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ORIGINAL ARTICLE

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Renal transplant biopsy specimen adequacy in a paediatric population

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Abstract Updated guidelines on the diagnosis of acute allograft rejection including criteria for biopsy specimen adequacy were published in 1999. We sought to determine the adequacy of specimens in paediatric transplant patients and identify factors influencing adequacy. All renal transplant biopsies performed between 1998 and 2003 were classified as adequate (n=25), minimal (n=19) or inadequate (n=27) in accordance with the Banff 97 criteria, and the histological diagnoses were documented. The effect on specimen adequacy of grade of operator, method of sedation, age of child, needle gauge, number of cores and total core length was then investigated. Overall, a minimal or adequate specimen was obtained in 62% of cases. No histological diagnosis could be made in 30% of all specimens, just over half of which were inadequate. Higher rates of rejection were found in adequate (52%) than inadequate (33%) samples. The grade of operator (p=0.498), the age of the child at the time of biopsy (p=0.815) and type of sedation (p=0.188) did not affect adequacy. More than one core was obtained in 38 (54%) cases, and this was significantly associated with specimen adequacy (p<0.0005) as was longer total core length (p=0.002). Clinical features in isolation are not sufficient for the diagnosis of acute allograft rejection. Renal biopsy remains the gold standard and relies on adequate specimen collection. Our data shows that specimen adequacy according to the Banff 97 guidelines is achievable in children and that more than one core at the time of sampling significantly improves this achievement. Adequate sampling reduces the risk of an inconclusive histological diagnosis.

Keywords Banff · Adequacy · Biopsy · Kidney · Pediatric · Transplant

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Introduction

The gold standard for the diagnosis of renal allograft rejection is the kidney biopsy, as clinical criteria alone are insufficient. Historically, allograft biopsy reports were descriptive. The Banff Working Classification of Renal Allograft Pathology was published in 1993 after the initial meeting of pathologists and nephrologists in Banff, AB, Canada in 1991 [1]. An alternative classification, known as the Collaborative Clinical Trials in Transplantation (CCTT) schema was also in use in the early 1990s, supported by the National Institute of Health. The Banff 97 guidelines, published in 1999, combined the previous classifications and were adapted to take into account consensus opinions from the interval Banff conferences and the results from several large trials [2]. These guidelines include more emphasis on the importance of vascular changes and also include a modification to the definition of biopsy specimen adequacy, to allow more extensive examination of the vasculature. The grade of rejection has been correlated with clinical prognosis and graft outcome, highlighting the importance of an accurate histological diagnosis [3, 4, 5, 6]. Table 1 compares the Banff 93 and Banff 97 criteria for biopsy adequacy. In 2003, an addition to the Banff 97 guidelines was published, regarding antibody-mediated rejection, but this did not change the adequacy criteria [7].

Table 1 Comparison of Banff 93 and Banff 97 criteria for renal allograft biopsy specimen adequacy

	Banff 93	Banff 97
Adequate	≥7 glomeruli 1 artery	≥10 glomeruli ≥2 arteries
Minimal (marginal)	1–6 glomeruli 1 artery	≥7 glomeruli 1 artery
Inadequate	No glomeruli or arteries	<7 glomeruli or no arteries

There are no published data available to establish if these guidelines are achievable in children. This study was designed to assess the adequacy of renal allograft biopsy specimens in a paediatric population and to investigate factors that could affect adequacy.

Subjects and methods

All renal transplant biopsies obtained between January 1998 and January 2003, from children aged less than 19 years, were retrospectively reviewed by a single pathologist (A.H.). The number of cores submitted, total core length, number of glomeruli and arteries present and histological diagnosis were noted. The biopsies were classified according to the Banff 97 criteria as adequate, minimal or inadequate. A case note review was performed to establish the age at the time of biopsy, the grade of the doctor doing the biopsy, the type of sedation/analgesia used and the gauge of needle used. The number of cores taken was corroborated with the procedure note. Any adverse effects were noted.

