Acute kidney injury after transplantation

= 

Delayed graft function (DGF)

PD Dr. med. Bernd Schröppel

Section of Nephrology

University Hospital Ulm, Germany
DGF and donor source

Live donor: 3%
Standard criteria: 21%
Expanded criteria donor (ECD): 33%
Donation after cardiac death (DCD): 40%

SRTR 2008
DGF and transplant outcome

Cumulative probability of patient survival with graft function:

Log rank $P < 0.0001$

<table>
<thead>
<tr>
<th>Follow-up</th>
<th>No DGF</th>
<th>DGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 months</td>
<td>97.2</td>
<td>93.5</td>
</tr>
<tr>
<td>1-year</td>
<td>95.8</td>
<td>91.3</td>
</tr>
<tr>
<td>3-years</td>
<td>91.2</td>
<td>84.3</td>
</tr>
<tr>
<td>5-years</td>
<td>85.8</td>
<td>76.6</td>
</tr>
<tr>
<td>7-years</td>
<td>80.0</td>
<td>68.8</td>
</tr>
</tbody>
</table>

Number at risk:

<table>
<thead>
<tr>
<th>Follow-up</th>
<th>No DGF</th>
<th>DGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 months</td>
<td>38704</td>
<td>11542</td>
</tr>
<tr>
<td>1-year</td>
<td>33188</td>
<td>8308</td>
</tr>
<tr>
<td>3-years</td>
<td>26735</td>
<td>6303</td>
</tr>
<tr>
<td>5-years</td>
<td>20449</td>
<td>4752</td>
</tr>
<tr>
<td>7-years</td>
<td>14555</td>
<td>3323</td>
</tr>
<tr>
<td>10-years</td>
<td>9372</td>
<td>2104</td>
</tr>
<tr>
<td>15-years</td>
<td>4895</td>
<td>1135</td>
</tr>
<tr>
<td>20-years</td>
<td>1488</td>
<td>353</td>
</tr>
</tbody>
</table>

Tapiawala SN, JASN, 2010
Biological processes implicated in ischemia and reperfusion

- Vascular leakage
- No reflow phenomenon
- Cell death programs: Apoptosis, necrosis, autophagy-associated cell death
- Transcriptional reprogramming
- Autoimmunity: Autoantibody and complement activation
- Innate and adaptive immune activation

Eltzschig, Nat Med 2011
Innate immune system

Not a separate system, but rather an overlapping response to disturbed tissue integrity.

**Components:** Toll-like receptors

  Autophagy

  Complement
Concept of donor-derived inflammation:
Islet-derived MCP-1 attracts recipient immune cells

Schröppel, JASN 2005
Damage-associated molecular patterns (DAMPs) cause sterile inflammation

Arslan F, Nat. Rev. Cardiol. 2011
Islets release HMGB1 and TLR sense injury and mediate early graft failure after transplantation.
Donor Toll-like receptor 4 contributes to ischemia and reperfusion injury following human kidney transplantation

Bernd Krüger\textsuperscript{a,b}, Stefanie Krick\textsuperscript{a}, Navdeep Dhillon\textsuperscript{a}, Susan M. Lerner\textsuperscript{c}, Scott Ames\textsuperscript{c}, Jonathan S. Bromberg\textsuperscript{c}, Marvin Lin\textsuperscript{a}, Liron Walsh\textsuperscript{a}, John Vella\textsuperscript{d}, Michael Fischereder\textsuperscript{e}, Bernhard K. Krämer\textsuperscript{b}, Robert B. Colvin\textsuperscript{f}, Peter S. Heeger\textsuperscript{a,c}, Barbara T. Murphy\textsuperscript{a,c}, and Bernd Schröppel\textsuperscript{a,c,1}

PNAS 2009
TLR4 is up-regulated in deceased donor compared to living donor kidneys

Kruger, B, PNAS 2009
What are the endogenous TLR ligands?

Fibronectin/ Fibrinogen
Heparan sulfate
Defensins
Heat shock proteins
Histones
High mobility group box protein (HMGB1)
What are the endogenous TLR ligands?

