Pathology of Kidney Allograft Dysfunction

B. Ivanyi, MD
Department of Pathology,
University of Szeged, Szeged, Hungary
The renal biopsy is a powerful tool in the diagnostic evaluation of allograft dysfunction
Evaluation procedure

**Standard**

- At least two cores whenever possible
- Light microscopic stainings on serial sections (H&E, PAS, trichrome, methenamine silver)
- Immunostaining for complement 4d - *indicator of antibody-mediated rejection*
• **LM**: elastin staining - *chronic rejection-induced intimal fibrosis*

• **IF**: IgG, IgA, IgM, C3, C1q - **GN**
  tubular HLA-DR expression - *acute T-cell-mediated rejection*

• **EM** - *chronic rejection, GN*

• Implantation biopsy for comparison
Objective

To review

• the pathology of rejection, CNI toxicity, polyomavirus nephropathy, and post-transplantation glomerulonephritis

• factors leading to end-stage kidney allograft disease

Solez et al. Banff 07 Classification of Renal Allograft Pathology. AJT 8:753, 2008
Inflammatory response initiated by alloantigen recognition

**Acute**
- T-cell mediated
- Alloantibody mediated

**Chronic**
- Alloantibody mediated
- T-cell mediated
Inflammatory response against HLA class II antigens expressed on

- peritubular and glomerular capillary endothelial cells
- tubular epithelial cells
- interstitial dendritic cells
- mesangial cells
Acute T-cell-mediated rejection

Inflammatory response against HLA class II antigens expressed on:
- peritubular and glomerular capillary endothelial cells
- tubular epithelial cells
- interstitial dendritic cells
- mesangial cells

<table>
<thead>
<tr>
<th>Effector mechanisms</th>
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<tr>
<td>CD8+ CTL-mediated cytotoxicity</td>
<td>+++</td>
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<tr>
<td>Delayed type hypersensitivity</td>
<td>+</td>
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<tr>
<td>Antibody-dependent cytotoxicity</td>
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</table>
Acute T-cell-mediated rejection

- Peritubular capillaries
- Interstitium
- Tubules
  - Tubulointerstitial rejection

± Arteries
  - Vascular rejection

± Glomeruli
  - Transplant glomerulitis
Tubulointerstitial rejection

**Peritubular capillaritis**: accumulation of lymphocytes + monocytes
Peritubular capillaritis on EM
Hypertrophy of endothelial cells, accumulation and emigration of ly-s into the interstitium; CTL-mediated lysis
Tubulointerstitial rejection
Interstitial infiltrates rich in CTLs, oedema, tubular HLA-DR expression, and tubulitis
**Tubulitis:** tubular expression of chemokines induce CTLs to invade the tubules; CTLs injure tubular epithelial cells
Subsiding tubulointerstitial rejection:
nodular infiltrates around newly formed lymphatic vessels;
Tregs, CD4+, CD8+, CD68+; scanty tubulitis; oedema Ø
Differential diagnosis of tubulointerstitial rejection

- **Acute pyelonephritis**  
  Infiltrates rich in neutrophils, neutrophilic tubulitis
Differential diagnosis of tubulointerstitial rejection

- **Acute pyelonephritis**
  - neutrophilic tubulitis

- **Polyomavirus nephropathy**
  - nuclear inclusion bodies

- **Drug-induced TIN**
  - faint tubular HLA-DR staining

- **Post-transplant lymphoproliferative disease**
  - predominance of B-lymphocytes
**Vascular rejection**

- Infiltration of the intima by lymphocytes + monocytes
- Involves the large arteries more frequently than the interlobular arteries
Differential diagnosis

Intimal arteritis is pathognomonic for acute T-cell-mediated rejection
**Transplant glomerulitis**

Infiltration of the capillary loops by lymphocytes ± monocytes

**Isolated transplant glomerulitis:**
in 10% of cases with acute T-cell-mediated rejection
<table>
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<tr>
<th>Differential diagnosis</th>
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<tbody>
<tr>
<td><strong>Recurrent or <em>de novo</em> proliferative GN (rejection: no glomerular immune deposits)</strong></td>
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</table>
Clinical correlation of acute T-cell-mediated rejection

- Most common cause of a graft dysfunction in the first 3 months after Tx
- Sudden asymptomatic rise in the serum creatinine level
Tubulointerstitial rejection responds well to high-dose iv. steroid therapy.

Vascular rejection can be reversed with anti-lymphocyte antibody preparations.

