Le Rejet Humoral Chronique en 2010: Histoire naturelle et problématiques

“CAMR in 2010: natural history and perspectives”

Alexandre Loupy
1. Introduction

2. CAMR: the missing link

3. Natural history of CAMR

4. New definitions of CAMR in 2010?

5. Conclusions / Perspectives
Introduction

« Homo transplants differ not only in the strength of rejection, but also in the nature and location of the phenomena induced by rejection. This conclusion must not be regarded as a cause for discouragement. On the contrary, it should spur further research ».  

- Jean Hamburger

Cellular rejection

Hyperacute rejection
The changing picture of rejection

Jean Dausset
Nobel price 1980

Paul I. Terasaki
The changing picture of rejection

- 1970 - 1990: $Ac = \text{epiphenomena}$
- Underlying mechanisms of Ab mediated injury = ?
- No connection between Ab and pathology
Publications related to C4d

The changing picture of rejection

Nombre de publications

Pub\textbf{Med}

Halloran

Feucht

AMR Banff

SAMR

AHR Collins

AMR Banff

2009
### CAMR definition in 2007

<table>
<thead>
<tr>
<th>Clinical</th>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNN / Monocytes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Thrombi capillaires</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterite transmurale</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necrose fibrinoide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IHC C4d + CPT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Histological</th>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>IF/TA cg cv ptc lam</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>NTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSA</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

« National Conference to Assess AMR in Solid Organ Transplantation »

Banff 2007
Introduction

CAMR: the missing link?

Natural history of CAMR

New definitions of CAMR in 2010?

Conclusions / Perspectives
CAMR 2010: missing link?

Lefaucheur et al., JASN 2010

Graph showing graft survival over time post transplant (months) with two lines representing No AMR and AMR conditions. The survival rates decrease over time, with the AMR condition showing a more pronounced decrease. The number at risk is given for each time point:

<table>
<thead>
<tr>
<th>Time post transplant (months)</th>
<th>No AMR</th>
<th>AMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>370</td>
<td>32</td>
</tr>
<tr>
<td>12</td>
<td>338</td>
<td>29</td>
</tr>
<tr>
<td>24</td>
<td>323</td>
<td>26</td>
</tr>
<tr>
<td>36</td>
<td>256</td>
<td>24</td>
</tr>
<tr>
<td>48</td>
<td>172</td>
<td>12</td>
</tr>
<tr>
<td>60</td>
<td>121</td>
<td>9</td>
</tr>
<tr>
<td>72</td>
<td>89</td>
<td>7</td>
</tr>
<tr>
<td>84</td>
<td>61</td>
<td>6</td>
</tr>
<tr>
<td>96</td>
<td>41</td>
<td>4</td>
</tr>
</tbody>
</table>

Significant difference is noted with p<0.0001.
CAMR 2010: the missing link

AMR = 0

Graft survival (%)

<table>
<thead>
<tr>
<th>Time post transplant (months)</th>
<th>DSA -</th>
<th>DSA +</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>316</td>
<td>54</td>
</tr>
<tr>
<td>12</td>
<td>290</td>
<td>48</td>
</tr>
<tr>
<td>24</td>
<td>279</td>
<td>45</td>
</tr>
<tr>
<td>36</td>
<td>219</td>
<td>38</td>
</tr>
<tr>
<td>48</td>
<td>146</td>
<td>27</td>
</tr>
<tr>
<td>60</td>
<td>100</td>
<td>22</td>
</tr>
<tr>
<td>72</td>
<td>73</td>
<td>17</td>
</tr>
<tr>
<td>84</td>
<td>48</td>
<td>14</td>
</tr>
<tr>
<td>96</td>
<td>33</td>
<td>10</td>
</tr>
</tbody>
</table>

Number at risk

p = 0.02

Lefaucheur et al., JASN 2010
CAMR 2010: the missing link

Graft Function

Graft Pathology
1 Introduction

2 CAMR: the missing link

3 Natural history of CAMR

4 New definitions of CAMR in 2010?

5 Conclusions / Perspectives
Study population:
KTR with high immunological risk (DSA+)

Induction
CNI: FK506
MMF
Steroids
IVIg 2g/Kg bw over 96h 4 cures
Anti CD 20 (rituximab) 375 mg/m²
Plasmaphérèses
# Natural history of CAMR

