Surveillance biopsies in children post-kidney transplant

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Abstract Surveillance biopsies are increasingly used in the post-transplant monitoring of pediatric renal allograft recipients. The main justification for this procedure is to diagnose early and presumably modifiable acute and chronic renal allograft injury. Pediatric recipients are theoretically at increased risk for subclinical renal allograft injury due to their relatively large adult-sized kidneys and their higher degree of immunological responsiveness. The safety profile of this procedure has been well investigated. Patient morbidity is low, with macroscopic hematuria being the most common adverse event. No patient deaths have been attributed to this procedure. Longitudinal surveillance biopsy studies have revealed a substantial burden of subclinical immunological and non-immunological injury, including acute cellular rejection, interstitial fibrosis and tubular atrophy, microvascular lesions and transplant glomerulopathy. The main impediment to the implementation of surveillance biopsies as the standard of care is the lack of demonstrable benefit of early histological detection on long-term outcome. The considerable debate surrounding this issue highlights the need for multicenter, prospective, and randomized studies.

Keywords Pediatric · Kidney transplantation · Protocol renal allograft biopsies

Introduction

The surveillance biopsy, also known as the 'protocol biopsy,' is defined as the sampling of renal tissue in patients with stable allograft function at predetermined time points [1, 2], typically between 1-12-months post-transplantation. Surveillance biopsies are increasingly used to diagnose subtle (i.e., subclinical) acute and chronic pathology in renal allografts. In some centers, they are also performed to evaluate baseline histology at implantation (i.e., 'donor' or 'implantation' biopsies) or to determine the efficacy of acute rejection (AR) therapy (i.e., 'follow-up' biopsies) [3]. The main justification for this procedure is to detect early and presumably modifiable renal allograft injury. However, in the pediatric renal transplant community, considerable debate about the clinical utility of this invasive procedure remains, particularly in the low immunological risk recipient [2, 4–10]. Similarly, in the absence of obvious graft dysfunction at predetermined time points, private insurers may be reluctant to provide coverage for this procedure.

Rationale for surveillance biopsies in pediatric renal transplant recipients

Several unique factors merit a higher index of suspicion for subclinical renal allograft injury in pediatric recipients. The first is the large mass of the adult-sized kidney (ASK) relative to the small pediatric recipient [11]. In one study in the pre-surveillance biopsy era, less than 50% of young pediatric recipients with acute rejection on biopsy actually manifested an appreciable increase in their baseline sCr values [12]. In the original Winnipeg pediatric cohort, AR, diagnosed on surveillance biopsy but without functional deterioration (i.e., subclinical acute rejection, SCR), was

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observed in 19% of low immunological risk patients managed on antibody, steroids, tacrolimus, and mycophenolate mofetil [13]. In this cohort, neither the estimated GFR (eGFR), nor the presence of proteinuria was predictive of interstitial fibrosis and tubular atrophy (IF/TA) [13], formerly known as 'CAN' [14].

Pediatric renal transplant recipients also exhibit a high degree of immunological responsiveness. Young peritoneal dialysis patients manifest higher total lymphocyte counts, CD4/CD8 ratios and increased blastogenesis when compared to their older counterparts [15]. Similarly, following sensitizing events such as blood transfusions, pediatric patients are five times more likely to develop anti-HLA antibodies than older patients [16]. Thus, it has been postulated that the large renal mass of an ASK may conceal incipient acute and chronic renal allograft injury in the pediatric recipient [3, 10, 13]. Since children have more robust immunological responses, they are potentially at higher risk for SCR [10, 13].

Biopsy procedure

The surveillance biopsy is typically performed under conscious sedation in an outpatient unit [17]. Depending on center expertise, the procedure is performed by a pediatric nephrologist or an interventional radiologist. Conscious sedation (e.g., intravenous midazolam or propofol) is administered by an anesthesiologist or an intensivist. Specimen adequacy is determined by a histopathology technician who is also present during the procedure.

A renal pole situated away from the main transplant vessels is localized with ultrasound in real-time. Ideally, two tissue cores are obtained using an 18-gauge or a 16-gauge disposable needle [17]. While the utilization of a larger diameter needle improves specimen adequacy (at least seven glomeruli and two arteries) [18], its use is associated with a higher incidence of post-biopsy hemorrhage [17, 19]. Patients are recovered for a minimum of 4 h, as the majority of biopsy-related complications manifest within the first 4 h of biopsy [17].

