Observational Study

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The role of protocol biopsies after pediatric kidney transplantation

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Abstract

Data on protocol biopsies (PBs) after pediatric kidney transplantation are rare.

We evaluated 6-month post-transplantation renal function in 86 children after PB as observational study. Patients were divided into 3 groups:

1. PB pathological findings absent, no intervention (n = 44);
2. pathological findings but stable serum creatinine so no intervention (n = 27);
3. pathological findings (borderline rejection (borderline) Banff classification (Banff) Ia or IIa), increased serum creatinine 20%, therapy initiated (n = 15).

Glomerular filtration rate (GFR) and delta GFR were determined.

1. Group 1: Mean GFR was 79 mL/min/1.73 m^2 body surface area (BSA) (± 23) at time of biopsy. Six months after PB GFR was 75 mL/min/1.73m^2 BSA (± 24), delta GFR –4.7 and remained stable until 24 months when it decreased to 64 mL/min/1.73m^2 BSA (± 23), delta GFR –15.3.
2. Group 2: Mean GFR was 83 mL/min /1.73m^2 BSA (± 26). 12 months after PB mean GFR decreased slightly (79 mL/min/1.73m^2 BSA (± 29), delta GFR –5.1) and by 24 months had decreased to 75 mL/min/1.73 m^2 BSA (± 27), delta GFR –9.6 (1 vs 2 $P = .54$).
3. Group 3: Mean GFR was lower, 59 mL/min/1.73m^2BSA (± 23). Six and 12 months after PB mean GFR increased, but by 24 months it had decreased to 51 mL/min/1.73m^2 BSA (± 12), delta GFR +2.2 (1 vs 3 $P = 0.009$, 2 vs 3 P = .035).

PBs 6 months post-kidney transplantation did not influence the clinical course in stable pediatric patients and are therefore of questionable value. Decreased kidney function may however be stabilized by therapeutic intervention according to results of PB.

Abbreviations: BANFF = Banff classification, borderline = borderline rejection, BSA = body surface area, eGRF = estimated glomerular filtration rate, EVR = everolimus, GFR = glomerular filtration rate, IF/TA = interstitial fibrosis and tubular atrophy, PB = protocol biopsy.

Keywords: children, immunosuppression, kidney transplantation, protocol biopsy, rejection

1. Introduction

The benefit of conducting protocol biopsies (PBs) for future graft function after kidney transplantation is not entirely clear. In a review of the role of PBs, Chapman emphasized that their value must be weighed against the risks, but concluded that a PB seems to be a valuable opportunity for monitoring and personalizing immunosuppression.[1] Zachariah et al showed that the finding of subclinical acute rejection and interstitial fibrosis and tubular atrophy (IF/TA) in early PB during the first year after transplantation has no influence on baseline estimated glomerular filtration rate (eGFR) or rate of eGFR change. But subclinical acute rejection and IF/TA in late (between 12 and 24 months) PB can predict a decrease in eGFR.[2]

Gordillo and colleagues also assessed the advantages and disadvantages of PB in their review.[3] They conclude that the benefit of PB programs is early diagnosis of allograft injury due to medical intervention.[4] The disadvantage is the procedural risk, for example arteriovenous fistulas leading to regional hypoperfusion, paranchyma loss, and renin-mediated hypertension.

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The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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Table 1

Clinical data.

Total (N = 86)	Without pathological findings (N = 44)	With pathological findings without intervention (N = 27)	Pathological findings and intervention (N = 15)
Age at biopsy, yr (mean)	11 ± 4.5	10 ± 4.2	11 ± 4.2
Sex (M/F), N	22/22	15/12	7/8
Underlying disease, N			
Dysplasia/urinary tract malformation	17	8	4
Glomerular	8	8	7
Tubular	0	1	0
Immunological/infection	6	1	1
cystic	10	7	2
Other	3	2	1
Dialysis (yes/no), N	25/19	16/11	10/5
Organ source (deceased vs living donor), N	19/25	21/07	09/06
Donor/recipient body weight ratio (mean)	236 ± 145	218 ± 185	208 ± 180

N = number.