## GFR steady state

### Microcirculation inflammation
- glomerulitis / ptc

### Ab deposition
- C4d+

### Circulating Ab
- DSA+

= 31% of patients with preformed DSA+

New Entities

Lerut et al.
Gloor et al., AJT
Haas et al., AJT
Natural history of CAMR

New Entities

\[ \text{g/ptc at 3 months or 1 year} = 92.3\% \]

Persisting microcirculation inflammation

Loupy et al AJT 2009
Natural history of CAMR

Increase of chronic lesions

Loupy et al AJT 2009
Natural history of CAMR

3 months post TX = SAMR

glomerulitis

capillaritis

Edema

Creatinine 82 μmol/L
Natural history of CAMR

1 year post TX

glomerulitis

capillaritis

IF/TA

Creatinine 124 µmol/L
Natural history of CAMR

3 years post TX

glomerulitis

capillaritis

IF/TA

TG

cv

Creatinine
168 µmol/L
What are the determinants of CAMR?

- Graft pathology
- DSA
- C4d

Manuscript in preparation
STUDY DESIGN

Kidney transplantations recipients 2002 - 2009 (n = 900)

KTR with preformed DSA (n = 80)

- Protocol biopsies (n=170)
- C4d staining (0,1,2,3)
- DSA testing (Luminex® SA)
- GFR measurement

Manuscript in preparation
Protocol biopsies
N=170

- C4d Negative
  N=70 (41.2%)

- C4d Minimal
  N=32 (18.8%)

- C4d Focal
  N=33 (19.4%)

- C4d Diffuse
  N=35 (20.6%)

Manuscript in preparation
Morphological and immunological parameters according to C4d status

- g score
- ptc score
- cv score
- cg score

Manuscript in preparation
Morphological and immunological parameters according to C4d status

Class I DSA MFI

Class II DSA MFI

Manuscript in preparation
Risk factors of CAMR

Humorality is a dynamic and multidirectional process

C4d=3

C4d=2

C4d=1

C4d=0

0 Negatif, 1 minimal, 2 focal, 3 diffus

Manuscript in preparation
Risk factors of CAMR

81 DSA+ KTR, 170 protocol biopsies

Manuscript in preparation
Risk factors of CAMR

* p<0.05 compared to negative C4d
## Risk factors of CAMR

### Association between CAMR and subgroups defined by C4d pattern, MI and DSA status at 3 months

<table>
<thead>
<tr>
<th>Parameters at 3 months</th>
<th>n</th>
<th>RR for CAMR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4d Negative</td>
<td>38</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C4d minimal</td>
<td>15</td>
<td>4.7</td>
<td>1.3 - 17</td>
<td>0.02</td>
</tr>
<tr>
<td>C4d focal</td>
<td>11</td>
<td>18.6</td>
<td>3.1 - 112</td>
<td>0.001</td>
</tr>
<tr>
<td>C4d diffuse</td>
<td>13</td>
<td>12</td>
<td>2.8 - 52</td>
<td>0.001</td>
</tr>
<tr>
<td>Microcirculation inflamation = 0</td>
<td>21</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Microcirculation inflamation &gt;0</td>
<td>56</td>
<td>6.2</td>
<td>1.6 - 23</td>
<td>0.007</td>
</tr>
<tr>
<td>No DSA</td>
<td>28</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DSA class I</td>
<td>17</td>
<td>3.2</td>
<td>0.8 - 12</td>
<td>0.09</td>
</tr>
<tr>
<td>DSA class II</td>
<td>30</td>
<td>6</td>
<td>1.8 - 20</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Manuscrit en préparation
## Risk factors of CAMR

### Association between CAMR and subgroups defined by C4d pattern, MI and DSA status at 3 months

<table>
<thead>
<tr>
<th>Parameters at 3 months</th>
<th>n</th>
<th>RR for CAMR (adjusted for C4d)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcirculation inflamation = 0</td>
<td>21</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Microcirculation inflamation &gt;0</td>
<td>56</td>
<td>4</td>
<td>1.01 - 16</td>
<td>0.05</td>
</tr>
<tr>
<td>No DSA</td>
<td>28</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DSA class I</td>
<td>17</td>
<td>2.8</td>
<td>0.7 - 11</td>
<td>0.15</td>
</tr>
<tr>
<td>DSA class II</td>
<td>30</td>
<td>4.11</td>
<td>1.1 - 15</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Manuscript in preparation
Natural history of CAMR: summary

