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ORIGINAL ARTICLE

Timing and value of protocol biopsies in well-matched kidney transplant recipients – a clinical and histopathologic analysis

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Summary

The role and timing of protocol biopsies after kidney transplantation are controversial. We changed our protocol biopsy policy and compared the predictive value of biopsies at different time-points. Protocol biopsies at 6 months ($n = 45$) were obtained during 2001–2004, and at 3 and 12 months from 2004 ($n = 41$). Donor biopsy was available from 70 patients. Histopathologic changes were described with chronic allograft damage index (CADI) and Banff 1997. Follow-up was for 18 months. Chronic allograft nephropathy (CAN) was present in 12%, 51%, and 34% and borderline or subclinical rejection in 9.8%, 8.9%, and 7.3% of patients at 3, 6, and 12 months. CAN at 6 and 12 months was associated with reduced graft function ($P = 0.001$). Semiquantitative CADI scores at all time-points significantly correlated with glomerular filtration rate (GFR) at 18 months. Strongest correlation existed with CADI at 12 months ($P < 0.001$). Change in CADI between 0–6 and 0–12 months, but not between 0–3 and 3–12 months, correlated with GFR at 18 months ($P = 0.03$, $P = 0.01$). Subclinical rejections were rare and chronic changes mild at 3 months. In our well-matched population, the predictive value of a biopsy at 3 months was inferior to biopsies at 6 or 12 months, both of which were effective in predicting long-term graft function.

Introduction

The role of protocol biopsies in the follow-up of renal transplant recipients is still a matter of debate [1]. Perhaps the strongest argument in favor of protocol biopsies is the possibility to detect subclinical rejection, usually defined as signs of acute or borderline cellular rejection in protocol biopsy specimens without concomitant signs of graft dysfunction [1]. Subclinical rejection is associated with progression of chronic histopathologic changes [2] and inferior long-term outcome [2,3], and treatment of subclinical rejection by intensification of immunosuppression may be beneficial [4,5]. A recent study reported that also persistent inflammation not fulfilling the Banff criteria of interstitial inflammation recorded in the biopsies is associated with progression of histopathologic changes and inferior outcome [6]. The incidence of subclinical rejection in kidney transplant recipients varies substantially with the population analyzed and immunosuppressive protocol used. Incidence figures up to 60% at 1 month, 45% at 3 months, 45% at 6 months, and 25% at 12 months, including both borderline and acute rejection grade Ia, have been reported [2,4,7].

A second argument in favor of protocol biopsies is clinical evidence from several studies showing analysis of chronic histologic changes, such as the semiquantitative chronic allograft damage index (CADI), as strong predictors of graft outcome long before clinical signs of graft deterioration are evident [8,9]. Also a quantitative analysis of histologic changes compared to baseline donor

histology seems to be a good surrogate marker of allograft prognosis [10,11]. The histologic changes in protocol biopsies at different time-points have been well described in studies with sequential protocol biopsies [4,12,13]. The optimal timing of a biopsy, however, was not the main focus of these studies, and no consensus exists on when to take protocol biopsies. Protocol biopsies are invaluable for research purposes, not only to help understand the pathophysiology and progression of chronic changes in the graft, but also as surrogate markers for clinical immunosuppressive and other drug studies. A recent consensus meeting also stated the usefulness and valuable role of protocol biopsies in the follow-up of kidney transplant recipients [14].

The kidney transplant population in Finland differs somewhat from that of all previous studies; almost all grafts are from cadaveric donors and well-matched with still relatively short cold ischemia time. Despite relatively conservative immunosuppressive treatment, acute rejection rates are low (10%) (L. Kyllönen, pers. commun., Helsinki University Hospital, Helsinki). The policy of our clinic until 2004 was to obtain a protocol biopsy at 6 months. To diagnose possible subclinical inflammatory responses, we changed our policy to protocol biopsies at 3 and 12 months at the beginning of 2004. The aim of this study was to evaluate these strategies in detail; to describe the progression of histopathologic changes using both CADI score and Banff 1997 classification [15], and correlate these histopathologic findings with later graft function, and try to find a time-point for protocol biopsies with optimal prognostic value in a well-matched Finnish kidney transplant population.