<table>
<thead>
<tr>
<th>Ligands</th>
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<tr>
<td>Fibronectin/Fibrinogen</td>
</tr>
<tr>
<td>Heparan sulfate</td>
</tr>
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<td>Defensins</td>
</tr>
<tr>
<td>Heat shock proteins</td>
</tr>
<tr>
<td>Histones</td>
</tr>
<tr>
<td>High mobility group box protein (HMGB1)</td>
</tr>
</tbody>
</table>
HMGB1 is only expressed in decreased donor kidneys
Kidneys with “loss-of-function” TLR4 mutation have higher rate of immediate graft function

<table>
<thead>
<tr>
<th></th>
<th>n=267</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$P$</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>TLR4 mutant</td>
<td>0.018</td>
<td>3.89 (1.26-12.05)</td>
</tr>
<tr>
<td>Donor Age (y)</td>
<td>0.015</td>
<td>0.96 (0.93-0.99)</td>
</tr>
<tr>
<td>Donor Type</td>
<td>0.22</td>
<td>2.72 (0.55–13.45)</td>
</tr>
<tr>
<td>CIT (min)</td>
<td>0.23</td>
<td>0.99 (0.99-1.0)</td>
</tr>
<tr>
<td>Center</td>
<td>0.78</td>
<td>1.14 (0.46–2.88)</td>
</tr>
</tbody>
</table>
HMGB1/TLR4 are critical mediators in IR injury

Donor TLR4 affects graft failure in liver transplant recipients. 
Dhillon, J Hepatol 2010; Oeting, Liver Transpl 2012

Neutralization of HMGB1 protects against renal IR injury.
Li J, NDT 2011

HMGB1 contributes to kidney IR injury.
Wu, H, JASN 2010

TLR4 regulates endothelial activation during ischemic AKI.
Chen J, Kidney Int. 2011

HMGB1 mediates liver IR injury.
Tsung, JEM 2010

HMGB1/TLR4 mediates cardiac IR injury.
Zhu, Transplantation 2013
Oxidation or selective mutation of Cys106 abolished the HMGB1-induced activities.

BoxA, a HMGB1 inhibitor, interferes with leukocyte recruitment but not with activation.

Anderson, Ann Rev Imm 2011
Kamaza, Immunity 2008; Yang, PNAS 2010, Venereau JEM 2012
TLRs as therapeutic targets

OPN-305, Humanised Monoclonal Antibody blocking TLR2

• Start Feb 2013; Phase 2 (n = 278); NCT01794663
• Intravenous infusion for 1 hour at start of transplant procedure
Autophagy
‘auto’- (self), ‘phagy’ (eating)

• Quality control & removal of disfunct organelles

• Energy source during starvation

• Effector and regulator of innate and adaptive immunity

• Cell survival and death

Schröppel B. Autophagy: Basic Principles and Relevance to Transplant Immunity, Am J Transplant 2014
Autophagy and apoptosis

“autosis”

caspase-dependent

Schröppel, B. Am J Transplant 2014
Dynamic changes of autophagy in proximal tubules

Renal tubular cells of CAG-RFP-EGFP-LC3 mice - puncta outside the autolysosome

Resolution of autophagy is accompanied by activation of mTOR and by tubular repair

Ling Li et al. JASN 2014
Autophagie is protective during IR injury

Liu, Autophagy 2012
Autophagy in Renal Ischemia-Reperfusion Injury: Friend or Foe?

P. Decuypere, AJT 2014
Autophagy in Renal Ischemia-Reperfusion Injury: Friend or Foe?
Autophagy as therapeutic target?

**PRO**

• Huge clinical interest

(Crohn's disease, cancer, aging, diabetes, infection, Alzheimer).

**CON**

• No validated clinical markers

• Lack of safe and selective inducers or inhibitors
Complement
Terminal complement activation is critical in IR injury