All biopsies were obtained using an automated biopsy gun (Biopty, Covington, GA, USA) with either a 16-guage or 18-gauge needle. Ultrasound assistance, by a consultant radiologist, was used in all cases, but this varied between real-time ultrasound and ultrasound guidance to locate the appropriate biopsy site immediately before the needle was inserted. The exact ultrasound technique used could not be established in all patients, due to inadequate documentation in the case notes and was therefore not entered into the analysis. In addition to the sedation administered pre-procedure, local anaesthetic was infiltrated to the kidney capsule in all cases. Specimens were processed as per the Banff 97 guidelines, with seven slides containing multiple sequential sections, cut at 3–4 micron intervals. Three slides were stained with hematoxylin and eosin, three with silver stain and one with trichrome stain.

Chi-squared tests of homogeneity were used to investigate the effect on biopsy adequacy (adequate, minimal or inadequate) of each of the following variables in turn: grade of operator (consultant or trainee); method of sedation (IV or general anaesthesia); number of cores (1 or ≥2); total core length (0–9, 10–19 or ≥20 mm); age of child (<10, 10–14 or ≥15 years) and gauge of biopsy needle (16 or 18).

Results

Seventy-one biopsies were performed on 31 patients with a mean age of 12.2 years (range 4.4–18.4). Twenty-five (35%) were classified as adequate, 19 (27%) minimal and 27 (38%) inadequate. Table 2 shows how the distribution of biopsy adequacy grades was affected by the other variables assessed. Neither grade of operator, method of sedation nor age of the child significantly affected the distribution of grades of biopsy adequacy. Taking more than one core, or increasing total core length, significantly and substantially improved the percentage of adequate specimens. Seven biopsies were recorded as being done with a 16-gauge needle and 31 with an 18-gauge needle, but in 33 cases the needle gauge was not documented. In view of this, no statistical comparison was made, but the sample results suggest that there is little if any effect of

Table 2 Effect of operator grade, sedation type, core number, core length, age and gauge of needle on biopsy specimen adequacy

	Adequate	Minimal	Inadequate	p value (χ^2 test)
Total	25 (35%)	19 (27%)	27 (38%)	
Grade of operator				
Consultant (42)	17 (40%)	11 (26%)	14 (33%)	0.492
Trainee (29)	8 (28%)	8 (28%)	13 (45%)	
Method of sedation				
IV sedation (62)	20 (32%)	16 (26%)	26 (42%)	0.188
General anaesthesia (9)	5 (56%)	3 (33%)	1 (11%)	
Number of cores				
1 (33)	4 (12%)	10 (30%)	19 (58%)	<0.0005
>1 (38)	21 (55%)	9 (24%)	8 (21%)	
Total core length				
0–9 mm (12)	0 (0%)	2 (17%)	10 (83%)	0.002
10–19 mm (38)	13 (34%)	11 (29%)	14 (37%)	
≥20 mm (21)	12 (57%)	6 (29%)	3 (14%)	
Age of child				
<10 years (18)	7 (39%)	5 (28%)	6 (33%)	0.815
10–14 years (35)	11 (31%)	11 (31%)	13 (37%)	
≥15 years (18)	7 (39%)	3 (17%)	8 (44%)	
Gauge of needle:				
16 (7)	2 (29%)	2 (29%)	3 (43%)	n/a
18 (31)	9 (29%)	9 (29%)	13 (42%)	
Not documented (33)	-	-	-	

Table 3 The histological diagnoses

Diagnosis	Adequate (25)	Minimal (19)	Inadequate (27)
Rejection (total)	13 (52%)	7 (37%)	9 (33%)
Suspicious (6)	2	1*	3
Type I (16)	8*	4	4
Type II (6)	2	2	2*
Treated (1)	1	0	0
Acute tubular necrosis (14)	6*	6*	2
Polyoma virus (2)	0	1	1*
Tacrolimus toxicity (1)	1	0	0
Chronic allograft nephropathy (2)	2*	0	0
Tubular atrophy (1)	0	1	0
Ischaemic necrosis (1)	0	0	1
No histological abnormality (21)	5	5	11
No renal tissue (4)	0	0	4

* More than one diagnosis

choice of needle gauge on the distribution of biopsy adequacy grades.