Patients and methods

Patients

All the adult patients of Helsinki University Hospital district who received a kidney transplant between December 2001 and October 2005, who had a protocol biopsy performed according to the policy of our clinic, and who were followed up for at least 1 year were included in this retrospective study. From the beginning of 2004, patients were prospectively analyzed for the purpose of this study. Baseline immunosuppression was usually a triple-drug regimen with Cyclosporine A, mycophenolate mofetil and steroids, and in patients with re-transplantation, previous sensitization, poor mismatch, long waiting time, cyclosporine was replaced by tacrolimus. Biopsy proven acute rejections of grades I–II were treated with high doses of i.v. methylprednisolone, OKT3 or plasmapheresis. All the biopsies analyzed in this study were taken according to our clinical follow-up protocol, and as no extra biopsy or blood samples were taken for the purpose of this retrospective study, approval of the ethics committee was not required.

Protocol biopsies

Before the year 2004, the policy of our clinic was to perform a protocol biopsy at 6 months, and from the beginning of 2004, protocol biopsies were taken at 3 and 12 months. Donor biopsies were obtained from donor kidneys during the donor operation or in a minority of cases after revascularization in the recipient operation. Protocol biopsies were performed under ultrasound guidance using either Bard Magnum® or Bard Biopty® devices and 18 gauge Biopty-cut® needles. For light microscopy, serial tissue sections were stained with hematoxilin and eosin, periodic acid-schiff (PAS), methenamine silver, and Masson's trichrome. An ultrasound and Doppler sonography was performed to every patient after the biopsy to detect early complications. All the biopsies were scored according to the CADI [8], with the individual parameters scored from 0 to 3 according to Banff 1997 classification [15], except for the percentage of globally sclerosed glomeruli, which is not included in the Banff classification (0, no globally sclerosed glomeruli; 1, <15%; 2, 16 to 50%; and 3, >50% globally sclerosed glomeruli). CADI results from the sum of the following six histopathologic parameter scores: interstitial inflammation, tubular atrophy, interstitial fibrosis, arterial fibrointimal thickening, glomerular mesangial matrix increase, and the percentage of globally sclerosed glomeruli. The biopsies were interpreted by one of three pathologists. In uncertain cases, and in all cases of subclinical rejection or borderline changes, the biopsies were reviewed by two pathologists and a consensus was reached. Biopsies with ≥7 glomeruli were considered adequate, biopsies with 2–6 glomeruli marginal, and biopsies with 0–1 glomeruli were considered inadequate and not included in the analysis. The progression of histopathologic changes (ΔCADI) was estimated by calculating the difference between the CADI score in the protocol biopsies and in the donor biopsies, or the differences in CADI scores between 3- and 12-month biopsies. Chronic allograft nephropathy (CAN), the sum score of tubular atrophy and interstitial fibrosis (IF/TA, each scored from 0 to 3), and acute rejection were diagnosed in the biopsies according to the Banff classification [15,16]. The impact of biopsy result on the treatment of the patient was analyzed from patient files. Subclinical rejections and borderline rejections were treated with intensification of baseline immunosuppression (increased trough level target for cyclosporine or conversion to tacrolimus), or controlled with a biopsy or more intensive clinical follow-up. In stable patients with no signs of immune activation in the 6- or 12-month biopsy,

and especially in patients with problems with osteoporosis or glycemic control, steroids were withdrawn slowly during the second post-transplant year. However, a team of clinicians responsible for the treatment of the recipient made every decision of a possible consequence of a biopsy individually in each case. As no evidence-based therapy to CAN exists, the consequence of each such finding was similarly individually assessed.