CAMR
- cg
- IF/TA
- Accelerated arteriosclerosis

SAMR
- Microcirculation inflammation
- C4d
- DSA

Graft dysfunction

TX
1 Introduction

2 CAMR: the missing link

3 Natural history of CAMR

4 New definitions of CAMR in 2010?

5 Conclusions / Perspectives
“Truncated” forms of CAMR

Antibody-Mediated Microcirculation Injury Is the Major Cause of Late Kidney Transplant Failure

Endothelial transgenic mice were generated using a transactivation response element (TARE) promoter to drive expression of the Fas ligand (Fasl) and a dominant negative mutant of C4d-negative antibody (Ab)-mediated injury. Banu Sis and Philip J. Shaw

Complement-mediated Endarteritis and Transplant Arteriopathy in Mice

Outcome of Subclinical Antibody-Mediated Rejection in Kidney Transplant Recipients with Preformed Donor-Specific Antibodies

= CAMR may be underdiagnosed
1/ C4d positivity is not the gold marker of CAMR
1/ C4d positivity is not the gold marker of CAMR

- Banff 2007: C4d (neg - minimal - focal - diffuse)
- Necker ChL: 20% of CAMR are C4d NEG
- P Halloran: “ABCD tetrad” : 64% TG = C4d NEG
- B SIS: Microarray / AMR C4d NEG
- RB Colvin: “C4d NEG CAMR” murine model of heart TX”
2/ Arterosclerosis may mimic “banal” aging lesions

3 mois

1 an = cv “banal”
2/ Arterosclerosis may mimic “banal” aging lesions

Normal Aging Kidney

DSA + at 12 months Post-Tx
3/ IF/TA is a non specific feature of CAMR++
Graft dysfunction

CAN ≠ CNI toxicity

Accelerated arteriosclerosis

Microcirculation inflammation

A specific diagnosis is mandatory

TX
4/ CAMR with MI: significance?

>80% of CAMR

Define therapeutic strategies
Treatment of CAMR: what are the options?

IVIg
PP
Anti CD 20
Bortezomib
Anti-C5
Treatment of CAMR: what are the options?

Necker experience = Day-0 prophylactic strategy (n=54)

Loupy et al transplantation 2010
Treatment of CAMR: what are the options?

Patient survival 96% follow-up 3 years (NS)

Graft survival 89% follow-up 3 years (NS)

AMR = 19.6% vs 16.6% (NS)

Loupy et al transplantation 2010
## Treatment of CAMR: what are the options?

### Histological course at 1 year according to treatment

<table>
<thead>
<tr>
<th></th>
<th>IVIg</th>
<th>IVIg/PP/Ritux</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>g</td>
<td>1.1 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>ns</td>
</tr>
<tr>
<td>ptc</td>
<td>1.6 ± 0.2</td>
<td>1 ± 0.2</td>
<td>0.01</td>
</tr>
<tr>
<td>C4d</td>
<td>1.1 ± 0.2</td>
<td>0.8 ± 0.3</td>
<td>ns</td>
</tr>
<tr>
<td>cg</td>
<td>0.4 ± 0.1</td>
<td>0 ± 0</td>
<td>0.02</td>
</tr>
<tr>
<td>IF/TA</td>
<td>1.7 ± 0.2</td>
<td>1.1 ± 0.3</td>
<td>0.09</td>
</tr>
<tr>
<td>cv</td>
<td>1.1 ± 0.2</td>
<td>1 ± 0.3</td>
<td>ns</td>
</tr>
<tr>
<td>SAMR (%)</td>
<td>44%</td>
<td>7%</td>
<td>0.02</td>
</tr>
<tr>
<td>CAMR (%)</td>
<td>43%</td>
<td>13.3%</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Loupy et al transplantation 2010*
Treatment of CAMR: what are the options?

Immunological course at 1 year according to treatment

Loupy et al transplantation 2010
Introduction

CAMR: the missing link

Natural history of CAMR

New definitions of CAMR in 2010?

Conclusions / Perspectives
Clinical relevance of screening biopsies

Function

Pathology

+ C4d
+ DSA

Natural history / stratification of the risk of CAMR
1/ CAMR= emerging and underdiagnosed entity

2/ SAMR progress to CAMR

3/ CAMR criteria are underwearing a dynamic reexamination
   - C4d Banff score 2/3 = tip of the iceberg
   - C4d NEG forms are also relevant
   - The significance of CAMR + MI needs to be evaluated
   - Cv + FIAT are NON specific.

