Chronic Renal Allograft Dysfunction
An unsolved problem

Johan (Hans) W. de Fijter, MD, PhD
Professor of Medicine and Nephrology
Director of the Kidney and the Pancreas Transplant Program

Leiden University Medical Center
Leiden, The Netherlands

Catalan Transplantation Society, Barcelona March 18th, 2015
Kidney Transplantation

Long-term outcomes remain poor and inadequate


Graft Loss
Death with function dominated by Cardiovascular Disease

75% No Graft Loss

14.5% Graft Failure incl. PNF

10.5% Death with Graft Function

28.2% Cardiovascular
15.2% Infections
13.8% Malignancies
11.6% Other
31.2% Unknown

Mean follow-up: 50.3 ± 32.6 months

Robust association between GFR and CV-mortality
One-year eGFR <50 mL/min/1.73m² is associated with inferior outcome

Relationship between eGFR and graft failure over 10-years follow-up
(Cox proportional hazard adjusted for multiple covariates)

GFR a robust risk factor for cardiovascular mortality

Standardized Cardiovascular Event Rate (GP mean follow-up 2.8 years)

Non-Renal Solid Organ Transplant recipients

High risk of renal failure

No. at risk

<table>
<thead>
<tr>
<th>Organ</th>
<th>No. at risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>36,849</td>
</tr>
<tr>
<td></td>
<td>28,495</td>
</tr>
<tr>
<td></td>
<td>24,041</td>
</tr>
<tr>
<td></td>
<td>19,508</td>
</tr>
<tr>
<td></td>
<td>15,724</td>
</tr>
<tr>
<td></td>
<td>12,564</td>
</tr>
<tr>
<td></td>
<td>9,844</td>
</tr>
<tr>
<td></td>
<td>7,345</td>
</tr>
<tr>
<td></td>
<td>5,292</td>
</tr>
<tr>
<td></td>
<td>3,614</td>
</tr>
<tr>
<td></td>
<td>2,261</td>
</tr>
<tr>
<td>Lung</td>
<td>7,643</td>
</tr>
<tr>
<td></td>
<td>5,633</td>
</tr>
<tr>
<td></td>
<td>4,316</td>
</tr>
<tr>
<td></td>
<td>3,184</td>
</tr>
<tr>
<td></td>
<td>2,327</td>
</tr>
<tr>
<td></td>
<td>1,629</td>
</tr>
<tr>
<td></td>
<td>1,136</td>
</tr>
<tr>
<td></td>
<td>745</td>
</tr>
<tr>
<td></td>
<td>468</td>
</tr>
<tr>
<td></td>
<td>258</td>
</tr>
<tr>
<td></td>
<td>133</td>
</tr>
<tr>
<td>Heart</td>
<td>24,024</td>
</tr>
<tr>
<td></td>
<td>19,885</td>
</tr>
<tr>
<td></td>
<td>17,238</td>
</tr>
<tr>
<td></td>
<td>14,687</td>
</tr>
<tr>
<td></td>
<td>12,341</td>
</tr>
<tr>
<td></td>
<td>10,022</td>
</tr>
<tr>
<td></td>
<td>7,997</td>
</tr>
<tr>
<td></td>
<td>6,104</td>
</tr>
<tr>
<td></td>
<td>4,526</td>
</tr>
<tr>
<td></td>
<td>3,096</td>
</tr>
<tr>
<td></td>
<td>1,991</td>
</tr>
</tbody>
</table>

Early CNI-reduction in Liver Transplant recipients
Impressive improvement in native renal function

Evolution of Renal Function over time
(On-Treatment population)

Renal endpoints at 24-months
(ITT population)

Saliba F. Presented at the AASLD, 2012; Boston
**BENEFIT: long-term extension study**

Belatacept vs. CNI: Renal Function at 5 Years

Rostaing L et al., Am J Transplant 2013;13: 2875-83
Chronic Renal Allograft Dysfunction
Immunosuppression: Too much or not enough?