Clinical and laboratory variables

The following pre- and perioperative data were obtained from patient files: recipient and donor age and gender, human leukocyte antigen (HLA)-A, -B, and -DR mismatch, cold ischemia time, time on dialysis and dialysis modality, donor and recipient cytomegalovirus (CMV) serostatus, and primary renal disease. Delayed graft function was defined as the need for dialysis treatment during the first week after transplantation. Furthermore, the following clinical data after transplantation were collected from patient files during the 18-month follow-up at months 3, 6, 12 and 18 after transplantation: doses and the type of immunosuppressive medication, the number and the type of blood pressure medications, the use of statins and angiotensin-converting enzyme (ACE)-inhibitors (ACEI) or angiotensin receptor blockers (ARB), the number and type of acute rejection episodes, and cytomegalovirus and polyomavirus infections. Plasma creatinine, proteinuria from 24-h urine collections, glycosylated haemoglobin A1c, total and low-density lipoprotein cholesterol, cyclosporine trough levels, tacrolimus trough levels, C-reactive protein, albumin, parathyroid hormone, phosphorus and ionized calcium were collected from the laboratory database. In addition, glomerular filtration rate (GFR) was estimated from plasma creatinine with the Cockcroft–Gault formula [17].

Statistical analyses

All data are expressed as mean ± 1 SD, unless otherwise indicated. Difference in the distribution of continuous and ordinal variables was assessed using the nonparametric Mann–Whitney's *U*-test. Comparisons between more than two groups were calculated with the nonparametric Kruskal–Wallis one-way analysis, and significances between groups were assessed with the Dunn test. Correlation between binary variables was calculated with the Fisher's exact test. Correlation between continuous and ordinal variables was calculated with the nonparametric Spearman's correlation (R_s), and multiple linear regression was used to rule out confounding factors. The calculations were performed with SPSS statistical software (version 12.0.1., SPSS Inc, Chicago, IL, USA). Two-tailed *P*-values lower than 0.05 were considered statistically significant.

Results

Patients characteristics

Of the 169 patients from Helsinki University Hospital district transplanted during the study period, 45 patients had a protocol biopsy taken at 6 months after transplantation (group 1), and 41 patients had protocol biopsies performed at 3 and 12 months (group 2). Only patients with true protocol biopsies (i.e. no clinical indication and stable graft function) with a maximum delay of 1 month from the protocol were included in the analysis. The reasons for delay or not taking protocol biopsies in remaining the 83 patients transplanted during the study period were: surgical complications (wound healing problems, lymphocele, ureter stricture, etc.) after transplant operation and follow-up at the transplantation surgery clinic in 32 patients (39%), infectious problems in 12 (14%), problems with anticoagulation or antithrombotic medication or other increased risk of bleeding in 11 (13%), patient refusal in 10 (12%), nonfunctioning graft or patient deceased at the time of biopsy in 4 (5%), living donor transplantation in 3 (4%), and miscellaneous reasons (participation in clinical immunosuppressive drug trial, logistical problems, complications of previous biopsy, recent acute rejection) in 11 patients (12%). A baseline donor biopsy was available from 34 patients in group 1 and from 36 patients in group 2. Altogether 197 biopsy specimens were analyzed for this study. No differences existed between the groups in any of the pre- or perioperative data analyzed, or in immunosuppressive or other medication used (Table 1). Follow-up data up to 18 months after transplantation were available from all patients. One patient died in both groups during the 18-month follow-up. Mean estimated GFR (eGFR) at 18 months was 66.12 ± 20.35 ml/min in group 1 and 64.99 ± 16.44 ml/min in group 2 (P = NS). No differences between the groups were recorded in any of the laboratory values analyzed. No cases of polyomavirus nephropathy were found in this cohort. Of the 83 patients transplanted during the study period with no protocol biopsy, 4 patients died and 1 patient returned to dialysis during the study period. Mean eGFR in these patients with a functioning graft at 18 months was 73.26 ± 22.94 ml/min. No significant differences were recorded in graft function or survival between patients with no protocol biopsies or patients with protocol biopsies taken. Altogether five patients received azathioprine instead of mycophenolate mofetil at some point after transplantation for gastrointestinal side-effects of MMF (two patients in group 1, 3 patients in group 2). Three of them (two in group 1, one patient in group 2) were later switched back to MMF.

Table 1. Demographic data of the patients.

