Learning Objectives:
1. Describe the pathophysiology of renal transplant rejection
2. Define subclinical rejection and the role of the protocol biopsy in renal transplantation
3. Review current literature regarding the utility of treatment of subclinical rejection in renal transplant recipients
4. Develop an evidence-based recommendation regarding the treatment of subclinical rejection in renal transplant recipients
A. Renal transplantation\textsuperscript{1}
   a. Treatment of choice for end stage renal disease
   b. Substantial benefit in comparison to long-term dialysis
      i. Mortality
      ii. Quality of life
      iii. Cost

B. Goals of immunosuppression\textsuperscript{1,2}
   a. Prevent acute rejection of allograft
   b. Prevent early graft loss

C. 1-year graft rejection has decreased and graft survival has increased steadily since the 1960s, correlating with development and release of new immunosuppressive agents\textsuperscript{2-6}

\textbf{Figure 1. 1-year graft survival and rejection rates, 1960-2013}\textsuperscript{3}

D. Despite advances in immunosuppression and an impressive decrease in rejection rates within the first year post-transplant, long-term renal allograft survival has not significantly benefited\textsuperscript{3}
   a. Graft failure still remains roughly 4\% per year
   b. Chronic rejection is the second leading cause of graft failure
   c. Acute rejection is an infrequent cause of graft failure
   d. Epidemiologic studies have found a strong correlation between acute rejection episodes and development of chronic allograft damage and allograft failure
e. Number, severity, and type of acute rejection episodes are strongly correlated with early graft failure

**Figure 2. Long-term Renal Transplant Graft Survival**

RENAL ALLOGRAFT REJECTION\(^{1-2, 7-9}\)

A. Graft rejection may be cellular and/or humoral\(^{1-2, 7-9}\)
   a. Although both pathways are intertwined, damage to the graft is dependent on cell pathway orchestrating the immune response

B. Cellular graft rejection – “three signal model” of alloimmune responses\(^{1,2,9}\)
   a. Antigen-presenting cells (APC) identify non-self in graft and process graft antigen for presentation
      i. Engage alloantigen-reactive T-cells and memory T-cells
   b. Signal 1
      i. Antigen processed and presented by APC triggers T-cell activation through T-cell receptor
   c. Signal 2
      i. Also known as “co-stimulation”
      ii. Combination of signal 1 and 2 triggers signal transduction pathways that lead to production of important inflammatory molecules
         1. Interleukin-2
         2. Tumor necrosis factor alpha
      iii. These molecules play a role in activation of signal 3
   d. Signal 3
      i. Trigger for T-cell proliferation
      ii. Leads to
         1. Nucleotide synthesis
         2. Differentiation of T-cells into various effector T-cells
   e. Effector T-cells and graft damage
      i. Effector T-cells emerge from lymphoid organs and orchestrate inflammatory response
ii. Infiltrate graft

iii. Activates several cell lines, including macrophages, B cells, plasma cells, and chemokines

iv. Typical graft damage involves
   1. Infiltration of the kidney tubules by mononuclear cells and intima of small arteries
   2. Direct cell lysis from cytotoxic T-cells and activated effector cells

C. Humoral graft rejection\(^\text{1,7-9}\)
   a. Also commonly known as antibody-mediated rejection (AMR)
   b. Antigen presented to B cells, which differentiate and mature into active antibody-producing plasma cells
   c. Antibodies produced by plasma cells mark cells for destruction
      i. Activates complement cascade
         1. Binding of C1q to antigen-antibody complex on graft endothelium triggers activation of complement cascade
         2. End-product is membrane attack complex (MAC) which attacks and destroys integrity of phospholipid bilayer of cells leading to cell death
      ii. Forms immune complexes which target cell for destruction via macrophages or natural killer cells
   d. Transplant recipients may develop donor-specific antibodies (DSA) to donor human leukocyte antigen (HLA)