The histological diagnoses are shown in Table 3. Nine of the 71 biopsies were diagnosed as rejection despite being inadequate samples. This diagnosis was based on the presence of an interstitial inflammatory infiltrate, with evidence of tubulitis with or without arteritis. There were four specimens with no renal tissue and a repeat biopsy was performed in three of these cases, with an adequate specimen being obtained. Two of these children had evidence of rejection. The fourth patient's creatinine returned to baseline spontaneously. Two patients with histological evidence of acute tubular necrosis also had features consistent with rejection, specifically tubulitis, and demonstrated improvement in renal function following alteration of immunosuppressant therapy. Another 11 inadequate specimens had no obvious histological diagnosis. It would be impossible to exclude rejection in these cases, but on review of the clinical histories, all the children recovered their baseline creatinine without an increase in their immunosuppression and maintained this level for at least 2 months following the biopsy. In addition to the three children with no renal tissue on initial biopsy, one further child underwent a second biopsy because of an inadequate specimen initially.

Of the 46 minimal or inadequate samples, 21 were because of paucity of glomeruli alone; six were due to insufficient arteries alone; and 19 were due to both insufficient glomeruli and arteries, including four specimens with no renal tissue. There were seven episodes of gross haematuria, none of which required a blood transfusion or surgical intervention. In all these cases, more than one core had been taken. One patient developed an arteriovenous fistula, which required a coil insertion 1 year later.

Discussion

The renal allograft biopsy is essential to establish a diagnosis of rejection. The Banff 97 guidelines set out diagnostic criteria for rejection, and, to ensure that this diagnosis can be made accurately, there are minimal requirements for biopsy specimens. These requirements were met in 62% of our samples, and this figure approached 80% when more than one core was taken, as recommended in the Banff 97 guidelines. These results are comparable with the biopsy adequacy rates reported in the adult literature [8, 9, 10, 11], but we are not aware of any paediatric transplant data addressing this issue, as paediatric studies have combined allograft with native kidney biopsies when looking at adequacy for histological diagnosis [12]. Many of the older studies allowed lower minimal requirements for the number of glomeruli and did not require the presence of arteries to establish a histological diagnosis in biopsy samples, potentially overestimating the achieved adequacy rates. One previous adult study comparing the impact of the change of adequacy requirements between the Banff 93 and Banff 97 specimen adequacy criteria found that 86% of biopsies were at least minimal according to Banff 97 [13]. These results are somewhat better than ours and could be attributed to the routine use of a dissecting microscope at the time of biopsy to count the number of glomeruli obtained, allowing further cores to be taken if the initial sample is found to be inadequate. We do not have this facility available in our institution. Since 40 out of 46 minimal or inadequate samples in our series had insufficient glomeruli, the routine use of a dissecting microscope at the time of biopsy would be one possible method of improving biopsy adequacy.

Beckingham et al. compared blind vertical-pass biopsy, ultrasound guided biopsy and ultrasound guided biopsy with immediate microscopic examination of the specimen [14]. They obtained cortical renal tissue in 75%, 91% and 100% of specimens, respectively; however, even with immediate microscopy, there was a mean number of only 9.3 glomeruli per specimen, which would be below the requirements in the Banff 97 guidelines. Our institution has adopted the use of direct ultrasound guidance for biopsies, but this was not consistent during the study period.