	Group I (6-month biopsy group, n = 45)	Group II 3 and 12-month biopsy group, n = 41)
Recipient age (years)	49 ± 13	47 ± 11
Donor age (years)	48 ± 13	49 ± 13
Diabetic nephropathy as primary disease	13 (29)	15 (37)
Time on dialysis before transplantation (months)	24 ± 20	23 ± 19
HLA-mismatch (A, B, and DR)	2.0 ± 1.0	2.2 ± 0.9
Cold ischemia time (h)	20 ± 5	21 ± 4
Delayed graft function	14 (31)	18 (44)
Patients on tacrolimus (vs. cyclosporine)	9 (22)	11 (27)
Patients on azathioprine (vs. mycophenolate mofetil)	2 (4)	3 (7)
CMV infection(s)	5 (11)	8 (20)
Acute rejection episode(s)	8 (18)	9 (22)
On statins at 6 months	18 (40)	24 (59)
On angiotensin-converting enzyme inhibitor or angiotensin receptor blocker at 6 months	8 (18)	4 (10)
Number of blood pressure medications	1.5 ± 0.9	1.3 ± 0.8
eGFR at 6 months (ml/min)*	68.11 ± 22.39	69.83 ± 15.75
eGFR at 18 months (ml/min)*	66.11 ± 20.35	64.98 ± 16.44
C0-level at 6 months (μg/l)	112 ± 29	119 ± 24
Low-density lipoprotein cholesterol at 6 months (mmol/l)	2.5 ± 0.9	2.4 ± 0.5

*eGFR, glomerular filtration rate estimated with the Cockcroft–Gault formula.

Data expressed as mean ± SD, or number of patients (and percentage). All differences are NS. Values given within parentheses are represented in percent.

Donor biopsies

Findings in the donor biopsies are summarized in Table 2. Glomerulosclerosis was present in 47% of the donor biopsies in group 1 and in 39% of the biopsies in group 2, and arterial fibrointimal thickening in 15% and 17%, respectively (All differences nonsignificant, P = NS). Mean number of sclerosed glomeruli in the donor biopsies with glomerulosclerosis was 0.9 ± 0.9 (range: 1–4) and percentage of sclerosed glomeruli in biopsies with glomerulosclerosis was 9.9 ± 7.9% (range: 4.8–25%). Donor age correlated positively with CADI score at baseline (R_s = 0.411, P = 0.001).

Findings in protocol biopsies

Findings and CADI scores in the protocol biopsies are depicted in Table 2. CAN was present in 12% (n = 4) of the biopsies at 3 months, 50% (n = 23) at 6 months, and 34% (n = 14) at 12 months (the presence of CAN at 3 vs. 6 months: $P < 0.001$, 6 vs. 12 months: P = NS). CAN was grade Ia in all except four cases (grade IIa was found in two biopsies at 6 months and two biopsies at 12 months). Subclinical acute rejection was present in one biopsy at 3 months (grade Ia, 2.4% of the biopsies), in one biopsy at 6 months (grade Ia, in 2.2% of the biopsies), and in one biopsy at 12 months (grade Ib, 2.4% of the biopsies) (P = NS). Borderline acute rejection was present in three biopsies at 3 months (7.3%), in three biopsies at 6 months (6.7%), and in two biopsies at 12 months (4.9%) (P = NS). Of all the biopsies, 24% were only marginally representative. Mean CADI score was significantly higher at 6 months compared with 3 months (P = 0.006). Patients with borderline or subclinical acute rejections at 3 months did not show higher CADI scores at 12 months compared with recipients without subclinical acute rejection (data not shown). Donor and recipient age, and cold ischemia time correlated significantly with $CADI_{12}$, but not with $CADI_3$ or $CADI_6$ (data not shown). Recipients with delayed graft function after transplantation had significantly higher $CADI_6$ at 6 months (3.4 ± 1.8 vs. 1.7 ± 1.6, P = 0.008), but no differences existed in $CADI_3$ or $CADI_{12}$ (data not shown). History of acute rejections, HLA-mismatch, time on dialysis before transplantation, trough levels of cyclosporine or tacrolimus, the presence of diabetes, or any of the laboratory values did not correlate with the CADI scores at any time-point (data not shown).