Renal tissue specimens are fixed in formalin and embedded in paraffin. For Banff scoring, paraffin sections are processed with hematoxylin and eosin (H&E), periodic acid Schiff (PAS), periodic acid methenamine Schiff (PAMS) and Masson's trichrome (MT) stains [18]. To facilitate the diagnosis of antibody-mediated rejection (AMR), most centers also perform C4d staining on frozen tissue. When the protocol biopsy is used for research purposes, upon procurement, a portion of the core (e.g., 1/3 or 1/2) is snap-frozen in liquid nitrogen and stored at −80°C for future analyses [19].

Adverse events related to surveillance biopsies

The safety profile of surveillance biopsies has been documented in more than 1,900 adult and 250 pediatric recipients who underwent approximately 5,000 and 700 biopsy procedures, respectively (Table 1). Importantly, the incidence of major adverse events such as allograft loss is extremely low and no deaths have been reported in these series [17, 20–25]. Macroscopic hematuria was the most-commonly reported adverse event. Its frequency increased with the use of a larger diameter needle (16-gauge vs. 18-gauge) [17, 20] and the penetration of renal medulla or highly inflamed arteries [21]. It should be noted that since many centers do not routinely perform post-biopsy ultrasound, the incidence of perinephric hematomas and arteriovenous fistulae is likely to be under-reported. In all of these series, major (i.e., highly invasive) post-biopsy interventions were rare [17, 20–25] (Table 2).

Surveillance biopsies for the detection of subclinical acute cellular rejection

Definition of subclinical acute cellular rejection

Rush et al. [26] originally described the condition, "subclinical rejection" (SCR), in which one-third of adult renal transplant recipients managed on steroids, cyclospor-

Table 1 Incidence of adverse events following surveillance biopsies in adult and pediatric kidney transplant recipients [17, 20–25]

Adverse event	Incidence (%)	
	Adult kidney transplant recipients	Pediatric kidney transplant recipients
Macroscopic hematuria	2.8-3.1	2.7-8.8
Perinephric hematoma*	3.3	13.4
Arteriovenous fistula*	9.0	1.3
Bowel perforation	0.04	0
Vasovagal reaction	0.8	0
Allograft loss	0.04-0.3	0
Death	0	0

*May be under-reported

Table 2 Post-biopsy interventions in adult and pediatric kidney transplant recipients [17, 20–25]

Intervention	Incidence (%)	
	Adult kidney transplant recipients	Pediatric kidney transplant recipients
Blood transfusion	0.1-0.7	0
Bladder catheterization	0.3-0.6	2.3
Radiological procedures	0.04	2.3
Surgical procedures	0.09-0.4	0
Prolonged hospitalization	2.0%	3.5%

ine, and azathioprine had acute cellular rejection on surveillance biopsy. Notably absent was a concomitant increase in their baseline serum creatinine (sCr) values. These findings led to the implementation of biopsies 1, 2, 3, 6, and 12 months post-transplantation as standard of care for renal allograft monitoring [27].

In the last decade, the definition of SCR has been subclassified to include: (1) Acute subclinical rejection (A-SCR), in which the degree of cellular interstitial and tubular infiltration reach Banff criteria for AR ($\geq$ i2t2, respectively) (Figs. 1a and 2). Borderline subclinical rejection (B-SCR), characterized by milder degrees of inflammation (i0-1 and/or t1-t3) [2, 28] (Fig. 1b). However, as the differences in cellular infiltration (e.g., activated macrophages) and pro-inflammatory gene expression (e.g., tumor necrosis factor alpha, interleukin (IL)-1 beta, transforming growth factor beta, interferon gamma, IL-2, IL-4, IL-10, and IL-15, granzyme B, perforin, Fas ligand, and CD152 costimulation molecule) are quantitative rather than qualitative, it is likely that A-SCR and B-SCR merely represent different potencies of the same acute inflammatory process [19, 29, 30].

Epidemiology of subclinical acute cellular rejection

The incidence of all forms of SCR is influenced by both the amount and potency of immunosuppression [2, 28]. In the cyclosporine/azathioprine era, SCR was detected in approximately 30% adult recipients during the first 3 months post-transplant [26, 27]. Similarly, in the landmark Australian report of adult kidney-pancreas recipients undergoing 1,000 surveillance biopsies over 10 years, the incidence of SCR was 46% at 3 months post-transplant. By 1 year post-transplant, the incidence had decreased to 18% [2], possibly due to the phenomenon of accommodation. The use of more potent immunosuppressive medications such as antibody induction, tacrolimus, and mycophenolate mofetil has led to a marked reduction in the overall prevalence of SCR (3-5% for A-SCR and 11% for B-SCR) [31, 32]. The paucity of acute inflammation reported in these recent studies have led some investigators to question the value of the surveillance biopsy in the low immunological risk adult recipient [31, 32].