If not: fibrous obliteration of arteries $\Rightarrow$ graft loss.
Acute alloantibody-mediated rejection

Donor-specific HLA class I antibodies

Complement-mediated cytotoxic injury to the endothelial cells
Donor-specific HLA class I antibodies

- Complement 4d degradation product binds to PTC endothelial cells
- This stable molecule is detected by IF

Complement-mediated cytotoxic injury to the endothelial cells
Evaluation of C4d

Immunofluorescence on frozen sections is more sensitive than immunohistochemistry on paraffin sections.
Diagnostic criteria of acute alloantibody-mediated rejection: C4d along PTCs; evidence of tissue injury; demonstration of donor-specific antibodies

Evidence of tissue injury
1) ATN-like, minimal inflammation.
2) Capillary-NG margination and/or thromboses
3) Transmural arteritis and/or arterial fibrinoid necrosis

Cytotoxic injury to PTC endothelial cells manifests in lysis + apoptosis

Liptak et al. AJT 5:2870, 2005
Differential diagnosis

- TMA secondary to CNI toxicity
- Anti-cardiolipin sy
- Recurrent HUS
- Viral infection due to CMV or parvovirus B19
Differential diagnosis

- TMA secondary to CNI toxicity
- Anti-cardiolipin sy
- Recurrent HUS
- Viral infection due to CMV or parvovirus B19

Clue
None of these displays C4d positivity along the peritubular capillaries
Clinical correlation

- Infrequent (2-8%)
- Most common in the first few weeks after Tx; oligoanuria develops within days
- Therapeutic efforts may reverse the rejection process
- Poor prognosis
Chronic rejection

Ongoing, smouldering alloimmune damage to the allograft, mediated predominantly by alloantibodies

Vascular lesions are characteristic
- Transplant arteriopathy
- Transplant glomerulopathy
- Transplant capillaropathy
Vascular lesions of chronic rejection are associated with interstitial fibrosis and tubular atrophy (IF/TA).
Tx arteriopathy: new-onset intimal fibrosis

Absence of elastosis
**Tx arteriopathy** is more pronounced in the arcuate and larger arteries than in the small arteries of the superficial cortex.
Consequence

Chronic progressive ischemic injury to the graft parenchyma
Tx glomerulopathy: double-contoured loops; EM: newly formed BM layer(s)
Consequences

Proteinuria

Focal glomerular obsolescence
Tx glomerulopathy may resemble to recurrent or de novo MPGN; no immunocomplexes on IF
**Tx capillaropathy:** circumferentially laminated BM; pathognomonic: 5 or more BM layers; C4d+ along PTCs
**Tx capillaropathy on light microscopy:** thickened and laminated BMs; EM is more sensitive in the verification of TxC
Consequence

A progressive decrease in the number of PTCs
Diagnostic triad of chronic antibody-mediated rejection

1) TxA, TxG, TxC either alone or in combination

2) Diffuse C4d deposition in PTCs

3) Presence of donor-specific antibody

Solez et al. AJT 7:1-9, 2006
Chronic active T-cell-mediated rejection

Lymphocytes in arterial intimal fibrosis
Clinical correlation of chronic rejection

- Major cause of graft loss after 1 year
- Insidious, progressive decline in the GFR, frequently accompanied by proteinuria (often in the nephrotic range) and hypertension
Cyclosporin and tacrolimus can cause acute or chronic nephrotoxicity; the lesions are identical.

**Acute toxicity**
- Toxic tubulopathy
- Vascular toxicity
  - Acute arteriolopathy
  - TMA

**Chronic toxicity**
- Hyaline arteriolopathy

Toxic tubulopathy
Isometric vacuolization of the proximal straight tubules
The features of this tubulopathy cannot be distinguished from those of

radiocontrast nephrotoxicity or

osmotic nephrosis,

conditions to be considered while making the diagnosis
Acute arteriolopathy: SMC injury; replacement of damaged myocytes by rounded plasma protein insudates
Clinical correlation

- Acute dysfunction
- The serum drug level is usually elevated
- Toxic tubulopathy is reversible
- Acute arteriolopathy may be irreversible
TMA: thrombi in glomerular capillary loops
Differential diagnosis

• CNI-induced TMA cannot be differentiated from other forms of TMA by morphology alone

• Pronounced arterial changes are not typical of CNI-induced TMA

• Acute humoral rejection (C4d+)
• Recurrent HUS
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<th>Clinical correlation</th>
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<tr>
<td>• Rare</td>
</tr>
<tr>
<td>• Resembles the HUS</td>
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<tr>
<td>• If the lesions are associated with extensive thrombosis, graft loss develops</td>
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</table>
Chronic toxicity: hyaline arteriolopathy