Immunologic factors
- Poor HLA matching and previous sensitization
- Delayed graft function
- Episodes of acute rejection
- Subacute and chronic alloimmune response
- Late ABMR
- Noncompliance of patient
- Suboptimal immunosuppression

Disease recurrence
- BK nephropathy

Nonimmunologic factors
- Older donor or poor graft quality
- Brain-death injury, preservation injury, or ischemic injury
- Acute peritransplantational injuries
- Delayed graft function
- Hypertension
- Hyperlipidemia
- Chronic toxic effects of cyclosporine or tacrolimus

Late cellular and/or humoral rejection
- Transplant glomerulitis-/opathy
- C4d deposition in peritubular capillaries

CNI-induced nephrotoxicity
- Hyalinosis of arterioles
- Focal glomerulosclerosis

Anti-HLA Abs detected after transplantation
Inferior long-term renal allograft outcome

Kidney allograft survival according to HLA-Abs status (N=1014)

- No anti-HLA antibodies (n=712)
  - Graft survival: 83%
  - Time after HLA antibody testing, years: 5

- DSA (n=93)
  - Graft survival: 70%
  - Time after HLA antibody testing, years: 5

- NDSA (n=209)
  - Graft survival: 49%
  - Time after HLA antibody testing, years: 5

Antibody-Mediated Rejection
Represents a small but definite component of renal transplant failure

Kidney Transplant Recipients n=1317

- 75% No Graft Loss
- 2% ABMR
- 14.5% Graft Failure incl. PNF
- 10.5% Death with Graft Function

- 4.6% Unknown
- 11.7% Acute rejection
- 16.3% Medical
- 30.7% IF/TA
- 36.6% Glomerular

~15% Recurrent Disease
~15% Transplant Glomerulopathy

Evidence from Histology:
Chronic tissue injury (any 2 of below):
- Arterial intimal fibrosis w/o elastosis
- Duplication of the GBM
- Multi-laminated PTC basement membrane
- IF/TA

Evidence from Tissue staining:
Ab action/deposition (e.g. Cd4 in PTC)

Evidence from Serology:
Anti-HLA or other anti-donor antibody

Mean follow-up: 50.3 ± 32.6 months

C4d-staining for the diagnosis of ABMR
Neither completely specific nor sensitive enough¹,²

- Progression to TGP in DSA-positive patients with micro-vascular inflammation, but w/o C4d deposition³

- High endothelial cell-specific gene expression leading to ABMR in renal transplant biopsy samples with DSA but w/o C4d⁴

- Positive C4d occurs with recurrent glomerular diseases (LN; ICX-GN)⁴

De novo donor-specific HLA-antibodies
Evidence derived from Histology, C4d-staining and Serology

Microvascular inflammation
C4d-positive PTC staining

Luminex-based assays also have limitations:
- What is the association between dnDSA and risk of ABMR?
- What is the DSA-threshold for the diagnosis DSA-positive?
- Role of IgG isotypes and/or the ability to bind complement?

Donor-specific anti-HLA Abs after transplantation
Complement fixing or non-complement fixing antibodies

Consecutive patients, population-based study (N=1016)¹

Allograft survival and DSA-status

Allograft survival DSA+C1q-status

The distribution of graft-injury phenotypes and rates of allograft survival were similar across all classes. Both class I and class II of donor-specific anti-HLA antibodies after transplantation were harmful²

Complement-binding DSAs
Association with Tissue damage/Inflammation

Graft histopathology (N=1016) according to HLA-Dsap status

A Microvascular inflammation

![Bar chart showing mean Banff score for microvascular inflammation across DSA- and DSA+/C1q+ groups.](chart)

B C4d graft deposition

![Bar chart showing percent of patients with C4d graft deposition across DSA- and DSA+/C1q+ groups.](chart)

C Transplant glomerulopathy

![Bar chart showing mean Banff score for transplant glomerulopathy across DSA- and DSA+/C1q+ groups.](chart)

D Interstitial inflammation and tubulitis

![Bar chart showing mean Banff score for interstitial inflammation and tubulitis across DSA- and DSA+/C1q+ groups.](chart)

E Interstitial fibrosis and tubular atrophy

![Bar chart showing mean Banff score for interstitial fibrosis and tubular atrophy across DSA- and DSA+/C1q+ groups.](chart)

F Arteriosclerosis

![Bar chart showing mean Banff score for arteriosclerosis across DSA- and DSA+/C1q+ groups.](chart)

Data based on 1016 kidney-allograft biopsies performed in the first year after transplantation. (845 at 1-year and 171 during acute rejection in the first year).

Antibodies not binding complement
Equally associated with inferior graft survival

Graft survival in RTRs tested for anti-HLA immunoglobulin subclasses (n=274; 2008)

Cumulative survival

Years after transplantation

Complement fixing Antibodies (p<0.001*)
Non-complement fixing Antibodies (p=0.002*)

Antibodies at the last date of testing
* log rank test vs. no-antibodies.