In contrast, in children, the incidence and prevalence of SCR remains high. In a recent report of pediatric recipients managed on basiliximab induction, steroids, tacrolimus or sirolimus and mycophenolate mofetil, 29% patients had either A-SCR or B-SCR at 3 months post-transplant [33]. However, in this study, the dosage of mycophenolate mofetil (600–900 mg/m^2/day) was lower than a recent task force dosage recommendation of 1,200 mg/m^2/day [34].
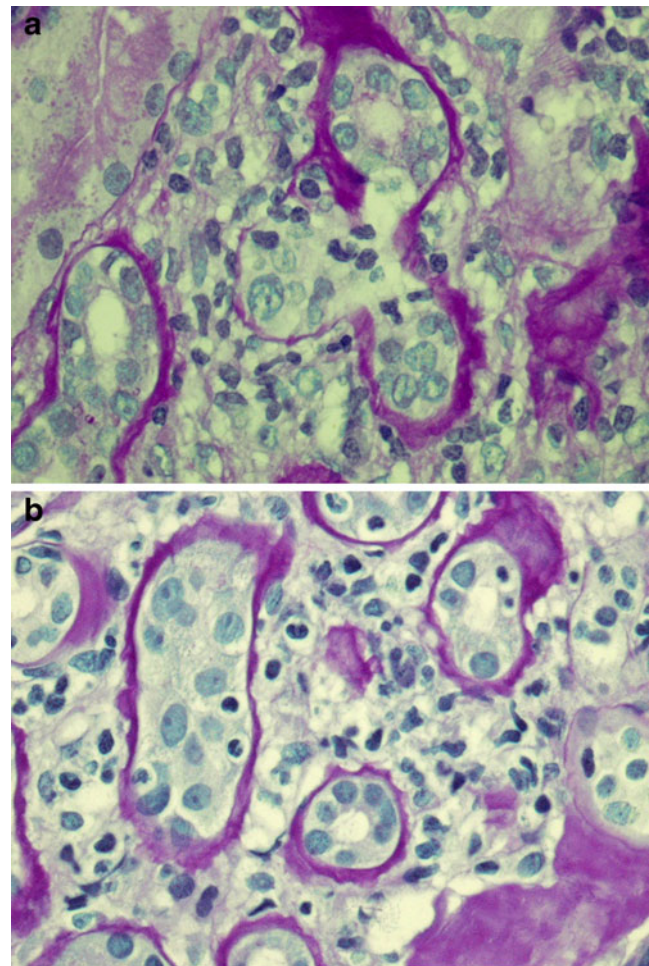

Fig. 1 **a** Surveillance renal allograft biopsy showing acute cellular subclinical rejection (A-SCR) with tubulitis (t2), PAS stain. **b** Surveillance renal allograft biopsy showing borderline cellular subclinical rejection (B-SCR) with minimal interstitial infiltrates (i1) and mild tubulitis (t1), PAS stain
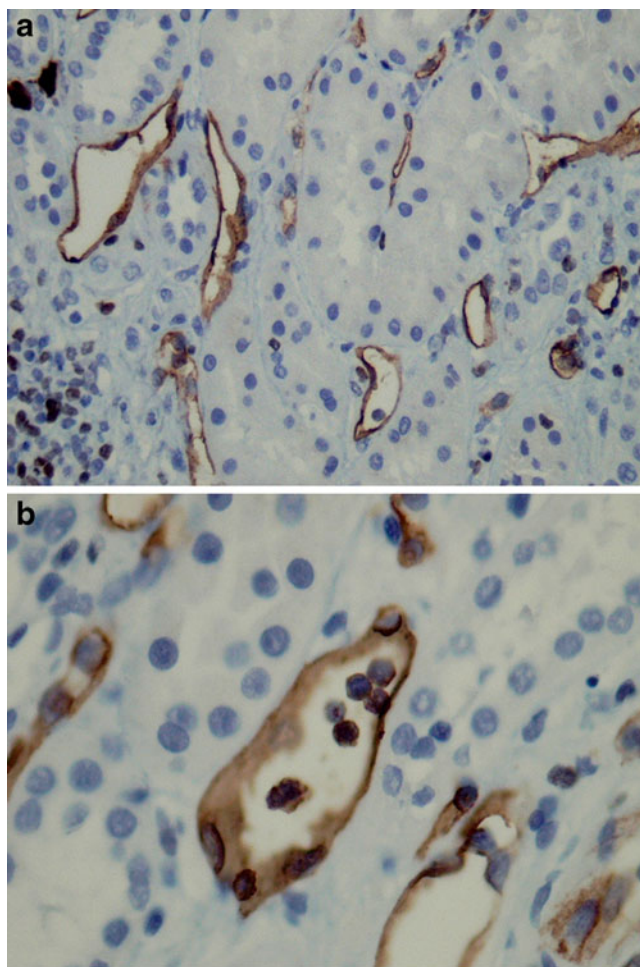
Fig. 2 a Surveillance renal allograft biopsy showing subclinical AMR with diffuse C4d+staining of peritubular capillaries and b Peritubular capillaritis (ptc2)

Thus, the higher prevalence of SCR observed in this particular study may reflect under-immunosuppression.