They speculate that risks in pediatric patients are underdocumented and rare in the literature.

A further argument for PBs is that children are at particular risk of subclinical rejection due to their developing immune system. They have a more pronounced response to antigenic stimulation. Furthermore, traditional biomarkers of rejection like increased serum creatinine are difficult to detect in the setting of low recipient body mass and high nephron mass when adult donors are used.[5]

Zotta et al postulated in 2018 that pediatric patients receiving treatment returned to a "standard" condition and thus potentially improved graft function.[6] However, our group suggested this in 2010 when we published the findings from the clinical course of 57 children after PB-based intervention that led to significantly better graft function.[7] However, over subsequent years we identified several children who had a stable GFR but also pathological findings after PB which required treatment. We therefore decided to re-evaluate a larger number of patients with and without interventions after pathological findings in PBs as compared to patients with normal PB in order to clarify whether treatment based on pathological PBs improved future graft function.

2. Patients and methods

Between 2002 and 2017, we performed PBs in our cohort of 86 children 6 months after renal transplantation without loss of follow up. Informed consent was obtained from the parents/legal guardians and approval was given by the local Ethics Committee. Demographic data are shown in Table 1.

Patients were divided into 3 different groups. Children in the first group (n = 44) had stable kidney function and no pathological findings after PBs. This group did not undergo any intervention. In the second group (n = 27) patients experienced stable kidney function but showed abnormalities in PB (Banff ≥ Borderline). Because of stable kidney function no interventions were required. The third group (n = 15) presented with a serum creatinine increase >20% at the time point of already scheduled PB. In this group, all biopsies showed pathological findings (Table 2 and Fig. 1).

Biopsies were performed by ultrasound guidance using an automated biopsy gun with a 16-gauge needle. At least 1 biopsy core was obtained respectively. Patients were kept in hospital for 1 night after the procedure with bed rest for 24 hours to reduce hematoma formation. Duplex-ultrasound evaluation of the transplant kidney was conducted before and after biopsy mainly to assess for hematomas and arteriovenous fistulas.

Biopsies were scored according to the Banff 2017 classification by either one of 2 local pathologists.[8] Patients were subdivided according to biopsy findings into 4 groups: biopsies without pathological findings, borderline findings, rejection > Banff Ia, IF/TA. Pathological findings are shown in Table 2 and Figure 1. GFR was compared during the 2-year observation period[9] between all 3 groups at the time point of PB, and 6, 12, and 24 months after PB. Delta GFR was also calculated at the same time points (6, 12, 24, months). Statistical significance of Delta GFR was calculated by Kruskal–Wallis test followed by Conover test for pairwise comparisons.

In addition, we investigated the relationship between donor and recipient bodyweights to evaluate the influence of nephron mass on stable serum creatinine baseline. To evaluate the possible impact of a weight mismatch between donors and recipients kidney size, we calculated donor weight/recipient weight × 100. Then we divided patients into 3 groups: small donor kidney < 75%, weight matched kidney 75% to 125%, large donor kidney >125%.[10] Kruskal–Wallis test was used and 5.99 was supposed as critical worth.

Table 2

Pathological findings in biopsy.

	Borderline	Banff Ia	Banff Ib	Banff IIa	IF/TA 5%	IF/TA 10%	IF/TA 20%	IF/TA 40%
Group 2 pathological findings no intervention	n = 19	n = 6	n = 1	n = 1	n = 11	n = 1	n = 1	n = 1
Group 3 pathological findings + intervention	n = 2	n = 9	n = 0	n = 3	n = 4	n = 0	n = 2	n = 0

BANFF = Banff classification, IF/TA = interstitial fibrosis and tubular atrophy.