Adult studies have compared different needle gauges [10, 15]. Cahen found that a mean of 13 glomeruli were obtained with a 14-gauge needle compared to seven glomeruli using an 18-gauge needle, with no statistical

difference in complication rates [10]. A prospective study comparing 14-, 16- and 18-gauge needles found that the mean number of glomeruli obtained was 15, 11 and 9, respectively [15]. The use of the 14-gauge needle was associated with more pain, but there was no difference in macroscopic haematuria rates. More recently, pediatric data have shown higher macroscopic haematuria rates with the use of 16-gauge needles compared with 18-gauge needles [16]. We were unable to statistically analyse our data with respect to the needle size used, as in many cases it was not documented, although it was routine practice to use an 18-gauge needle.

Similar to previous findings, in adult studies, we did not detect any difference in the adequacy rate when comparing the grade of operator, and there were no differences in complication rates [14, 17].

The Banff guidelines recommend that two biopsy cores be taken, preferably from different sites. Our results would support this. In the past, only one core was taken to minimise the potential for bleeding complications, but it is now recognised that the need to make an accurate diagnosis outweighs the risks of taking more than one core. The seven children (9.8%) in this study who developed macroscopic haematuria had at least two cores of renal tissue taken. Our rate of macroscopic haematuria is above the rate of just under 3% quoted in the more recent literature [16, 18]. In one case, only medulla was obtained, and this is known to be associated with increased macroscopic haematuria rates. Four children, who developed macroscopic haematuria, had three cores taken, as the initial two cores were thought to be inadequate. On review, it would appear that an adequate sample had been obtained with two cores, adding to the argument for the use of a dissecting microscope at the time of biopsy. No child required a blood transfusion or surgical intervention. We did not routinely screen for perinephric haematoma, but rates of 36% are suggested in the literature [16].

Our data showed no difference between sedation techniques on adequacy rates. Only a very small number of our children received general anaesthesia, as it was used only in very young children or in children who had not previously responded to IV sedation.

Although a diagnosis was made in the majority of our samples, there were 11 inadequate specimens in which a diagnosis could not be made. Although on follow-up at 2 months none of the children had clinical rejection, we accept that we have a small study population and that the potential to miss rejection with an inadequate specimen is real. Similarly, it is difficult to interpret these biopsies as being completely normal, despite the clinical histories. Obviously, the histological findings should be correlated with the clinical findings and a repeat biopsy should be obtained when clinically indicated. There were ten adequate or minimal biopsies in which there was no histological diagnosis, and these were thought to be normal biopsies, though there is the potential for sampling error within the kidney. Interestingly, 52% of the adequate samples showed rejection compared with 33–37% of the minimal or inadequate samples, suggesting under-diagnosis in the latter two categories, although the clinical histories do not support this. There were two biopsies with acute tubular necrosis in addition to borderline or type I rejection. One of these biopsies was of minimal adequacy, with 15 glomeruli, but only one artery, and the other was adequate. As an arteritis could not be excluded histologically, both children received a brief increase in immunosuppression, followed by a rapid taper, resulting in a rapid return to baseline creatinine.

A correct diagnosis also relies on the interpretation of the biopsy, and there have been concerns recently regarding the extent of interobserver variability in Banff scores of rejection between pathologists [19, 20, 21]. This problem was overcome in this study by review of all biopsies by a single pathologist.

This is the first study examining the adequacy of renal allograft biopsy specimens, with respect to the Banff 97 criteria, in children. Overall, we obtained reasonable adequacy for our biopsy specimens, meeting at least the minimal Banff criteria in almost 80% when more than one core was taken. In 15% of the specimens, a histological diagnosis was not possible because of inadequate renal tissue. It is hoped that with direct ultrasound guidance the adequacy rates will improve further. Secondly, as the most common reason for specimen inadequacy was insufficient glomeruli, we propose that this could be rectified by the implementation of a dissecting microscope at the time of biopsy to allow a rapid assessment of the number of glomeruli present and to take further cores if necessary.

In conclusion, the Banff 97 guidelines for specimen adequacy are achievable in the majority of children. More than one core should be sampled, to maximise total core length, as this almost doubles the rate of successful biopsy-specimen adequacy. Improved tissue sampling should result in more accurate diagnoses, but correlation with the clinical picture is still required.

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