D. Types of rejection in renal transplantation\(^\text{1-2}\)
   a. Hyperacute rejection
      i. Occurs when recipient’s immune system immediately rejects the donor organ
      ii. Typically occurs upon reperfusion of the newly transplanted organ, but can be delayed up to 3 days post-transplant
      iii. Characterized by rapid, widespread vascular thrombosis affecting arteries, arterioles, and glomeruli
      iv. With the development of detailed HLA phenotyping techniques and donor-recipient cross-matching, incidence of hyperacute rejection is rare
   b. Acute rejection
      i. Most commonly refers to acute cellular rejection (ACR)
      ii. Can occur at any time post-transplant
      iii. 2 predominant forms
         1. Cellular rejection (most common)
         2. Humoral rejection
      iv. Cellular rejection
         1. Most common form of acute rejection
         2. T-cell-mediated
         3. Inflammatory infiltrates typically diminish rapidly upon successful treatment, but edema, tubular inflammation, and tubular cell damage may persist
   v. Acute humoral rejection
      1. Mediated by antibody-producing plasma cells
2. Diverse histologic appearance
3. Characterized by diffuse peritubular capillary staining for complement component C4d, although not necessary
   a. Linked to presence of DSAs and humoral rejection

CHRONIC REJECTION\textsuperscript{1, 10-12}

A. Terminology and definition\textsuperscript{1, 10-12}
   a. Previously referred to as chronic allograft nephropathy (CAN)\textsuperscript{1}
   b. Due to presence of both alloimmune and non-alloimmune mechanism of progressive graft injury, the terms “chronic rejection” and “chronic allograft nephropathy” have been largely replaced by “interstitial fibrosis and tubular atrophy” (IFTA)\textsuperscript{1}
      i. Now commonly referred to as CAN/IFTA
   c. Progressive decline in allograft function gradually leading to allograft failure\textsuperscript{10-12}
      i. Multi-factorial
      ii. Involves immunologic as well as non-immunologic factors
         1. Immunologic
            a. Cellular rejection
            b. Humoral rejection
            c. Medication non-adherence
         2. Non-immunologic
            a. Delayed graft function (DGF)
            b. Infection
            c. Hypertension
            d. Post-transplant diabetes mellitus
            e. Calcineurin inhibitor (CNI) toxicity
      iii. Second leading cause of graft loss
d. Characterized by\textsuperscript{1, 11}
   i. Patch interstitial fibrosis with infiltrates of lymphocytes, plasma cells, and mast cells
   ii. Tubular atrophy or tubular dropout
   iii. Arterial wall thickening with intimal fibrosis
   iv. Glomeruli are often abnormal, constituting a lesion known as chronic transplant glomerulopathy
### Table 1. Risk Factors for the Development of CAN/IFTA\textsuperscript{10,12}

<table>
<thead>
<tr>
<th>Donor Derived</th>
<th>Recipient Derived</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deceased donor kidney</td>
<td>Obesity</td>
</tr>
<tr>
<td>Non-heart beating donor kidney</td>
<td>Polyomavirus nephropathy</td>
</tr>
<tr>
<td>Donor age &gt; 60</td>
<td>CNI toxicity</td>
</tr>
<tr>
<td>Female Donor</td>
<td>Recurrent renal disease or de novo glomerulopathy</td>
</tr>
<tr>
<td>Donor with prior cardiac history or vascular disease</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Cold ischemic time</td>
<td>Hyperlipidemia</td>
</tr>
<tr>
<td>DGF</td>
<td>Proteinuria</td>
</tr>
<tr>
<td></td>
<td>Diabetes</td>
</tr>
<tr>
<td></td>
<td>Medication non-compliance</td>
</tr>
<tr>
<td></td>
<td>HLA mismatch</td>
</tr>
<tr>
<td></td>
<td>Recipient pre-sensitization/panel reactive antibody (PRA)</td>
</tr>
<tr>
<td></td>
<td>Presence of donor specific antibody (DSA)</td>
</tr>
<tr>
<td></td>
<td>Acute rejection</td>
</tr>
<tr>
<td></td>
<td>Subclinical rejection</td>
</tr>
</tbody>
</table>

### TREATMENT OF REJECTION\textsuperscript{7-8, 10,13}

A. KDIGO Guideline for the Care of Kidney Transplant Recipients: Treatment for Rejection (2009)\textsuperscript{13}

   a. Cellular Rejection

      i. First line, mild-moderate: High-dose or “pulse-dose” corticosteroids

      ii. Steroid-resistant rejection or severe rejection: Rabbit antithymocyte globulin

   b. Humoral Rejection\textsuperscript{7-8, 13}

      i. Treatment is targeted at removing circulating DSA, B-cells, and antibody-producing plasma cells

      ii. Plasmapheresis and intravenous immune globulin (IVIG) serve as the backbone for antibody-mediated rejection treatment

         1. Rapidly removes circulating DSA

         2. IVIG replaces IgG that is removed during the plasmapheresis process, and is believed to have an effect on decreasing and preventing rebound DSA production

      iii. Targeted antibody therapies can be used in conjunction with plasmapheresis and IVIG, with or without corticosteroids