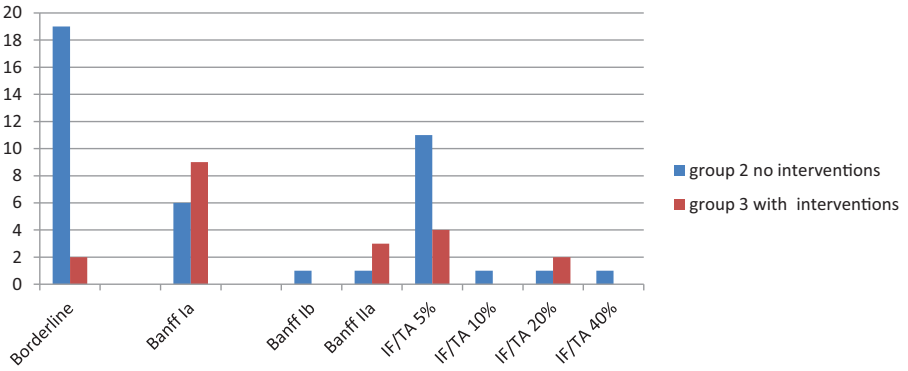

Figure 1. Pathological findings on biopsy.

Table 3

Development of glomerular filtration rate in all 3 groups.

Mean delta GFR (mL/min/1.73 m^2 BSF)	At PB	6 months after PB	12 months after PB	24 months after PB
Group 1: no pathological findings	79 (±23)	75 (±24)	74 (±25)	64 (±23)
Group 2: pathological findings no intervention	83 (±26)	83 (±25)	79 (±29)	75 (±27)
Group 3: pathological findings intervention	59 (±23)	68 (±25)	64 (±23)	51 (±12)

GFR = glomerular filtration rate, PB = protocol biopsy

3. Results

3.1. Group 1: children without any pathological findings in PB

Children in this group had stable serum creatinine at point of graft biopsy and no pathological findings in PB. Mean GFR at biopsy was 79 ± 23 mL/min/1.73m^2 BSA. Six months after biopsy children showed a slight decrease of mean GFR of 75 ± 24 mL/min/1.73 m^2 BSA, delta GFR –4.7. Twelve months after PB mean GFR was stable at 74 ± 25 mL/min/1.73 m^2 BSA, delta GFR –6.5. Twenty-four months after PB mean GFR decreased to 64 ± 23 mL/min/1.73 m^2 BSA, delta GFR –15.3 (Table 3 and Figs. 2 and 3). Donor/recipient body weight percentage showed median of 199% (quartile 148%/283% [P25/P75]) so that most of the children received a large donor kidney.

3.2. Group 2: children with pathological findings without intervention

In this group graft biopsies showed pathological findings (Table 2 and Fig. 1) but graft function was stable. Children with pathological findings and stable kidney function presented with a mean GFR 83 ± 26 mL/min/1.73m^2 at time point of graft biopsy. Mean GFR was stable with 83 ± 25 mL/min/1.73m^2 6 months after biopsy, delta GFR –0.9. Comparison of delta GFR between group 1 versus group 2 showed no significant difference 6 months after PB ($P = .434$). Twelve months after PB mean GFR slightly decreased to 79 ± 29 mL/min/1.73m^2 BSA, delta –5.1. There was no significant difference between delta GFRs in group 1 versus 2 after 12 months. Twenty-four months after biopsy mean GFR decreased further to 75 ± 27 mL/min/1.73m^2 BSA, delta GFR –9.6 but there was no significant difference between

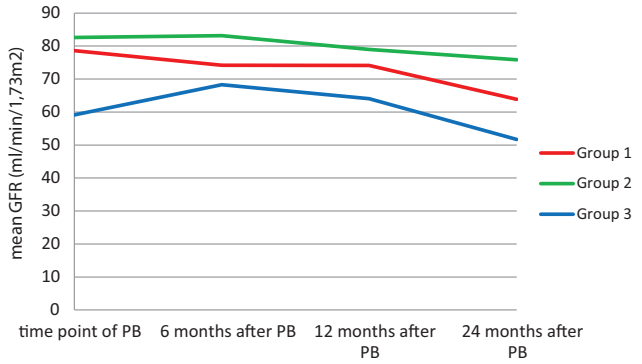

Figure 2. Mean glomerular filtration rate during observation time.