In the Winnipeg cohort (in which patients receive induction, steroids, tacrolimus, or sirolimus and mycophenolate mofetil 1,200 mg/m^2/day), by 7–12 months post-transplant, the incidence of A-SCR had declined to 8%, with an additional 13% 'spike' occurring in nonadherent adolescents after 3 years post-transplantation. Borderline SCR was observed in up to 20% of surveillance biopsies, with a peak incidence occurring at 7–12 months post-transplant [35]. In another pediatric report, A-SCR was seen in the late post-transplant period (19% at 3 years and 16% at 4 years post-transplant) [36].

Pathogenicity of subclinical acute cellular rejection

The potential for SCR to cause significant renal allograft injury continues to be debated. In both adult and pediatric recipients, A-SCR and B-SCR have been associated with the development and progression of IF/TA [13, 27, 35–41],

impaired glomerular adaptation [42], late allograft dysfunction [36–38] and decreased allograft survival [39, 43–46]. Given these findings, it is not unreasonable to assume that the immunosuppressive treatment of SCR would improve renal allograft outcomes. However, studies in adult recipients are collectively equivocal. In some reports, steroid treatment of SCR resulted in lower IF/TA scores at 6 months and 2 years post-transplant [40, 41, 47]; and fewer subsequent AR episodes and lower sCr values at 2 years post-transplant [47]. Other studies, however, showed no significant differences in chronic histology or in renal allograft function and survival [32, 48, 49]. These conflicting results reflect, in part, variable definitions of SCR and a lack of recognition of antibody-mediated rejection (AMR). To date, no pediatric studies have evaluated the immunosuppressive treatment of SCR using a randomized and prospective study design.

In one pediatric retrospective study, increasing the dosage of mycophenolate mofetil by 50% resulted in a significantly reduced prevalence of SCR from 44–29% [33]. However, this reduction was accompanied by a marked increase in polyoma (BK) viremia from 3–30%. Thus, in the management of SCR, the risk of pathogenicity must be weighed against the risk of over-immunosuppression, which includes opportunistic infections, and possibly, post-transplant lymphoproliferative disease.

Surveillance biopsies for the detection of chronic renal allograft injury

Patterns of chronic renal allograft injury

Longitudinal observational surveillance studies demonstrate that chronic renal allograft injury develops early after transplantation [35, 37], with 89% patients manifesting grade 1 or higher IF/TA by 7–12 months [35]. Both adult and pediatric surveillance biopsy studies reveal a biphasic pattern of pathological changes [35, 37]. Chronic tubulointerstitial injury develops within the first 12 months post-transplant, followed by chronic microvascular injury (vascular fibrous intimal thickening, arteriolar hyalinosis and glomerulosclerosis) at 2 years post-transplant and beyond [35, 37, 50–52].

Risk factors for chronic renal allograft injury

In children, multivariate analyses provide insights into the etiology of chronic renal allograft lesions. Interstitial fibrosis/tubular atrophy and arteriolar hyalinosis are associated with low recipient BSA, which is a surrogate for the renal hypoperfusion resulting from the transplantation of ASK's into small recipients [35, 50]. The development of glomerulosclerosis lesions parallels the onset of vascular lesions, implicating CNI-mediated ischemia as a contributing factor

[35, 51, 52]. Other potentially modifiable risk factors include AR and all types of SCR (IF/TA), donor hypertension (vascular fibrous intimal thickening) and post-transplant obesity (IF/TA) [35].

Surveillance biopsies for the detection of antibody-mediated rejection

Antibody-mediated rejection is characterized by the variable presence of: 1. Acute tissue injury, such as glomerulitis and peritubular capillaritis (ptc); 2. Complement degradation product (C4d) staining; and 3. Circulating donor-specific antibody (DSA) [53]. In a primate alloantibody model, the progression of AMR to renal allograft failure begins with the formation of DSA (most commonly anti-HLA Class II antibodies), followed by complement activation and C4d deposition in glomeruli and ptc. The sequelae of persistent glomerular inflammation is basement membrane duplication, mesangial matrix expansion and mesangial cell interposition, a condition known as transplant glomerulopathy (TG) [54, 55].