<table>
<thead>
<tr>
<th>Study</th>
<th>Species</th>
<th>Knock-out or treatment</th>
<th>IRI</th>
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<tbody>
<tr>
<td>Zhou et al.</td>
<td>Mouse</td>
<td>C3−/−</td>
<td>↓</td>
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<tr>
<td></td>
<td></td>
<td>C5−/−</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C6−/−</td>
<td></td>
</tr>
<tr>
<td>Zhou et al.</td>
<td>Mouse</td>
<td>C4−/−</td>
<td></td>
</tr>
<tr>
<td>De Vries et al.</td>
<td>Mouse</td>
<td>C5a mAb</td>
<td>↓</td>
</tr>
<tr>
<td>Arumugam et al.</td>
<td>Rat</td>
<td>C5aR antagonist</td>
<td>↓</td>
</tr>
<tr>
<td>De Vries et al.</td>
<td>Mouse</td>
<td>C5aR antagonist</td>
<td>↓</td>
</tr>
<tr>
<td>Castellano et al.</td>
<td>Swine</td>
<td>C1-inhibitor</td>
<td>↓</td>
</tr>
<tr>
<td>Moller-Kristensen</td>
<td>Mouse</td>
<td>MBL-A−/−</td>
<td>↓</td>
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<tr>
<td></td>
<td></td>
<td>MBL-C−/−</td>
<td></td>
</tr>
<tr>
<td>Thurman et al.</td>
<td>Mouse</td>
<td>Factor B−/−</td>
<td>↓</td>
</tr>
<tr>
<td>Thurman et al.</td>
<td>Mouse</td>
<td>Factor B mAb</td>
<td>↓</td>
</tr>
<tr>
<td>Zheng X et al.</td>
<td>Mouse</td>
<td>siRNA against C3, C5aR</td>
<td></td>
</tr>
</tbody>
</table>

*Damman, AJT 2011*
Renal cell and bone marrow cell expressed C3aR / C5aR are important

Peng Q et al. JASN 2012
Complement activation during brain death

Werkhoven, AJT 2013
Therapeutic interventions?

Pilot study, Mount Sinai: Eculizumab in DGF.

Complement Cascade

- Eculizumab binds with high affinity to C5
- Terminal complement – C5a and C5b-9 activity blocked
- Proximal functions of complement remain intact
  - Weak anaphylatoxin
  - Immune complex clearance
  - Microbial opsonization
Complement as therapeutic target

**C5a inhibitor Eculizumab**
- Start Mai 2014; Phase 2/3 (n = 283)
- IV infusion on day of transplant then 18-24 hours later

**C1 Esterase Inhibitor**
- Start Jul 2014; Phase 1/2 (n = 70)
- IVP administered on day of transplant, and another dose 24 hours post op.
Since activation of innate immune system occurs already before organ donation, complement-based strategies should start shortly after the diagnosis of brain death and extent into the recipient.
Barriers for DGF therapeutics

- To date no validated prognostic tools to predict allograft outcome of the *individual* donor kidney
- Defining *surrogate endpoints* is instrumental to test new treatments. No consensus on the definition of “DGF”.
Tubular Expression of KIM-1 Does not Predict Delayed Function After Transplantation

Bernd Schröppel,* † Bernd Krüger,* † Liron Walsh,* Melissa Yeung,§ ‖ Shay Harris,§
Krista Garrison,§ Jonathan Himmelfarb,§ Susan M. Lerner,† Jonathan S. Bromberg,†
Ping L. Zhang,‖ Joseph V. Bonventre,‖ Zhu Wang,** Alton B. Farris,‖ † Robert B. Colvin,‖ †
Barbara T. Murphy,* † and John P. Vella§

Schröppel, JASN 2010
“ATN-diagnosis” in the donor kidney is not helpful in predicting DGF or graft failure

1789 kidneys procured (933 potential donors)

964 kidneys biopsied (495 potential donors)

313 kidneys (32%) discarded (191 potential donors)

651 biopsy reports analyzed (369 donors)

P=0.3

P=0.9

P=0.01

Hall IE, CJASN 2014
“Danger and damage” is everywhere

POTENTIAL GRAFT INJURY AND ROLE OF INJURY BIOMARKERS

1. Donor identified
   - Identify injury during critical illness and brain death
   - Supplement expanded criteria and guide best care for the donor

2. Organ recovery surgery
   - Identify injury during organ procurement
   - Guide transport protocol and allowable cold time

3. Organ transport to transplant center
   - Identify injury during organ transport (storage media)
   - Guide transplant protocol for recipients

4. Transplant surgery in recipient
   - Identify delayed graft function at transplant surgery
   - Guide short-term treatment strategies and follow-up

5. Recipient follow-up
   - Identify worsening graft function, acute rejection
   - Guide best long-term treatment strategies

Hall IE, CJASN 2010
Glutathione S-transferase (GST) in perfusion solution

- 822 pumped kidneys before April 30, 2012 (411 Donors)
  - 220 kidneys pumped en bloc
  - 602 individually pumped kidneys (301 Donors)
    - 128 kidneys discarded
    - 42 kidneys with no perfusate sample collected
    - 4 kidneys with samples discarded due to protocol violation
- 428 transplanted kidneys with at least 1 GST value (239 Donors)