Progression of histopathologic changes

Changes in the CADI scores between the donor biopsies and protocol biopsies, and between the protocol biopsies at 3 and 12 months are presented in Fig. 1 ΔCADI was negative in seven (19%) patients between 0 and 6 months ($\Delta CADI_{0-6}$), in 8 (20%) patients between 0 and 3 months ($\Delta CADI_{0-3}$), and in 8 (20%) patients between 0 and 12 months ($\Delta CADI_{0-12}$). No significant differences existed between the ΔCADI scores at different biopsy time-points.

Correlation between histopathologic and clinical data

Renal function as measured by estimated GFR (eGFR) at 18 months, was not significantly lower in patients with CAN at 3 months (n = 4), compared with those with no CAN at 3 months (n = 37) (56.68 ± 14.77 vs. 65.71 ± 16.58 ml/min, respectively, P = 0.42). However, patients with CAN at 6 or 12 months (n = 23 and n = 14, respectively) had a lower eGFR at 18 months compared with patients with no CAN at 6 or 12 months

Table 2. Histopathologic changes in the protocol biopsies.

(A) Group I	No. of glomeruli	t	i	gs	mm	ci	ct	cv	CADI
Baseline (n = 34)	13.7 ± 6.3	0 ± 0	0 ± 0	0.4 ± 0.5	0 ± 0	0 ± 0	0.1 ± 0.2	0.2 ± 0.4	0.6 ± 0.6
6 months (n = 45)	9.0 ± 4.7	0.1 ± 0.4	0.5 ± 0.6	0.1 ± 0.3	0.3 ± 0.4	0.6 ± 0.6	0.6 ± 0.6	0.6 ± 0.6	2.2 ± 1.8
(B) Group II									
Baseline (n = 36)	15.3 ± 9.5	0 ± 0	0 ± 0	0.5 ± 0.6	0 ± 0	0 ± 0	0.1 ± 0.2	0.2 ± 0.4	0.7 ± 0.8
3 months (n = 41)	7.8 ± 3.4	0.1 ± 0.4	0.5 ± 0.6	0.1 ± 0.4	0.1 ± 0.2	0.2 ± 0.2	0.2 ± 0.4	0.2 ± 0.5	1.2 ± 1.2
12 months (n = 41)	8.8 ± 3.9	0.1 ± 0.5	0.5 ± 0.6	0.3 ± 0.5	0.1 ± 0.3	0.4 ± 0.6	0.3 ± 0.6	0.1 ± 0.4	1.9 ± 1.8

Group I = 6 months biopsy group.
Group II = 3- and 12-month biopsy group.
t, tubulitis; i, interstitial inflammation; gs, glomerulosclerosis; ci, interstitial fibrosis; ct, tubular atrophy; cv, vascular fibrointimal thickening; mm, mesangial matrix increase; CADI, chronic allograft damage index.
All data are expressed as mean ± SD.
All differences are NS.

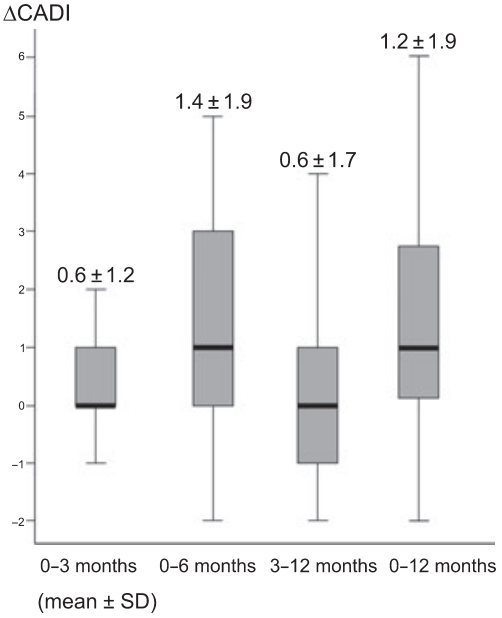

Figure 1 Change in chronic allograft damage index (ΔCADI) between protocol biopsy time-points.