In adult recipients, AMR is more deleterious than acute cellular rejection (35% renal allograft survival, compared to 100% renal allograft survival, respectively, at 4 years post-transplant) [56]. Similarly, in pediatric recipients, C4d-positive ptc is associated with a higher prevalence of TG and late renal allograft loss [57]. Subclinical acute AMR is increasingly recognized on surveillance biopsy (Figs. 2a and b), but its precise incidence is currently unknown. However, subclinical TG is well described in adult recipients, with a cumulative incidence of 3% (1 year), 6% (2 years), 9% (3 years) and 12% (5 years) [55]. By 1 year post-transplant, TG was already associated with reduced eGFR and increased proteinuria [55]. In the Winnipeg pediatric cohort, the overall prevalence of TG was 22% in patients with a mean follow-up of 44±5 months. Among these, 44% were C4d-positive on surveillance biopsy and 22% had DSA [35].

Limitations of surveillance biopsy findings

A typical biopsy core represents only 0.04% of the renal allograft [4]. Sampling error has been estimated to affect up to 25% of surveillance biopsies [58], leading to erroneous diagnoses. Noninvasive technologies (e.g., microarrays, proteonomics, and NMR spectroscopy) utilizing blood or urine can potentially obviate sampling error, but must be validated in large and heterogeneous populations. Thus, the surveillance biopsy, while flawed, remains the gold standard for the diagnosis of renal allograft pathology.

Surveillance biopsy data are also susceptible to the 'era effect.' The largest and most frequently cited surveillance biopsy study describing the natural history study of 'CAN' in kidney-pancreas recipients was performed before the routine use of C4d staining and solid-phase assays for DSA detection. In light of the subsequent observation of the negative impact of antibody-mediated injury on renal allograft survival, it is likely that some proportion of the IF/TA reported in this study actually resulted from AMR [59]. In adult recipients managed in the tacrolimus/sirolimus era, there is a growing body of evidence showing that mild IF/TA is minimally progressive [60] and in the absence of acute inflammation, it is not associated with renal allograft dysfunction or diminished graft survival [61].

Conclusions

Pediatric recipients are theoretically at increased risk for subclinical renal allograft injury due to their relatively large adult-sized kidneys and their higher degree of immunological responsiveness. In these patients, longitudinal surveillance biopsy studies have revealed a substantial burden of subclinical immunological and non-immunological injury, including acute cellular rejection, interstitial fibrosis and tubular atrophy, microvascular lesions and transplant glomerulopathy. The main impediment to the implementation of surveillance biopsies as standard of care is the lack of demonstrable benefit of early histological detection on long-term outcome. The considerable debate surrounding this issue speaks to the need for multicenter, prospective and randomized studies, which are currently lacking. In the absence of a direct benefit of longitudinal screening, the most pragmatic use of the surveillance biopsy is in guiding the post-transplant management of the higher immunological risk pediatric recipient.

Questions (answers are provided after references)

1) The following statements about subclinical acute cellular rejection (SCR) are true **except**:

   a) The incidence of SCR is dependent on the amount and potency of immunosuppression
   b) In adult renal transplant recipients, the incidence of SCR is decreasing
   c) In pediatric renal transplant recipients, the incidence of SCR is decreasing
   d) SCR is defined as Banff histology showing acute rejection in patients with stable allograft function

2) **True or False**: Acute subclinical rejection (A-SCR) is defined as acute rejection by Banff criteria (≥i2t2) with a concomitant increase in the serum creatinine.

3) **True or False**: Borderline subclinical rejection (B-SCR) is defined as acute rejection which does not meet Banff criteria (i0-i1 and/or t1-t3) without a concomitant increase in the serum creatinine.

4) The following are complications of surveillance biopsies **except**:

   a) Macroscopic hematuria
   b) Arteriovenous fistula
   c) Death
   d) Bowel perforation

5) All of the following increase the risk of post-biopsy hemorrhage **except**:

   a) Acute rejection with vascular involvement
   b) Adult-sized kidney (ASK)
   c) Penetration of the renal medulla
   d) Use of a 16-gauge needle

6) Which of the following lesions have been observed in surveillance biopsies?

   a) Acute cellular rejection
   b) Interstitial fibrosis and tubular atrophy (IF/TA)
   c) Transplant glomerulopathy (TG)
   d) All of the above

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Answers

1. c
2. False
3. True
4. c
5. b
6. d