. Confluent human pulmonary microvascular endothelial cells (HPMECs)
2. **Leukocyte supernatants** added
3. Resistance measured for 5 hours
#2. Heterogeneity in *Ex vivo* endothelial permeability

Human pulmonary endothelial cell permeability after exposure to LPS-stimulated leukocyte supernatants derived from patients with early sepsis.

Aleksandra Leiligdowicz, Lauren F Chun, Alejandra Jauregui, Kathryn Vessel, Kathleen D Liu, Carolyn S Calfee, and Michael A. Matthay Show less Authors

15 JUL 2016. https://doi.org/10.1038/srep29618

**Sum of area under the curve**

Resistance at 5 hours

Minimal permeability (9/40, 23%)

Intermediate permeability (22/40, 55%)

Maximal permeability (9/40, 23%)
#2. Endothelial permeability is proportional to leukocyte TNFα endotoxin response

\[ r_s = 0.94 \]
\[ p < 0.0001 \]
#3. Leukocyte RNA sequencing

- Analysis of protein-coding, non-mitochondrial genes
- Low expression genes removed
- Mixed effects (Limma, R) used to fit gene expression model
- Surrogate of endotoxin tolerance:
  - Fold change in cell culture supernatant TNFα
#3. Leukocyte RNA sequencing

LPS-Stimulated leukocytes

Unstimulated leukocytes

Immunosuppression
Endotoxin tolerant

Immunosuppression
Endotoxin tolerant
#4. Host inflammatory state: Plasma biomarkers

Systemic immune response

Immune response

Endothelial & epithelial injury, coagulopathy

Tissue breakdown & repair

Endothelial injury

Epithelial injury

Coagulopathy
#4. High plasma inflammation is associated with immune suppression
#4. High plasma inflammation is associated with immune suppression

\[ r_s = -0.43 \]
\[ p = 0.006 \]

- Minimal permeability
- Intermediate permeability
- Maximal permeability
Summary: Heterogeneity in leukocyte function in early sepsis

Infection → ↑ Immune response → Immune exhaustion → Immunosuppression (Endotoxin tolerance)

Host inflammatory state: Plasma biomarkers
- Low TNFα
- Lipoxins
- Microvesicles
- ↑ IL-6
- ↑ IFNγ

Leukocyte RNA Seq
- LPS
- Neutrophil
- Macrophage
- T cell
- Low TNFα
- ↓ IL-6
- ↓ IFNγ

No increase in Endothelial permeability
Implications

- Immunosuppression is present in *early* sepsis, not only in the late phase
  - Only in subset of patients → Heterogeneity
- Endotoxin stimulation is required for developing an endotoxin tolerance transcriptomic signature
  - RNA Seq from *unstimulated* cells is *inadequate*
- Potential to *individualize treatment* based on immune profiling & enable enrollment in clinical trials of *immune-modulating therapies*
Acknowledgements

Matthay Lab

Michael Matthay
Jason Abbott
Alejandra Jauregui
Kathryn Vessel
Serena Ke
Lauren Chun
Xiaohui Fang
Hanjing Zhuo
Carolyn Calfee
Kathleen Liu

Collaborators

Chaz Langelier
Jack Kamm
Saharai Caldera
Paula Hayakawa Serpa
Fengyun Xu
Xiaoli Tian
Arun Prakash

Funding

UHN clinical associate
CSTP/CIP U of Toronto
CIHR/Banting fellowship
Thank you
## Patient clinical & ex vivo characteristics