- The damaged SMCs are replaced by beaded hyaline deposits that bulge into the adventitia
- The insudates are positive for IgM and C3
Hyaline material (H) is present at sites where media SMCs have dropped out previously. E - swollen endothelial cells, A - apoptotic SMCs.
Hyaline arteriolopathy is associated with striped IF/TA
Differential diagnosis

Hyalinosis in ageing, hypertension and diabetes:

- Mainly subendothelial and rarely extends into the adventitia
- Necrosis of the SMCs is not observed
Clinical correlation

- Chronic toxicity occurs several months after Tx; the incidence increases with time
- A slow, insidious rise in the serum creatinine level
- The kidney damage is irreversible
Ageing/hypertension: intimal fibroelastosis; subendothelial arteriolar hyalinosis; patchy segmental/global glomerulosclerosis; IF/TA
Polyomavirus nephropathy

- The BK polyomavirus exhibits tropism for the renal tubular epithelium, where it establishes latent infection.
- Vigorous immunosuppression can lead to reactivation of the infection and the development of PVN.
- The definitive diagnosis requires an allograft Bx.

*Drachenberg et al. Hum Pathol 36:1245-1255, 2005*
Viral replication results in cytopathic changes (nuclear enlargement, inclusion bodies, tubular cell injury)
Massive replication leads to cytolysis of tubular epithelial cells and interstitial inflammation
Immunostaining with SV40 large T-cell antigen confirms the diagnosis
EM is also diagnostic: the virions are 40 nm in diameter, arranged in a paracrystalloid structure.
Other viral infections (adenovirus: 70-90 nm; CMV: 100-110 nm)

Acute rejection (lack of nuclear inclusion bodies, intense tubular HLA-DR expression)

Chronic rejection (lack of nuclear inclusion bodies, negative confirmatory tests)

<table>
<thead>
<tr>
<th>Pattern A</th>
<th>Early stage</th>
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| Viral cytopathic changes with no or only minimal inflammation | • No dysfunction  
• Urinary decoy cells  
• Favorable prognosis |

*Hirsch et al. Transplantation 79:1277-1286, 2005*
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<tr>
<th><strong>Pattern A</strong></th>
<th><strong>Early stage</strong></th>
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<tr>
<td>Viral cytopathic changes with no or only minimal inflammation</td>
<td>No dysfunction</td>
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<td>Favorable prognosis</td>
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<thead>
<tr>
<th><strong>Pattern B</strong></th>
<th><strong>Fully developed stage</strong></th>
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<tbody>
<tr>
<td>Cytopathic and cytolytic changes with interstitial inflammation</td>
<td>A gradually decreasing renal function</td>
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<td></td>
<td>Graft loss can exceed 50%</td>
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</table>

*Hirsch et al. Transplantation 79:1277-1286, 2005*
Pattern A
Viral cytopathic changes with no or only minimal inflammation

Pattern B
Cytopathic and cytolytic changes with IS inflammation

Pattern C
IF/TA
Variable cytopathic and inflammatory changes

Early stage
• No dysfunction
• Urinary decoy cells
• Favorable prognosis

Fully developed stage
• A gradually decreasing renal function
• Graft loss can exceed 50%

Late/sclerosing stage
• A severe dysfunction
• Graft loss is likely

Hirsch et al. Transplantation 79:1277-1286, 2005
## Summary of acute lesions

<table>
<thead>
<tr>
<th>T-cell-mediated rejection</th>
<th>CNI toxicity</th>
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<tbody>
<tr>
<td>• Peritubular capillaritis</td>
<td>• Isometric vacuolation of tubules</td>
</tr>
<tr>
<td>• IS infiltrates rich in CTLs</td>
<td>• Early-stage hyalinization of individual myocytes in afferent arterioles</td>
</tr>
<tr>
<td>• Lymphocytic tubulitis</td>
<td></td>
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<tr>
<td>• + Intimal arteritis</td>
<td></td>
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<tr>
<td>• + Glomerulitis</td>
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### Alloantibody-mediated rejection
- C4d+ along PTCs
- Evidence of tissue injury:
  - arterial fibrinoid necrosis
  - microthrombi
  - neutrophilic capillaritis
  - ischemic tubular damage
### Summary of chronic lesions