Clinical Immunosuppression
Long-term outcome after kidney transplantation

**Diabetes; Hypertension; Hyperlipidemia**

**Inadequate Renal Allograft Function**
- *Structural vascular changes (Hyalinosis; Capillaritis; Glomerulitis)*
- *Irreversible renal changes (Fibrosis; Transplant Glomerulopathy)*
1: Reduced exposure to CNIs in RTRs

Lower rejection rates do not translate in better long-term outcome parameters

<table>
<thead>
<tr>
<th>Regimen tested</th>
<th>n</th>
<th>eGFR (C-G) mL/min</th>
<th>BPAR %</th>
<th>1-Yr GS %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard-dose CsA (150–300 ng/mL first 3 months &amp; 100–200 ng/mL thereafter)</td>
<td>390</td>
<td>57.1 ± 25.1</td>
<td>30.1</td>
<td>91.9</td>
</tr>
<tr>
<td>Low-dose CsA (50–100 ng/mL)</td>
<td>399</td>
<td>59.4 ± 25.1</td>
<td>27.2</td>
<td>94.3</td>
</tr>
<tr>
<td>Low-dose TAC (3–7 ng/mL)</td>
<td>401</td>
<td>65.4 ± 27.0</td>
<td>15.4</td>
<td>96.4</td>
</tr>
<tr>
<td>Low-dose sirolimus</td>
<td>399</td>
<td>56.7 ± 26.9</td>
<td>40.2</td>
<td>91.7</td>
</tr>
</tbody>
</table>


![Graph showing comparable eGFR results at 3-years](image)
Low acute rejection rate vs. excess BKV replication
Common in RTRs and associated with graft loss

Retrospective analysis of 34,937 RTRs (SRTR: 2004–2006)
Significant difference in overall graft survival at 3 years

After the first year, a history of acute rejection had no effect on outcome.

Empiric dose reduction/drug withdrawal
CNI- and/or CS-elimination with mTORis

**ZEUS trial**
CNI-elimination with EVR at 4.5 months
*Intention-to-treat population*

**CONCEPT trial**
CNI-elimination with SRL at 3-months
*Intention-to-treat population*

Adapted from Budde K et al. Lancet 2011;377:837-47

3: Risk for *de novo* DSA and ABMR

1. Incompatibility for HLA class-II antigens

Median 10-year graft survival for the 15% of patients with *de novo* DSA was 40% lower than those w/o DSA (59% vs 96%, *P*<0.0001)

Independent predictors for *de novo* DSA formation:

1) **HLA-DR mismatch**
2) Non-adherence (pediatric recipients)
3) History of rejection or protocol biopsy with SCR between month 0-6

Most dnDSA are HLA-DQ specific

Linkage disequilibrium between HLA-DR and -DQ

**AMR-free survival by DR & DQ matching**

**DQ & DR mismatch and corresponding DSA**

<table>
<thead>
<tr>
<th>Mismatch (n)</th>
<th>Patients (n, %)</th>
<th>Corresponding DSAs (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-DR</td>
<td>146 (28.9)</td>
<td>0</td>
</tr>
<tr>
<td>DR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-DR</td>
<td>263 (52.1)</td>
<td>20 (7.6)</td>
</tr>
<tr>
<td>2-DR</td>
<td>96 (19.0)</td>
<td>9 (9.4)</td>
</tr>
<tr>
<td>DQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-DQ</td>
<td>184 (36.4)</td>
<td>0</td>
</tr>
<tr>
<td>1-DQ</td>
<td>250 (49.5)</td>
<td>35 (14.0)</td>
</tr>
<tr>
<td>2-DQ</td>
<td>71 (14.1)</td>
<td>15 (21.1)</td>
</tr>
<tr>
<td>DR ≥1</td>
<td>DQ 0</td>
<td>108 (21.4)</td>
</tr>
<tr>
<td>DR ≥1</td>
<td>DQ ≥1</td>
<td>38 (7.5)</td>
</tr>
<tr>
<td>DR ≥1</td>
<td>DQ ≥1</td>
<td>284 (56.2)</td>
</tr>
</tbody>
</table>

Risk for Antibody-mediated Rejection
2. Non-adherence

For-cause biopsies (n=315) between 2004-2008 with documented failure (n=56) in 3 N-A Centers

- Polyoma virus nephropathy 7%
- Medical/Surgical conditions 11%
- Glomerulonephritis 18%
- Probably ABMR 9%
- Mixed rejection 5%
- ABMR 50%
- 64% ABMR, probably ABMR or Mixed rejection
- Non-adherent 47%
- Adherent 53%