Hall, IE, AJT 2014
Glutathione S-transferase at the end of perfusion correlates with DGF

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Time point</th>
<th>Adjusted for donor, transport and recipient variables³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pi-GST</td>
<td>Base</td>
<td>1.05 (0.96–1.16)</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>1.36 (1.14–1.63)</td>
</tr>
</tbody>
</table>

alpha-GST: released by damaged proximal tubule cells
pi-GST: released by distal tubules

Hall IE, AJT 2014
“Danger and damage” is everywhere

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*Hall IE, CJASN 2010*
Donor AKI is associated with kidney discard and DGF

Using AKI Network criteria quantified AKI in donors using donor information not available in UNOS database (i.e. changes in SCr)
Donor AKI is associated with kidney discard and DGF

• Donor AKI is common (25% of 1,369 kidneys)
• Donor AKI is associated with kidney discard and DGF
• 6-mo eGFR was similar across AKI categories but was lower for recipients with DGF
• Given acceptable 6-mo allograft function, clinicians should consider cautious expansion into this donor pool
• Currently testing whether novel AKI biomarkers provide added decision-making value in this context
The reality of DGF therapeutics

• Lots of excellent science **but**
• Many challenges in translation bench to bedside
  – Animal models
    (species/strain/gender; WIT/CIT; method of clamping, iso vs. allograft; timing of intervention relative to injury)
  – Logistics and ethics
  – The right timing, target cells, and duration of such therapy?
• Promising novel therapeutics
• DGF interventions **need to improve long-term outcomes**
Merci

Acknowledgments


**Transplant centers:** Yale-New Haven (PI Chirag Parikh); University of Pennsylvania, Barnabas Health, Mount Sinai, Harper Hospital.

**Supported** by NIH
Pi-GST am Ende der Perfusion ist mit DGF assoziiert

Table 2: Biomarker concentrations in allograft pump perfusate by DGF status

<table>
<thead>
<tr>
<th>Variable</th>
<th>All (N = 428)</th>
<th>DGF (N = 141)</th>
<th>Non-DGF (N = 287)</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base pi-GST, µg/L</td>
<td>191 (65–356)</td>
<td>244 (76–399)</td>
<td>176 (61–336)</td>
<td>0.03</td>
</tr>
<tr>
<td>Post pi-GST, µg/L</td>
<td>498 (321–905)</td>
<td>653 (394–980)</td>
<td>446 (299–870)</td>
<td>0.002</td>
</tr>
<tr>
<td>Delta pi-GST, µg/L³</td>
<td>314 (145–601)</td>
<td>368 (196–610)</td>
<td>296 (124–595)</td>
<td>0.05</td>
</tr>
<tr>
<td>Base alpha-GST, µg/L</td>
<td>147 (43–394)</td>
<td>154 (66–413)</td>
<td>140 (38–373)</td>
<td>0.25</td>
</tr>
<tr>
<td>Post alpha-GST, µg/L</td>
<td>414 (218–800)</td>
<td>506 (226–865)</td>
<td>372 (215–748)</td>
<td>0.06</td>
</tr>
<tr>
<td>Delta alpha-GST, µg/L³</td>
<td>203 (81–481)</td>
<td>220 (103–544)</td>
<td>189 (69–406)</td>
<td>0.07</td>
</tr>
<tr>
<td>Renal resistance at 4 h, mmHg/mL/min</td>
<td>0.23 (0.17–0.29)</td>
<td>0.23 (0.19–0.32)</td>
<td>0.22 (0.17–0.28)</td>
<td>0.12</td>
</tr>
<tr>
<td>Perfusate flow at 4 h, mL/min</td>
<td>114 (94–132)</td>
<td>111 (91–131)</td>
<td>115 (99–133)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Values are medians (interquartile range). DGF, delayed graft function; GST, glutathione S-transferase.

¹For all perfusate biomarkers, base values were missing in 10 kidneys and post values were missing in 59 kidneys. Renal resistance and perfusate flow were missing in 106 kidneys.

²Wilcoxon rank-sum test (DGF vs. non-DGF).

³Difference in post minus base values.