         1. Rituximab

         2. Bortezomib (not included in 2009 KDIGO Guidelines)

         3. Eculizumab (not included in 2009 KDIGO Guidelines)

   c. Chronic rejection\textsuperscript{10}

      i. No specific treatment modality has been identified

      ii. The best “treatment” for chronic rejection/IFTA is prevention

         1. Prompt diagnosis and treatment of acute rejection episodes
2. Management of post-transplantation hypertension, hyperlipidemia, diabetes, and proteinuria
3. Optimization of immunosuppressive medications, especially CNIs
4. Maintenance of medication adherence

THE PROTOCOL BIOPSY\textsuperscript{14-25}

A. Definition\textsuperscript{14-23}
   a. Biopsy performed at pre-specified post-transplant milestones regardless of allograft function
   b. Surveillance in nature
   c. Performed in less than 20\% of transplant centers in the U.S.\textsuperscript{26}

B. Serum creatinine: an old friend that’s not keeping up\textsuperscript{24-25}
   a. Serum creatinine and proteinuria lack sensitivity as measurement for kidney dysfunction\textsuperscript{24}
      i. Although widely used due to its ease of measurement and relatively low cost, using serum creatinine as measure of glomerular filtration rate (GFR) has several disadvantages
      ii. Serum creatinine has wide inter-patient variability with regard to
          - Sex
          - Age
          - Race
          - Muscle mass and body weight
      iii. Change in serum creatinine may not manifest until underlying pathology is advanced
           1. Wide inter-assay variability exists between labs\textsuperscript{25}
   b. Entities such as early acute rejection and recurrent focal segmental glomerulosclerosis develop within hours to days post-transplant and become clinically apparent early\textsuperscript{26}
      i. All will present as an increase in serum creatinine
      ii. Using serum creatinine as primary marker lacks specificity for the pathologic etiology
   c. Other causes of graft dysfunction develop over a longer period of time\textsuperscript{26,27}
      i. Can be preceded by a “subclinical phase”
      ii. Changes and damage to graft can elude routine laboratory monitoring
   d. Other markers, with greater sensitivity and specificity for monitoring graft function, have been proposed\textsuperscript{24,26}
      i. Urinary biomarkers such as cystatin c
      ii. Serial 24-hour urine creatinine collections and GFR monitoring
      iii. Protocol biopsies
      iv. None of these methods are without limitations

C. Advantages of protocol biopsies\textsuperscript{14-23}
   a. Detect incidental findings
      i. BK virus nephropathy
      ii. CNI toxicity
      iii. Chronic rejection
      iv. Primary disease recurrence
v. *De novo* glomerulonephritis
vi. Asymptomatic urinary tract infections

b. Identify early clinical rejection
c. Identify subclinical rejection (SubR)
d. Provide a more useful marker than serum creatinine alone in monitoring graft function and changes over time in high immunologic risk renal transplant recipients
   i. Identification of early subclinical rejection on protocol biopsy has allowed for the opportunity for prompt treatment and modification of maintenance immunosuppression to prevention of progression

D. Disadvantages of protocol biopsies
   a. Invasive
   b. Risk of complications
      i. Hematuria
      ii. Perinephric hematoma
      iii. Bowel perforation
      iv. Vasovagal reaction
      v. Graft loss
   c. Sampling error
   d. Inter- and intra-observer variability among pathologists
e. Cost
f. Patient quality of life

SUBCLINICAL REJECTION

A. Definition/Epidemiology
   a. Presence of histological features of acute rejection on renal biopsy in absence of a decline in renal function
   b. Clinically significant decline in renal function - increase in serum creatinine ≥ 10-25% of baseline
   c. Prevalence is highest early post-transplant
      i. Most common in first 6 months post-transplant
      ii. One of the earliest studies evaluating subclinical rejection reported an incidence of 30% within the first 3 months of transplant in the era of cyclosporine (CsA)-based immunosuppression
      iii. Declines to 18% at 12 months post-transplant
      iv. Incidence of subclinical rejection in the era of tacrolimus (TAC)-based immunosuppression has declined tremendously
         1. 6-8% within 1-3 months post-transplant