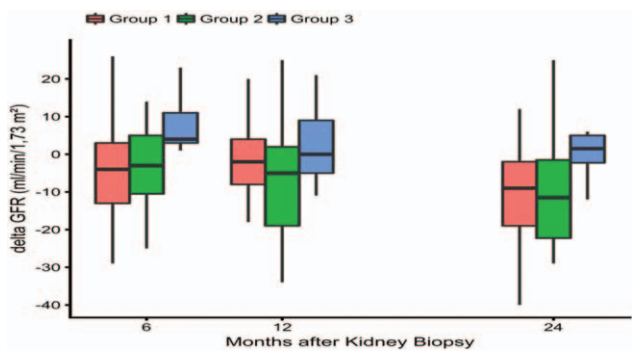

Figure 3. Delta glomerular filtration rate in all 3 groups.

Table 4

Without pathological findings.

Immunosuppression	
Group 1: Pred, CsA, EVR (N)	20
Level median (P25;P75)	CsA 58 (46/70); EVR 4.0 (3.4/5.3)
Group 2: Pred, TAC, MMF (N)	6
Level median (P25;P75)	TAC 7 (5.9/11.1)
Group 3: Pred, CsA, MMF (N)	17
Level median (P25;P75)	CsA 174 (115/206)
Group 4: Pred, TAC, EVR (N)	1
Level	TAC 2.8; EVR 3.1

CSA = cyclosporine A, EVR = everolimus, MMF = mycophenolate mofetil, N = number, Pred = prednisolone.

Table 5

With pathological findings without intervention.

Immunosuppression	
Group 1: Pred, CsA, EVR (N)	17
Level median (P25;P75)	CsA 61 (47/75); EVR 4.0 (3.3/4.9)
Group 2: Pred, TAC, MMF (N)	1
Level median (P25;P75)	TAC 6.0
Group 3: Pred, CsA, MMF (N)	9
Level median (P25;P75)	CsA 174 (115/206)
Group 4: Pred, TAC, EVR (N)	0
Level	

CSA = cyclosporine A, EVR = everolimus, MMF = mycophenolate mofetil, N = Number, Pred = prednisolone.

delta GFRs in group 1 versus 2 ($P = .538$) 24 months after PB. Delta GFR is shown in Figure 2.

Donor/recipient body weight percentage showed median value of 155% (quartile 95%/279% [P25/P75]), so even in this group children received large kidneys.

3.3. Group 3: children with pathological findings and with intervention

In this group serum creatinine increased ≥20% compared to baseline serum creatinine immediate before scheduled PB. Graft biopsy showed pathological findings (Fig. 1) and treatment was initiated (Tables 4–7). Eight children were switched from cyclosporine A to tacrolimus/rapamycin (Pat. 5–9 and 11–13, and 6 children were treated with prednisolone bolus therapy, as shown in Table 7. One child only received steroid bolus therapy. Immunosuppression was switched in 8 cases. None of the patients in this group received normal steroid withdrawal and were treated with prednisolone for a longer time (minimum of 1 year), what is in some cases the only intervention.

Mean GFR at time point of biopsy for these children was about 59 ± 23 mL/min/1.73m^2 BSA. Six months after PB mean GFR increased to 68 ± 25 mL/min/1.73 m^2 BSA, delta GFR +9.1. In contrast to group 1 versus 2 there was a significant difference in delta GFR between group 1 and 3 six months after PB ($P = .001$). Twelve months after PB mean GFR decreased slightly, but was still at a higher level than at time of PB (64 ± 23 mL/min/1.73 m^2 BSA, delta GFR +4.5). There was no statistically significant difference in delta GFRs between groups 1 and 3. Twenty-four months after PB mean GFR decreased again to 51 ± 12 mL/min/1.73 m^2 BSA, delta GFR +2.2, which is significant different compared to group 1 and group 2 (1 vs 3, $P = .009$ and 2 vs 3, $P = .035$). Delta GFR is shown in Figure 2. Donor/recipient body weight percentage showed median of 193% (quartile 153%/238% [P25/P75]), so that children in this group also received large kidneys.