(n = 22 and n = 27, 59.37 ± 15.97 vs. 72.25 ± 22.25 ml/min at 6 months P = 0.049; 52.09 ± 12.53 vs. 71.17 ± 14.51 at 12 months P < 0.001). Patients with borderline changes or subclinical acute rejection at 3, 6 or 12 months, did not have lower eGRF at 18 months compared to patients with no borderline or subclinical acute rejection (59.05 ± 21.10 vs. 66.22 ± 18.29, P = 0.47). Correlations of the CADI and ΔCADI, and IF/TA scores at different time-points to eGFR at 18 months are presented in Table 3. Correlation between baseline CADI in donor biopsies and eGFR did not reach a statistical significance. However, CADI scores at 3, 6 and 12 months correlated significantly to eGRF (P = 0.006 for $CADI_3$; P = 0.004 for $CADI_6$; and P < 0.001 for $CADI_{12}$). Similarly, $\Delta CADI_{0-6}$ (P = 0.03) and $\Delta CADI_{0-12}$ (P = 0.01) correlated significantly to eGFR. $\Delta CADI_{3-12}$ and $\Delta CADI_{0-3}$ did not correlate to eGFR. Donor and recipient age correlated also significantly with eGFR (R_s = –0.513 P < 0.001 for donor age; and R_s = –0.269, P = 0.017 for recipient age). In multiple linear regression analyses, the significant correlations observed between CADI scores at 3, 6 and 12 months and eGFR, and the correlation between ΔCADI scores and eGFR were independent of donor or recipient age (P = 0.005 for CADI at 3 months, P = 0.001 at 6 months, P = 0.005 at 12 months, P = 0.03 for $\Delta CADI_{0-6}$; P = 0.02 for $\Delta CADI_{0-12}$).

Table 3. Spearman's correlations (R_s) between chronic allograft damage index (CADI) scores, change in CADI (ΔCADI), or interstitial fibrosis and tubular atrophy scores (IF/TA) at different time-points and estimated GRF at 18 months. P-values <0.05 were considered statistically significant.

	R_s	P
CADI at baseline (n = 70)	–0.259	0.05
CADI at 3 months (n = 41)	–0.441	0.006
CADI at 6 months (n = 45)	–0.437	0.004
CADI at 12 months (n = 41)	–0.597	<0.001
ΔCADI 0–3 months (n = 36)	–0.232	0.22
ΔCADI 0–6 months (n = 34)	–0.391	0.03
ΔCADI 0–12 months (n = 36)	–0.463	0.01
ΔCADI 3-12 months (n = 41)	–0.200	0.24
IF/TA at baseline (n = 70)	–0.122	0.48
IF/TA at 3 months (n = 41)	–0.467	0.004
IF/TA at 6 months (n = 45)	–0.435	0.004
IF/TA at 12 months (n = 41)	–0.537	0.001

The IF/TA scores at 3, 6 and 12 months also significantly correlated with later graft function (P = 0.001 for IF/TA_3; P = 0.001 for IF/TA_6; and P = 0.001 for IF/TA_{12}.) In multiple linear regression analyses, the significant correlations observed between the IF/TA scores and eGFR were similarly independent of donor or recipient age (data not shown).

Complications

No grafts were lost because of complications, and no serious complications occurred. After three protocol biopsies (2.3% of the biopsies), the recipients experienced a short spontaneously resolving course of gross hematuria. Arteriovenous fistula was observed in one patient after the 12-month biopsy. In a controlled ultrasound 1 week after the biopsy, the arteriovenous-fistula had spontaneously resolved. No other complications were recorded.

Impact of biopsies on treatment of patients

No differences were observed in the impact of biopsy findings on treatment of the patient between the various biopsy time-points. The detailed consequences of biopsies to treatment, other than steroid withdrawal, are described in Table 4. Of the protocol biopsies taken at three months, four had direct impact on patient treatment. At 6 months, altogether six biopsies had direct impact on the treatment of the recipient, and at 12 months, similarly six biopsies had direct impact to the treatment of patients. Subclinical acute rejections were all treated with intensification of baseline immunosuppression or controlled with biopsy, but of the borderline changes, four were reacted to. Steroid was successfully withdrawn from 29 patients in group 1, and from 22 patients in group 2, (P = NS). Acute rejection occurred in one patient in group 2 six months after the withdrawal of steroid. This late acute rejection was successfully treated with high-dose of i.v. steroids and conversion from cyclosporine to tacrolimus.