B. Pathophysiology
   a. Alloimmune process similar to clinical acute rejection
   b. Key differences and similarities between subclinical and clinical rejection
      i. Macrophage activation marker allograft inflammatory factor-1 found to be 10-fold higher in patients with clinical rejection
         1. Detrimental effect on renal function in clinical rejection may be due to a vasoconstrictive or cytotoxic substances produced by macrophages
ii. Reduced chemokine, cytokine, and cytotoxic lymphocyte product amounts in comparison to clinical rejection

C. Risk factors\textsuperscript{37-39}
   a. HLA mismatch
      i. Class II HLA antigen mismatch (DR, DP, DQ) more closely associated with subclinical rejection in comparison to Class I HLA antigen mismatch (A, B, C)
   b. Prior sensitization
      i. Blood transfusion
      ii. Pregnancy
      iii. Previous organ transplant
      iv. Infection
   c. Previous clinical rejection
   d. Induction immunosuppression with interleukin-2 receptor antagonists (IL-2RA)\textsuperscript{39}

D. Correlation to acute and chronic rejection\textsuperscript{35, 40}
   a. Major clinical concern surrounding subclinical rejection pertains to potential implications on long-term graft damage and survival
      i. Previous literature demonstrated that a cohort of patients with a 7-day post-transplant biopsy showing subclinical rejection go on to develop clinical rejection if untreated\textsuperscript{46}
      ii. Of patients with borderline changes on surveillance biopsy, 20% experienced acute rejection within 6 months in a single-center study\textsuperscript{40}
      iii. Subclinical rejection found on 3-month protocol biopsy was a positive predictor of interstitial fibrosis at 1 year post-transplant\textsuperscript{35}

E. A new picture of graft damage\textsuperscript{26-27}
   a. With developing characterization of subclinical rejection and implications regarding acute and chronic rejection, there may be more that contributes to progressive allograft damage than previously speculated
   b. Subclinical rejection may represent a “behind the scenes” pathology that contributes to chronic rejection and acute rejection episodes

F. Treatment\textsuperscript{29-33}
   a. In the era of CsA-based maintenance immunosuppression, a single study has demonstrated benefit with early treatment of subclinical rejection on protocol biopsy\textsuperscript{32}
      i. Prospective, randomized trial of 72 patients demonstrated that patients who received early protocol biopsies and pulse steroids had significantly lower serum creatinine and higher GFR at 2 years post-transplant in comparison to patients who did not receive protocol biopsies
      ii. Patients randomized to protocol biopsies also had a lower incidence of clinical rejection episodes at 2, 3, and 7-12 months post-transplant
      iii. Patients in protocol biopsy group also had significantly lower chronic interstitial and tubular changes at 6 months post-transplant
   b. As incidence of subclinical rejection has declined significantly in the era of TAC-based maintenance immunosuppression regimens, benefit of treating subclinical rejection has been difficult to demonstrate\textsuperscript{33}
### G. Subclinical rejection in the TAC era

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Design</strong></td>
<td>Open-label, randomized, prospective, multi-center, parallel-group study in 11 centers in Canada and 1 center in the US</td>
</tr>
<tr>
<td><strong>Objective</strong></td>
<td>Assess the effect of treating subclinical rejection on protocol biopsy within the first 3 months post-transplant</td>
</tr>
</tbody>
</table>
| **Interventions** | Randomized 1:1 to:  
- Protocol biopsies at 1, 2, 3, and 6 months (Biopsy Arm, BA)  
- Biopsies at 6 months only (Control Arm, CA)  
All patients received TAC, mycophenolate mofetil (MMF), and prednisone for maintenance immunosuppression  
All clinical and subclinical rejection episodes were treated with a 2-week tapering course of prednisone starting at 200 mg, with the option for investigators to use methylprednisolone and anti-lymphocyte agents at their discretion |
| **Endpoints** | **Primary:** Prevalence of chronic histology at 6 months  
**Secondary:**  
- Prevalence of subclinical rejection at 6 months  
- Frequency of biopsy-confirmed or suspected acute rejections within months 0-6  
- Renal function at 6 months post-transplant |
| **Results** | **ITT Patient Population:** 218 patients included (111 in BA, 107 in CA)  
- Mean age 47.7 years, 67-71% male, 73.8-79.3% Caucasian  
- No significant (NS) difference between arms with regard to baseline characteristics  
**Primary:** NS difference in chronic histology scores between both groups (p = 0.09), although both experienced significant increases in scores between implantation and month 6  
**Secondary:** Prevalence of SubR at 6 months 9% in the BA vs. 6% in the CA, p = 0.48  
- Acute rejection rate NS different between arms, with 12 episodes in the BA and 8 episodes in the CA, respectively (P = 0.44)  
- NS difference in mean serum creatinine and estimated creatinine clearance between arms |
| **Author Conclusions** | There is no benefit to the procurement of early protocol biopsies in renal transplant patients receiving TAC, MMF, and prednisone, at least in the short term, likely due to the low prevalence of SubR |
| **Critique** | **Strengths:**  
- All patients treated with TAC-based immunosuppression  
- Multi-center, randomized, controlled trial  
**Limitations:**  
- Small sample size  
- Lack of true control group  
- Lack of long-term follow-up |
| **Bottom Line** | In the era of TAC-based immunosuppression, there may be a lower incidence of subclinical rejection than previously reported in CsA-based regimens. More frequent protocol biopsies do not prevent subclinical rejection at 6 months. This calls into question utility of more frequent surveillance biopsies to detect subclinical rejection, let alone whether or not it should be treated. |
CLINICAL QUESTION: IS IT WORTHWHILE TO TREAT SUBCLINICAL REJECTION?