4. Discussion

The literature shows that PBs might be important for improving long-term outcome in pediatric allograft recipients.[11] In this study we evaluated the PBs of 86 pediatric patients. We could show that in patients without increase of serum creatinine (groups 1 and 2) delta GFR did not significantly differ over 24 months, independent of biopsy result. Our conclusion from this retrospective study is that intensification of immunosuppression seems unnecessary in all patients with pathological findings in PB as long as serum creatinine remains stable. Our results confirm that PBs in stable pediatric transplant recipients have no additional value if performed 6 months after. This stands in opposite to the opinion of other groups.[6,12]

In our third group, eGFR could be stabilized although steroid bolus therapy and switch of immunosuppression were not initiated in every patient (Table 5) and only steroid withdrawal was omitted. However, the interventions we performed led to a stabilization of GFR with similar 2-year results as in stable patients. This kind of 6-month biopsy, classified somewhere between a protocol biopsy and a biopsy by cause, helps to detect

Table 6

With pathological findings with intervention.

Immunosuppression			After intervention
Group 1: Pred, CsA, EVR (N)	6	6	
Level median (P25;P75)	CsA 59 (52/70); EVR 3.8 (3.6/4.3)		CsA 62 (51/71); EVR 4.4 (3.7/5.2)
Steroid bolus therapy (N)	2		
Group 2: Pred, TAC, MMF (N)	0		
Group 3: Pred, CsA, MMF (N)	9		
Level median (P25;P75)	CsA 100 (90/121)	CsA 97 (89/190)	N = 7 CsA-> TAC N = 1 CsA-> Rapa, N = 1 only steroid bolus
Steroid bolus therapy (N)	6		
Group 4: Pred, TAC, EVR (N)	0		

CSA = cyclosporine A, EVR = everolimus, MMF = mycophenolate mofetil, N = number, Pred = prednisolone.

Table 7

Group 3 pathological findings intervention.

Patient	IS level at time point of biopsy	Dosage of IS at time point of biopsy	IS level 3 months after biopsy	Dosage of IS 3 months after biopsy	Additional therapy	Biopsy
Pat. 1	CsA 87, EVR 6.8	Pred 1x5 mg, CSA 2x60 mg, EVR 2x1.1mg	CsA 67, Ev.r 4.0	Pred 1x5 mg (for 2.3 years), CsA 2x60 mg, EVR 2x1.1 mg	Steroid bolus therapy	Banff IIa
Pat. 2	CsA 73, EVR 3.8	Pred 1x4 mg, CsA 2x70 mg, EVR 2x1mg	CsA 54, EVR 3.6	Pred 1x4 mg (for 3 years), CsA 2x70 mg, EVR 2x1mg	None	Banff Ia
Pat. 3	CsA 60, EVR 3.8	Pred 1x3 mg, CsA 2x45, EVR 2x0,5mg	CsA 57, EVR 4.7	Pred 1x 3 mg (for 5 months until death of patient), CsA 2x50 mg, EVR 2x0.6mg	Steroid bolus therapy, rituximab, igg	Banff IIa
Pat. 4	CsA 50, EVR 3.2	Pred 1x5 mg, CsA 2x70 mg, EVR 2x0.9mg	CsA 119, Evr 6.0	Pred 1x5 mg (for 11 months), CsA 2x100 mg, EVR 2x1.0mg	Steroid bolus therapy	Banff Ia
Pat. 5	CsA 87	Pred 1x5 mg, CsA 170–180 mg, MMF 2x500mg	TAC 8.5	Pred 1x 5 mg (3 years until transfer), TAC 2x6 mg, MMF 2x250mg	Steroid bolus therapy	Banff IIa
Pat. 6	CsA 224	Pred 1x 5 mg, CsA 2x160 mg, MMF 2x750mg	TAC 9.9	Pred 1x5 mg (4 years until transfer), TAC 2x6 mg, MMF 2x250mg	Steroid bolus therapy	Banff IIa
Pat. 7	CsA 126	Pred 1x3 mg, CsA 140/150 mg, MMF 2x500mg	SIR 6.2	Pred 1x10 mg (3 years until death of patient), MMF 2x500 mg, SIR 2x2mg	None	Banff IIa
Pat. 8	CsA 93	Pred 1x5 mg. CsA 2x 135 mg, MMF 2x 500mg	TAC 15.9	Pred 1x 5 mg (4 years until transfer), TAC 5mg/6 mg, MMF 2x250 mg	Steroid bolus therapy	Banff Ia
Pat. 9	CsA 115	CsA 2 x 150 mg, MMF 2 x 750mg	TAC 13.3	TAC 2 x5 mg, MMF 2x250mg	None	Banff Ia
Pat. 10	CsA 97	Pred 1x5 mg, CsA 2x170 mg, MMF 2x1000 mg	CsA 244	Pred 1x5 mg (3 years until transfer), CsA 2x150 mg, MMF 2x1000mg	Steroid bolus therapy	Banff Ia
Pat. 11	CsA C2 1196	Pred 1x2,5 mg, CSA 2x75mg	TAC 7.2	Pred 1x2.5 mg (13 years to date), TAC 2x3.5mg	Steroid bolus therapy	Borderline
Pat. 12	CsA 84	Pred 1x5 mg. CsA 170/180 mg, MMF 2x500mg	TAC 8.5	Pred 1x5 mg (1 year), TAC 2x5 mg, MMF 2x250mg	Steroid bolus therapy	Borderline
Pat. 13	CsA 132	Pred 1x 5 mg, CsA 2x180 mg, MMF 2x750mg	TAC 6.7	TAC 2 x4 mg, Myfortic 2x360mg	Steroid bolus therapy	Banff Ia
Pat. 14	CsA 57, EVR 4.4	Pred 1x2,5 mg, CsA 2x50 mg, EVR 2x0.5mg	CsA 72, EVR 5.4	Pred 1x2.5 mg (3 years to date), CsA 2x50 mg, EVR 2x0.5mg	None	Banff Ia
Pat. 15	CsA 49, EVR 3.5	Pred 1x5 mg, CsA 2x50 mg, EVR 2x0,4mg	CsA 43, EVR 3.1	Pred 1x5 mg (2 years to date), CsA 2x40 mg, EVR 2x0.35mg	None	Banff Ia