4/ DSA monitoring in the post transplant period is mandatory
   In high risk patients

5/ Banff 2011 +++
Acknowledgments

NCK
- Ch Legendre
- E Thervet
- J Zuber
- F Martinez

HEGP
- D Nochy
- G S Hill
- P Bruneval
- JP Duong

SLS
- C Suberbielle
Risk stratification according to DSA MFImax in peak serum

<table>
<thead>
<tr>
<th>DSA MFImax</th>
<th>RR</th>
<th>P</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;465</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[465 - 1500]</td>
<td>24.8</td>
<td>&lt;0.001</td>
<td>[4.6 - 135]</td>
</tr>
<tr>
<td>[1500 - 3000]</td>
<td>23.9</td>
<td>&lt;0.001</td>
<td>[3.5 - 160]</td>
</tr>
<tr>
<td>[3000 - 6000]</td>
<td>61.3</td>
<td>&lt;0.001</td>
<td>[11.5 - 327]</td>
</tr>
<tr>
<td>&gt;6000</td>
<td>113</td>
<td>&lt;0.001</td>
<td>[30.8 - 414]</td>
</tr>
</tbody>
</table>

Lefaucheur et al., JASN 2010
Risk stratification according to DSA MFImax in peak serum

Cox model = MFI >3000 = RR of graft loss of 3.8

Lefaucheur et al., JASN 2010
**Proposition de stratification du risque de CAMR**

<table>
<thead>
<tr>
<th>Clinical AMR</th>
<th>C4d=3</th>
<th>Ab +++</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAMR</td>
<td>C4d=2</td>
<td>Ab ++  Forme silencieuse AMR</td>
</tr>
<tr>
<td></td>
<td>C4d=1</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions / Perspectives**
Perspectives

- Incorporation des “OMICS” dans les outils diagnostiques

- Mécanismes endothéliaux

- Mécanismes de l’accommodation

- Rôle du complément

- Nouvelles voies thérapeutiques (Bortezomib, anti C5)

- Associations de risque / corrélations / pronostic

- Monitoring DSA / seuils / TTT prophylactiques
C4d focal

C4d minimal et focal: Banff 2007

Score 1 (10-25%)

Score 2 (25-50%)

Score 3 (>50%)
Anti-HLA ab screening (LUMINEX®)

- Purified HLA molecules bound to microspheres
- Each microsphere characterized by a specific fluorescence

30 min.
C4d staining

Normal Progression of Arteriosclerosis with Age

Grade arteriosclerosis = -.3406 + .02030 * Age Don

Correlation: \( r = .38402, p = .00016 \)

Donor Age

Arteriosclerosis score at Day-0
Acceleration of Arteriosclerosis Post-Transplant

Progression of Arteriosclerosis in Normal Kidneys (Estimated from Day 0 Biopsies)

- **DSA+**
  - $p = .014$
  - Prog - 28.8 yrs/yr

- **DSA-**
  - $p = NS$
  - Prog = 7.9 yrs/yr
Increment in Grade of arteriosclerosis between 3 and 12 months according to morphologic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DSA +</th>
<th></th>
<th>DSA -</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>cv 3mo-1 yr</td>
<td>p</td>
<td>Number</td>
<td>cv 3mo-1 yr</td>
</tr>
<tr>
<td>g &gt;0</td>
<td>31</td>
<td>+ 0.45</td>
<td>0.04</td>
<td>11</td>
</tr>
<tr>
<td>cg &gt;0</td>
<td>11</td>
<td>+ 0.59</td>
<td>0.1</td>
<td>5</td>
</tr>
<tr>
<td>PTC &gt;0</td>
<td>20</td>
<td>+ 0.66</td>
<td>0.003</td>
<td>0</td>
</tr>
<tr>
<td>C4d &gt;0</td>
<td>23</td>
<td>+ 0.63</td>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td>SAMR +</td>
<td>23</td>
<td>+ 0.67</td>
<td>0.006</td>
<td>0</td>
</tr>
<tr>
<td>IF/TA &gt;0</td>
<td>30</td>
<td>+ 0.64</td>
<td>0.008</td>
<td>34</td>
</tr>
<tr>
<td>i &gt;0</td>
<td>29</td>
<td>+ 0.64</td>
<td>0.008</td>
<td>19</td>
</tr>
</tbody>
</table>
DETERMINANTS OF ACCELERATION

Donor age
Recipient age and comorbidities
Hypertension
CNI toxicity

= No significant association with acceleration