**DSA occur with all types of immunosuppressive regimen**

3. Inadequate Overall Immunosuppression

<table>
<thead>
<tr>
<th>Immunosuppression</th>
<th>N</th>
<th>DSA posttransplantation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CsA 43% (n=129)<em>; Tac 35% (n=106)</em> w/AZA 16% (n=50)<em>; w/MMF 34% (n=102)</em></td>
<td>1014</td>
<td>29.8% (n=302): de novo HLA antibodies 9.2% (n= 93): de novo DSA</td>
<td>Lachmann N, et al. <em>Transplantation</em> 2009; 87:1505–13</td>
</tr>
<tr>
<td>CNI (CsA or Tac) Prednisone MMF</td>
<td>203</td>
<td>26.6% (n=54) de novo DSA - 16.6% (n=34) *de novo DQ-DSA - 9.8% (n=20) *de novo DQ-DSA + other DSA</td>
<td>Freitas MA, et al. <em>Transplantation</em> 2013;95:1113–19;</td>
</tr>
<tr>
<td>MMF (2 g/d); Prednisone Tac (8–10 ng/mL: first 3 mo; 6–8 thereafter CsA (C2: 800–1200 ng/mL first 3 mo; 600–800 thereafter)</td>
<td>1016</td>
<td>31.1% (n=316) de novo DSA at 4.8 yrs. (0.2-7) 7.6% (n= 77) C1q+</td>
<td>Loupy A, et al. <em>N Engl J Med.</em> 2013;369:1215–26;</td>
</tr>
<tr>
<td>CsA (n=129); Tac (n=57)</td>
<td>189</td>
<td>25% (n=47) *de novo DSA within 10 years</td>
<td>Everly MJ, et al. <em>Transplantation.</em> 2013;95:410–17</td>
</tr>
<tr>
<td>MMF/MPA + CsA + Pred MMF/MPA + Tac + Pred Aza + CsA + Pred CsA/Tac + Sir + Pred</td>
<td>78</td>
<td>16% de novo DSA within first year 19% de novo DSA within first year 5% de novo DSA within first year 3% de novo DSA within first year</td>
<td>Banasik M, et al. <em>Transplant Proc.</em> 2013;45:1449–52;</td>
</tr>
<tr>
<td>Tac+MMF+glucocorticoids: (n=48) CsA+MMF+glucocorticoids: (n=4) EVR+CsA+glucocorticoids: (n=3)</td>
<td>55</td>
<td>10% de novo DSA at 1 year 28% *de novo DSA at 3 years</td>
<td>Libri I, et al. <em>Am J Transplant.</em> 2013;13:3215–3222</td>
</tr>
</tbody>
</table>

*Data reflects proportion and numbers of patients with HLA antibodies post-transplantation.*
Inadequate control over immune reactivity
CNi-elimination (mo 4.5) followed by Steroid-withdrawal in 60%

Retrospective single-center analysis, pooled data from participation in two (comparable) trials

Incidence of DSA
(Luminex)

Incidence of late ABMR
(For-cause biopsy)

Independent risk factors:

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Everolimus (=CNi/CS stop) arm</td>
<td>5.35 (1.11-25.70)</td>
</tr>
<tr>
<td>Living donor</td>
<td>5.78 (1.44-23.16)</td>
</tr>
<tr>
<td>HLA-mismatch ≥4</td>
<td>5.10 (1.39-18.72)</td>
</tr>
<tr>
<td>Treated AR first year</td>
<td>10.22 (2.56-40.87)</td>
</tr>
</tbody>
</table>

# 4: Transplant Glomerulopathy

Antibodies against non-HLA antigens

<table>
<thead>
<tr>
<th>Variable</th>
<th>No TG</th>
<th>TG</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>527</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Recipient age (years)</td>
<td>51 ± 14</td>
<td>46 ± 17</td>
<td>0.02</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>34.9</td>
<td>30.9</td>
<td>NS</td>
</tr>
<tr>
<td>Dialysis pretransplant (%)</td>
<td>55</td>
<td>62</td>
<td>NS</td>
</tr>
<tr>
<td>PRA peak (%)</td>
<td>2.4 ± 12.7</td>
<td>6.5 ± 19.6</td>
<td>0.004</td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>42 ± 13</td>
<td>40 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>Donor type (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Living related</td>
<td>50</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>Living unrelated</td>
<td>27</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Deceased</td>
<td>23</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>First transplant (%)</td>
<td>68</td>
<td>52</td>
<td>0.03</td>
</tr>
<tr>
<td>Hepatitis C antibodies (%)</td>
<td>1.7</td>
<td>7.3</td>
<td>0.007</td>
</tr>
<tr>
<td>Acute rejection (AR)</td>
<td>75 (14.2%)</td>
<td>18 (32.7%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AR type (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borderline</td>
<td>9.9</td>
<td>16.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cell mediated</td>
<td>3.8</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>Antibody mediated</td>
<td>0.6</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>Follow-up months</td>
<td>41 ± 16.5</td>
<td>43 ± 15.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