CLINICAL DATA

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Design</td>
<td>Retrospective analysis of kidney transplant recipients at a single center between 01/01/2001 and 12/31/2003</td>
</tr>
<tr>
<td>Objective</td>
<td>Assess the effect of treating subclinical rejection on protocol biopsy within the first 3 months post-transplant</td>
</tr>
</tbody>
</table>
| Population | **Inclusion:**  
- Adult kidney transplant recipient  
- Combined kidney-pancreas transplant recipient  
- Protocol biopsies performed at 1 and 3 months post-transplant  
- CNI-based immunosuppression for the first year post-transplant  

**Exclusion:**  
- Both 1 and 3 month protocol biopsies not performed  
- Participation in a clinical drug trial  
- Loss to follow-up within 3 months post-transplant  
- Non-CNI-based maintenance immunosuppression  
- Graft loss within first 3 months post-transplant |
| Interventions | **Maintenance immunosuppression:**  
- Kidney transplant recipients  
  - CsA microemulsion 10 mg/kg/day (initial) or TAC 0.2 mg/kg/day (initial), then adjusted to center goal levels  
  - MMF 2 gm/day  
  - Prednisolone 20 mg/day for 3 months, then tapered to 10 mg daily by 6 months  
- Kidney-pancreas transplant recipients  
  - CsA microemulsion 10 mg/kg/day or TAC 0.2 mg/kg/day adjusted to center goal levels  
  - MMF 3 gm/day  
  - Prednisolone 30 mg/day for 3 months, then tapered to 10 mg daily by 6 months  

**Treatment of subclinical rejection:**  
- Intravenous methylprednisolone 500 mg daily x 3 days OR increase of prednisolone dose to 1 mg/kg/day x 5 days (no maximum dose), then tapered down to baseline dose  
  OR  
- Increase in overall immunosuppression (IMS)  
  - Increase in baseline CsA or TAC dose  
  - Increase in MMF dose  
  - Switching CsA to TAC |
| Endpoints | Subclinical rejection at 1 or 3 months post-transplant, defined as histological evidence of acute rejection or borderline changes in patients with stable renal function (< 25% change in serum creatinine from baseline) |
Subclinical rejection stratified:
- Acute (A-SubR) – at least Banff grade 1A
- Borderline (B-SubR) – foci of mild tubulitis and at least 10-25% mononuclear cell interstitial inflammation of the cortex

Results

Patient Population:
- 88 patients included (59 kidney transplant alone, 29 kidney-pancreas)
- Mean age 41.3 ± 12.1 years, 57.9% male
- 45 patients received CsA-based immunosuppression vs. 43 TAC-based

Subclinical Rejection:
- 46.6% overall incidence of subclinical rejection (n = 41/88)
  - A-SubR: 12.5% (n = 11/88)
  - B-SubR: 34.1% (n = 30/88)
  - Of the 41 episodes of SubR, 33 were identified during the protocol-defined endpoints, and 8 were identified upon repeat biopsy
- 25% and 10.2% of SubR was identified at 1 month and 3 months post-transplant, respectively
- SubR occurred in 17 CsA-treated patients and 24 TAC-treated patients

Treatment of Primary Subclinical Rejection Episodes (n = 33)