BANFF = Banff classification, CSA = cyclosporine A, EVR = everolimus, MMF = mycophenolate mofetil, Pred = prednisolone.

early histological changes that might be improved by intervention. Dharnidharka et al underlined this by showing that a high percentage of the PBs performed under modern immunosuppression revealed abnormal findings even when fibrosis was excluded.[13]

It might be that the time point for a PB must be chosen more individually, for example due to the appearance of de novo donor-specific antibodies,[14] proteinuria or slightly increased serum creatinine baseline (less than 20%), with or without a link to other problems such as inconsistent immunosuppression levels or an increase in urinary tract infections. On the other hand, fixed time points for PBs miss creeping creatinine and thereby an indication for biopsy.

Moreover, there is speculation as to whether PB should primarily be performed in small children, with large transplanted kidneys.[10] However, there was no difference in donor to recipient size matching between our 3 groups, thus a different regime in patients with a large donor kidney does not seem necessary.

Our study has several limitations. The retrospective design limits the generalizability of the results. Despite the definition for steroid-pulse therapy, there was not structured protocol for intervention after PB, and a switch or increase of immunosuppression was decided by the individual physician.

5. Conclusion

Our retrospective data demonstrates no role for regular 6-month PBs in stable pediatric kidney recipients. However, regular biopsies performed 6 months post-transplantation in the case of serum creatinine increase ≥ 20% can help guide interventions to stabilize graft function. Future prospective randomized trials are required to confirm our findings.

Author contributions

Nele Kanzelmeyer performed the biopsies, reviewed the data, did the statistical analyses and wrote the manuscipt, Christian Lerch

helped with statistical analyses and reviewed the manuscript, Thurid Ahlenstiel-Grunow performed biopsies, Jan H. Bräsen evaluated all biopsies, Dieter Haffner took part in designing the study, Lars Pape designed the study and critically reviewed the manuscript.

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