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Whole cohort (n=40)</th>
<th>Minimal endotoxin response (n=14)</th>
<th>Intermediate endotoxin response (n=11)</th>
<th>Maximal endotoxin response (n=15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>65.7 (+17.6)</td>
<td>67 (+14.6)</td>
<td>74.7 (+20.7)</td>
<td>57.9 (+15.1)</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Sex (male, %)</strong></td>
<td>25 (63%)</td>
<td>9 (64%)</td>
<td>8 (73%)</td>
<td>8 (53%)</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>WBC (10⁹/mL)</strong></td>
<td>13.6 (10.3, 22.5)</td>
<td>13.4 (7, 24.3)</td>
<td>12.2 (10.8, 15.4)</td>
<td>14.5 (10.6, 28)</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Immunosuppression (n, %)</strong></td>
<td>14 (35%)</td>
<td>4 (28.6%)</td>
<td>3 (27.3%)</td>
<td>7 (46.7%)</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Shock (n, %)</strong></td>
<td>14 (35%)</td>
<td>6 (42.9%)</td>
<td>3 (27.3%)</td>
<td>5 (33.3%)</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>APACHE III</strong></td>
<td>87.4 (+35.4)</td>
<td>86 (+35.6)</td>
<td>92.5 (+41.5)</td>
<td>84.8 (+31.8)</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>SAPS II</strong></td>
<td>46.3 (+16.1)</td>
<td>46.7 (+18.2)</td>
<td>45.2 (+16.3)</td>
<td>46.6 (+14.9)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Hospital death (n, %)</strong></td>
<td>6 (15%)</td>
<td>3 (21.4%)</td>
<td>1 (9.1%)</td>
<td>2 (13.3%)</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Source of sepsis (n, %)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.9</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>23 (58%)</td>
<td>8 (57%)</td>
<td>8 (73%)</td>
<td>7 (46.7%)</td>
<td></td>
</tr>
<tr>
<td>Intraabdominal</td>
<td>9 (23%)</td>
<td>3 (21.4%)</td>
<td>2 (18.2%)</td>
<td>4 (26.7%)</td>
<td></td>
</tr>
<tr>
<td>Urinary tract</td>
<td>2 (5%)</td>
<td>1 (7.1%)</td>
<td>0 (0%)</td>
<td>1 (6.7%)</td>
<td></td>
</tr>
<tr>
<td>Skin and soft tissue</td>
<td>2 (5%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2 (13.3%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>4 (10%)</td>
<td>2 (14.3%)</td>
<td>1 (9.1%)</td>
<td>1 (6.7%)</td>
<td></td>
</tr>
<tr>
<td><strong>Organism (n, %)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram +ve</td>
<td>13 (33%)</td>
<td>4 (28.6%)</td>
<td>4 (36.4%)</td>
<td>5 (33.3%)</td>
<td>1</td>
</tr>
<tr>
<td>Gram -ve</td>
<td>11 (28%)</td>
<td>4 (28.6%)</td>
<td>4 (36.4%)</td>
<td>3 (20%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Virus</td>
<td>6 (15%)</td>
<td>3 (21.4%)</td>
<td>1 (9.1%)</td>
<td>2 (13.3%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Negative cultures</td>
<td>16 (40%)</td>
<td>5 (35.7%)</td>
<td>6 (54.6%)</td>
<td>5 (33.3%)</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Ex vivo variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFα fold change</td>
<td>65.5 (20.2, 448)</td>
<td>12.3 (8.7, 25.4)</td>
<td>55.6 (30.2, 138)</td>
<td>559 (369, 1118)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Endothelial permeability (n, %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Minimal permeability</td>
<td>9 (22.5%)</td>
<td>9 (64.3%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Intermediate permeability</td>
<td>22 (55%)</td>
<td>5 (35.7%)</td>
<td>11 (100%)</td>
<td>6 (40%)</td>
<td></td>
</tr>
<tr>
<td>Maximal permeability</td>
<td>9 (22.5%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>9 (60%)</td>
<td></td>
</tr>
<tr>
<td>Endothelial permeability (AUC)</td>
<td>0.42 (0.26, 0.64)</td>
<td>0.13 (0.07, 0.28)</td>
<td>0.4 (0.35, 0.49)</td>
<td>0.71 (0.63, 0.82)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Plasma CXCL9/MIG (pg/mL)</td>
<td>1423 (642, 2449)</td>
<td>2130 (1976, 3911)</td>
<td>1123 (571, 1659)</td>
<td>700 (391, 2778)</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Relationship between WBC & endotoxin tolerance

\[ r_s = 0.11 \]
\[ p = 0.49 \]

- Minimal permeability
- Intermediate permeability
- Maximal permeability

In Vitro: Fold change in supernatant TNF-\(\alpha\)

Ex vivo: WBC 10\(^9\)/L