<table>
<thead>
<tr>
<th></th>
<th>1 month (n = 22)</th>
<th>3 months (n = 9)</th>
<th>12 months (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase in IMS alone</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CsA → TAC alone</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Pulse steroids alone</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Pulse steroids AND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased IMS</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CSA→TAC</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OKT3</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>No Treatment</td>
<td>3</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

Progression of Subclinical Rejection
- Early chronic graft damage was observed in 29.5% (n = 26/88) of all 1-month protocol biopsies
- Higher incidence of chronic graft damage in patients with SubR on 1-month protocol biopsy (p < 0.005)
- Increased IFTA scores in biopsies with SubR vs. no SubR (p < 0.05) at 1 month post-transplant
- By 3 month protocol biopsy, inflammation had largely decreased in comparison to 1 month protocol biopsies
  - Significantly lower Banff scores (p < 0.05)
  - Chronic allograft damage was increased in the cohort of patients who were not treated for 1 month SubR compared to those who were treated (p < 0.05)

Author Conclusions
- SubR was common in the study cohort and associated with early chronic tubulointerstitial damage
- Histological progression of injury appeared to be stabilized by treatment with pulse corticosteroids combined with augmentation of baseline immunosuppression

Critique

Strengths:
- Included patients with TAC-

Limitations:
- Small sample size
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>design</td>
<td>Single center, prospective, randomized trial</td>
</tr>
<tr>
<td>objective</td>
<td>Evaluate benefit of early and late protocol biopsies Evaluate the impact of early treatment of discovered pathologies on allograft function at 2 and 3 years and long-term (5-year) graft survival</td>
</tr>
</tbody>
</table>
| population | Inclusion:  
- Age > 18 years  
- Stable graft function at 3 months post-transplant (serum creatinine < 3.39 mg/dL)  
- No clinical symptoms of rejection within 1 month prior to randomization  
- Maintenance CNI and MMF-based immunosuppression  
- Stable immunosuppressive trough levels  
  - TAC 5-15 ng/mL  
  - CsA 100-250 ng/mL  
- Good compliance to treatment |
| interventions | Patients randomly assigned under controlled allocation 1:1 to two biopsy regimens:  
- Protocol biopsies at 3 and/or 12 months (biopsy group) within 1 of 3 protocols:  
  - Protocol biopsy at 3 months only  
  - Protocol biopsy at 12 months only  
  - Protocol biopsies at both 3 and 12 months  
- Clinical surveillance only, no protocol biopsy (control group)  
Subclinical rejection: defined as histologic findings consistent with the occurrence of an acute rejection episode without associated graft dysfunction  
- Treated with a steroid pulse therapy |
| endpoints | Outcomes assessed include:  
- Treatment of subclinical rejection has impact on the progression of chronic damage within the renal allograft  
- Optimal time for protocol biopsy for detection of subclinical rejection is within 3 months post-transplant  
- SubR incidence at 1 month post-transplant suggest benefit of protocol biopsies earlier on in the post-transplant course than 3 months  
- The optimal treatment regimen for SubR has yet to be elucidated |
Graft function at 3 months, 1 year, and annually up to 5 years post-transplant, measured as estimated GFR (eGFR) according to the Cockcroft-Gault method and the Modification of Diet in Renal Disease (MDRD) formula
- Descriptive results of protocol biopsies
- Safety of protocol biopsy as defined by incidence of biopsy-related adverse events

### Results

**Patient Population:**
- Five-year follow-up data available on 145 patients (N=113 protocol biopsy, N=51 control)
- Majority male (56% and 58% in protocol biopsy and nonbiopsy groups, respectively), mean age 44 years
- NS difference in baseline characteristics, including HLA mismatch, proportion of patients with PRA ≥ 50%, and induction therapy used
- Patients randomized 1:1 to TAC:CsA within groups for maintenance immunosuppression
  - Protocol biopsy group: 59 TAC, 53 CsA
  - Control group: 25 TAC, 24 CsA

**Graft Function:**
- NS difference in eGFR at 1 and 2 years post-transplant between groups
- Significantly lower serum creatinine (1.80 ± 0.51 mg/dL in protocol biopsy group vs. 2.45 ± 1.05 mg/dL in control group, p=0.003) and greater eGFR observed in protocol biopsy arm (46.0 ± 13.8 mL/min/1.73 m² in protocol biopsy group vs. 35.4 ± 15.0 mL/min/1.73 m² in control group, p=0.002) at 3 years post-transplant

**Subclinical Rejection**
- 65 cases of subclinical acute rejection identified by 1 year post-transplant all treated with pulse steroids
- Numerically higher amount of SubR identified at 12 months post-transplant, but not statistically significant (p = 0.08)