<table>
<thead>
<tr>
<th>Rejection</th>
<th>CNI toxicity</th>
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<tr>
<td>• Arterial intimal fibrosis</td>
<td>• Hyaline arteriolopathy</td>
</tr>
<tr>
<td>• Double-contoured glomerular</td>
<td>• Patchy glomerular sclerosis</td>
</tr>
<tr>
<td>capillary loops</td>
<td>• Striped IF/TA</td>
</tr>
<tr>
<td>• PTC BM lamination</td>
<td></td>
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<tr>
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<td></td>
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<td>• C4d+ along PTCs</td>
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<th>Ageing/hypertension</th>
<th>Polyomavirus NP</th>
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<tr>
<td>• Intimal fibroelastosis in</td>
<td>• Cytopathic effects</td>
</tr>
<tr>
<td>arteries</td>
<td>(inclusion bodies and</td>
</tr>
<tr>
<td>• Nonspecific arteriolar</td>
<td>tubular epithelial injury)</td>
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<tr>
<td>hyalinosis</td>
<td>• A varying degree of IS</td>
</tr>
<tr>
<td>• Patchy glomerular sclerosis</td>
<td>inflammation, IF/TA</td>
</tr>
<tr>
<td>• IF/TA</td>
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</table>

- **Rejection**
  - Arterial intimal fibrosis
  - Double-contoured glomerular capillary loops
  - PTC BM lamination
  - IF/TA
  - C4d+ along PTCs

- **CNI toxicity**
  - Hyaline arteriolopathy
  - Patchy glomerular sclerosis
  - Striped IF/TA

- **Ageing/hypertension**
  - Intimal fibroelastosis in arteries
  - Nonspecific arteriolar hyalinosis
  - Patchy glomerular sclerosis
  - IF/TA

- **Polyomavirus NP**
  - Cytopathic effects (inclusion bodies and tubular epithelial injury)
  - A varying degree of IS inflammation, IF/TA
Post-transplantation glomerulonephritis

- Recurrences of GN tend to occur in the first few weeks after Tx
- *De novo* GN usually manifests at least a year after Tx
Post-transplantation glomerulonephritis

- FSGS, MPGN, IgAN and idiopathic diarrhea-negative HUS recur frequently
- Membranous nephropathy, FSGS, anti-GBM nephritis in Alport patients, and drug-induced TMA are the most common de novo diseases
- Recurrent or de novo GN often coexists with acute and/or chronic rejection and/or chronic CNI-toxicity, and contribute together to allograft loss

Recurrent FSGS
Proteinuria developed some days after the implantation.
1st Bx, EM: effacement of foot processes.
2. Bx (3 mo later). The FSGS lesion in two glomeruli
Coexistence of transplant GP and membranous NP
Bx performed because of nephrotic sy that developed 26 mo after Tx.
Kidney allograft from a deceased donor

~10 years later: end-stage allograft disease
Kidney allograft from a deceased donor

Early insults

Late insults

Additional insults

The insults cumulate: end-stage allograft disease

Nankivell BJ, Chapman JR. Transplantation 2006;81:643-654
Early insults
- Ischemia reperfusion injury
- Acute tubular necrosis
- Acute/subclinical rejection
- Acute CNI toxicity

Late insults

Additional insults

End-stage allograft disease
Early insults
- Ischemia reperfusion injury
- Acute tubular necrosis
- Acute/subclinical rejection
- Acute CNI toxicity

Late insults
- Chronic rejection
- Chronic CNI toxicity
- Polyomavirus NP
- Hypertension
- Recurrent/de novo glomerular disease

Additional insults

End-stage allograft disease
Early insults
- Ischemia reperfusion injury
- Acute tubular necrosis
- Acute/subclinical rejection
- Acute CNI toxicity

Late insults
- Chronic rejection
- Chronic CNI toxicity
- Polyomavirus NP
- Hypertension
- Recurrent/de novo glomerular disease

Additional insults
- Architectural degradation
- Cortical ischemia
- Persistent chronic inflammation
- Accelerated ageing
- Cytokine excess
- Epithelial-mesenchymal transition and fibrosis

End-stage allograft disease
Renal allograft removed for end-stage kidney allograft failure
The kidney is shrunken, the cortex is no longer separated from the medulla, and the large arteries display severe atherosclerosis
Conclusions

The application of

- light microscopic stainings on serial sections
- the full immunofluorescence panel ($C_4d$, HLA-DR, IgG, IgA, IgM, C3)
- tissue sampling for optional EM
- comparison with a time-zero biopsy

enables the pathologist to achieve etiologic diagnoses