Gloor et al., Am J Transpl, 2007;7:2124
HLA-identical Kidney Transplant Recipients (siblings)

Impact of pre-transplant %-PRA (HLA & non-HLA antigens)

1-yr Graft survival according to PRA

10-yr Functional graft survival according to PRA

<table>
<thead>
<tr>
<th>Number of transplants</th>
<th>No PRA</th>
<th>1-50% PRA</th>
<th>&gt;50% PRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-yr Graft survival</td>
<td>3001</td>
<td>803</td>
<td>244</td>
</tr>
<tr>
<td>10-yr Graft survival</td>
<td>2914</td>
<td>2864</td>
<td>235</td>
</tr>
<tr>
<td>Functional graft</td>
<td>2774</td>
<td>2765</td>
<td>229</td>
</tr>
<tr>
<td>survival (%)</td>
<td>223</td>
<td>223</td>
<td>215</td>
</tr>
</tbody>
</table>

Opelz et al., Lancet 2005, 365:1570-6

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Preformed PRA</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None (n=3001)</td>
<td>1014 (34%)</td>
<td></td>
</tr>
<tr>
<td>1-50% PRA (n=803)</td>
<td>361 (45%)</td>
<td></td>
</tr>
<tr>
<td>&gt;50% PRA (n=244)</td>
<td>154 (63%)</td>
<td></td>
</tr>
<tr>
<td>Proportion with retransplant</td>
<td>159 (5%)</td>
<td></td>
</tr>
<tr>
<td>Mean (SE) pretransplant</td>
<td>3.47 (0.15)</td>
<td></td>
</tr>
<tr>
<td>blood transfusions</td>
<td>112 (14%)</td>
<td></td>
</tr>
<tr>
<td>Mean (SE) pretransplant</td>
<td>6.01 (0.43)</td>
<td></td>
</tr>
<tr>
<td>blood transfusions</td>
<td>97 (40%)</td>
<td></td>
</tr>
<tr>
<td>Mean (SE) pretransplant</td>
<td>10.7 (1.12)</td>
<td></td>
</tr>
<tr>
<td>blood transfusions</td>
<td>84 (65%)</td>
<td></td>
</tr>
</tbody>
</table>

Table: Association of preformed PRA with various characteristics of patients

p<0.0001
Agonistic antibodies against the AT1-R
Refractory vascular rejection in RTRs (n=33)

Angiotensin II Type 1–Receptor Activating Antibodies in Renal-Allograft Rejection

Pretransplant MHC class I–related chain A antibodies
Early kidney graft loss and Tissue expression

Pre-transplant antibodies against MICA non HLA-sensitized RTRs

Sequential kidney biopsies pre-implantation and at day-7 stained with MICB antibody

(A) Very low levels of tubular MICB expression on the renal tubules in the donor kidney biopsy

(B) Up-regulated MICB expression 7 days post-transplant in proximal and distal tubular cytoplasm

First graft/well matched/low-risk/non-sensitized


Chronic Renal Allograft Dysfunction

- Inadequate renal (allograft) function is a robust independent risk factor for excess cardiovascular mortality, especially with concomitant diabetes.

- ABMR is a relative small component of overall graft loss\(^1\), but a definite cause of premature graft failure\(^2\).

- De novo DSA have been documented in RTRs while treated according to all major immunosuppressive maintenance regimen\(^7,8\). Major risk factors include
  - Incompatibility for HLA-class II, and/or
  - Non-adherence, and/or
  - Inadequate overall immunosuppression

- Clinical immunosuppression is further challenged by:
  - Low acute rejection rates vs. excess BKV-replication
  - Prolonged CNi- and CS-use vs. unfavourable CV risk profile (GFR/DM/HT)
  - Inappropriate (empiric) dose reduction(s) and chronic/late (humoral) rejection