**Safety:**
- 7 patients experienced biopsy-related complications
  - All patients were discharged after the 4-hour post-biopsy observation time

### Author Conclusions
- Protocol biopsy is an excellent method for the early diagnosis of disorders in the transplanted kidney and to monitor the effects of immunosuppression
- The protocol biopsy, followed by appropriate treatment, promotes preservation of kidney allograft function and therefore improves long-term graft survival

### Critique

**Strengths:**
- Prospective, randomized study
- All subclinical rejection episodes treated the same, with pulse steroid therapy
- Long-term follow-up
- Inclusion and randomization of patients to TAC and CsA

**Limitations:**
- Single center, small sample size
- No definition of graft dysfunction
- Lack of pre-defined outcomes
- No stratification based on CNI
- No stratification of results based on protocol biopsy regimen used
- “Pulse steroids” is ambiguous

**Bottom Line**
- Use of protocol biopsies within the first year post-transplant, as well as
prompt treatment of subclinical rejection has beneficial effect on long-term (> 2 years) renal graft function
- There appears to be no beneficial effect on allograft function at 1 year post-transplant

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Design</strong></td>
<td>Single center, retrospective study</td>
</tr>
<tr>
<td><strong>Objective</strong></td>
<td>Evaluate the incidence of early subclinical rejection revealed by protocol biopsy at 1 months post kidney transplantation, factors potentially involved in the manifestation of SubR, and evaluate the association between SubR previously treated with low-dose intravenous steroids and long-term graft survival</td>
</tr>
</tbody>
</table>
| **Population** | **Inclusion:**
- Primary kidney transplant
- Deceased donor
- Protocol biopsy at day 30 post-transplant

| **Exclusion:**
- Primary non function
- For-cause biopsy before day 30 post-transplant |
| **Interventions** | SubR defined as absence of functional deterioration and with presence of histologic findings indicative of rejection on the basis of tubulitis (t) and mononuclear cell infiltration (i) scores:
- t score ≥ 1 and i score > 0 in the absence of functional deterioration (< 15% decrease in serum creatinine) classified as SubR

Immunosuppression regimens:
- Induction: basiliximab
- Maintenance:
  - CNI (TAC or CsA) adjusted to levels:
    - TAC trough levels of 5-15 ng/mL
    - CsA C0 levels of 150-300 ng/mL
    - CsA C2 levels of 1400-1800 ng/mL early post-transplant and 800-1200 ng/mL later after transplant
  - MMF 1.5 gm/day
  - Steroids

Treatment:
- SubR: methylprednisolone 250 mg/day intravenous for 3 days
- Acute rejection: methylprednisolone 500 mg/day intravenous for 3 days
  - Anti-thymocyte globulin was used as clinically and histologically indicated

Patients divided into normal, SubR, and acute rejection groups according to Banff classification on protocol biopsy for analysis |
| **Endpoints** | 10-year graft survival
- Cox-proportional hazards regression model to determine factors associated with graft survival |
| **Results** | Patient population:
- Italian
- 174 patients screened, 159 patients underwent protocol biopsy
- 59.8% male, mean age 46.15 ± 12.2 years
- 69 patients (39.7%) experienced DGF requiring ≥ 1 days on dialysis
- 113 of 174 patients received TAC as CNI (64.9%) |
### Outcomes of interest:

- **Protocol biopsy results** (n = 159)
  - Normal histology: 142 patients (89.3%)
  - SubR: 17 patients (10.7%), all treated with pulse steroids per protocol
- **10 patients with functional changes**
  - 2 with CsA toxicity
  - 8 with acute rejection
- **NS difference in SubR between patients treated with TAC vs. CsA** (9.8% treated with TAC vs. 9.7% treated with CsA, p = 0.202)
- **Factors associated with SubR (multivariate analysis):**
  - Donor age: OR 1.04 (95% CI 1.01-1.09)
  - DGF: OR 1.08 (95% CI 1.03-1.12)
- **10-year graft survival:**
  - No difference between SubR group and normal histology group
  - Acute rejection group had a significantly lower 10-year graft survival in comparison to both SubR and normal histology groups
  - Factors associated with 10-year graft failure (multivariate analysis):
    - Donor age: HR 1.03 (95% CI 1.01-1.05)
    - DGF: HR 1.57 (95% CI 1.04-2.22)
    - Acute rejection: HR 5.22 (95% CI 1.70-16.01)
    - Subclinical rejection WITH TREATMENT was not independently associated with 10-year graft failure

### Author Conclusions

An early protocol biopsy performed 1 month after renal transplantation is a useful tool to detect subclinical rejection, and anti-rejection treatment for SubR with low-dose i.v. pulse steroids could be an appropriate strategy to improve kidney transplant graft survival in the long term.

### Critique

#### Strengths:
- All patients with SubR received identical treatment
- Greater proportion of patient population received TAC, mimicking current clinical practice
- Long-term follow up

#### Limitations:
- No evaluation of graft function outside of overall graft survival
- Single-center, Italian patient population hinders external validity
- Retrospective
- No true control group

### Bottom Line

- Results support the practice of using early (< 3 month post-transplant) protocol biopsies to evaluate early subclinical changes in renal transplant recipients
- Standardized treatment of subclinical rejection with low-dose pulse steroids and lack of significant difference in graft survival at 10-years post-transplant suggests that pre-emptive treatment is able to attenuate or even reverse these subclinical changes
- Patients with treated subclinical rejection behave more like patients without rejection
- A prospective, randomized trial would be the gold-standard to assess this hypothesis
SUMMARY

- Incidence of SubR was drastically reduced with the introduction of TAC and MMF-based maintenance immunosuppression regimens
- Few well-designed studies have directly evaluated the benefit of treatment of SubR
- The optimal time for detection of SubR is within the first 3 months post-transplant
- Early treatment of SubR has negligible benefit on renal function at 1 year post-transplant, but is associated with significant benefit on long-term (> 2 year) renal function
- Pulse steroids are able to attenuate and reverse SubR episodes

RECOMMENDATION

- Patients at high immunologic risk for the development of CAN/IFTA should be monitored with protocol biopsies post-transplant
  - Positive crossmatch
  - ABO-incompatible
  - Delayed graft function
- For centers utilizing protocol biopsies after renal transplant, biopsies should be performed within 3 months post-transplant
- For centers performing routine protocol biopsies within the first year post-transplant, SubR should be treated if detected
  - Methylprednisolone 250-500 mg daily for 3 days (similar to treatment for acute rejection)
  - Optimization of immunosuppression

REFERENCES


Appendix A. Abbreviations

ACR: Acute cellular rejection  
AMR: Antibody-mediated rejection  
APC: Antigen-presenting cell  
A-SubR: Acute subclinical rejection  
B-SubR: Borderline subclinical rejection  
CAN: Chronic allograft nephropathy  
CNI: Calcineurin inhibitor  
CsA: Cyclosporine  
DGF: Delayed graft function  
DSA: Donor specific antibody  
eGFR: Estimated glomerular filtration rate  
GFR: Glomerular filtration rate  
HLA: Human leukocyte antigen  
IFTA: Interstitial fibrosis and tubular atrophy  
IL-2RA: Interleukin 2 receptor antagonist  
IVIG: Intravenous immune globulin  
MAC: Membrane attack complex  
MMF: Mycophenolate mofetil  
SubR: Subclinical rejection  
TAC: Tacrolimus

Appendix B. Banff ’97 Classification of Renal Allograft Pathology, 2009 Update

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Histologic Characteristics and Grading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal histology of biopsy sample</td>
</tr>
<tr>
<td>Borderline Changes</td>
<td>“Suspicious” for acute t-cell mediated rejection:</td>
</tr>
<tr>
<td></td>
<td>1. No intimal arteritis</td>
</tr>
<tr>
<td></td>
<td>2. Foci of tubulitis with minor interstitial infiltration</td>
</tr>
<tr>
<td></td>
<td>Interstitial infiltration with mild tubulitis</td>
</tr>
<tr>
<td>Acute T-cell-mediated rejection</td>
<td>IA. Interstitial inflammation (&gt; 25% of parenchyma) and foci of moderate tubulitis</td>
</tr>
<tr>
<td></td>
<td>IB. Interstitial inflammation (&gt;25% of parenchyma) and foci of severe tubulitis</td>
</tr>
<tr>
<td></td>
<td>IIA. Mild to moderate intimal arteritis</td>
</tr>
<tr>
<td></td>
<td>IIB. Severe intimal arteritis comprising &gt;25% of luminal area</td>
</tr>
<tr>
<td></td>
<td>III. Transmural arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells with accompanying lymphocytic inflammation</td>
</tr>
</tbody>
</table>