Discussion

When comparing the timing of protocol biopsies in our homogenous, relatively low-risk kidney transplant population in Finland, we found that CADI at 6 and 12 months correlated with later graft function at 18 months. Chronic changes in 3-month biopsies were mild, and the diagnosis of CAN at 3 months did not associate with poor graft function. Semiquantitative CADI score, on the other hand, correlated already at 3 months to later poor graft function. The sum of IF/TA scores predicted later graft function with comparable results to CADI scores. When analyzing the progression of histopathologic changes, the best correlation with graft function was observed in the progression of chronic changes between baseline and 12 months and between baseline and 6 months. Surprisingly, progression between baseline and 3 months and progression between two time-points after transplantation, 3 and 12 months, did not correlate to later graft function. Subclinical rejection was a rare finding in our material, and had no correlation to graft function, or progression of histopathologic changes. Steroids were successfully withdrawn from 51 patients, and we detected only one late acute rejection episode in these patients.

Chronic allograft damage index is a semiquantitative scoring system, which takes into account six histologic parameters [8]. Evidence shows that CADI serves as a good surrogate marker for long-term graft prognosis [8,10]. However, data about the optimal timing of a biopsy have not been reported. We found that CADI scores at 3, 6, and 12 months correlated with later graft

Table 4. Consequences of protocol biopsies at different time-points.

Patient no. and time of biopsy	Finding in protocol biopsy	Consequence of the biopsy
1. (3 months)	Subclinical acute rejection Ia	Control biopsy at 5 months with normal histology
2. (3 months)	Subclinical borderline rejection	Control biopsy at 6 months with normal histology
3. (3 months)	Subclinical borderline rejection	Control biopsy at 6 months with normal histology
4. (3 months)	Tubular vacualization	Tacrolimus dose reduction
5. (6 months)	Subclinical acute rejection Ib	Intensification of immunosuppression
6. (6 months)	Subclinical borderline rejection	Conversion from cyclosporine to tacrolimus
7. (6 months)	CAN	Conversion from azathioprine to mycophenolate
8. (6 months)	CAN	Conversion from azathioprine to mycophenolate
9. (6 months)	CAN	Angiotensin-converting enzyme-inhibitor therapy
10. (6 months)	CAN and interstitial inflammatory infiltrate	Control biopsy at 12 months with no inflammation
11. (12 months)	Subclinical acute rejection Ia	Conversion from cyclosporine to tacrolimus, control biopsy 1 month later with normal histology
12. (12 months)	Subclinical borderline rejection	Control biopsy 1 month later with normal histology
13. (12 months)	CAN	ACE-inhibitor therapy, cyclosporine dose reduction
14. (12 months)	CAN	ACE-inhibitor therapy, cyclosporine dose reduction
15. (12 months)	CAN, suspicion of cyclosporine toxicity	Cyclosporine dose reduction
16. (12 months)	Inflammatory infiltrates	Intensified clinical follow-up

CAN, chronic allograft nephropathy.

function, but the diagnosis of CAN at 3 months was not associated with reduced graft function, although a later diagnosis of CAN was associated with a lower estimated GFR. Early CADI score thus seems to be a more accurate predictor of long-term outcome compared with the binary diagnosis of CAN, although the low number of patients with CAN at 3 months may also explain the lack of correlation. The latest Banff classification recommends the use of the term IF/TA instead of the nonspecific diagnosis of CAN [16]. We also found that IF/TA scores at all time-points correlate to later eGFR with comparable prediction of later graft function to CADI score.

Evidence indicates also the progression of CADI score at 6 months and 2 years compared with baseline correlates with long-term graft function [10,11], and similarly, the progression of CAN between baseline and 3 months after transplantation correlates with long-term prognosis [13]. We found a correlation with ΔCADI scores and graft function only between baseline and 6 or 12 months, but not between baseline and 3, and between 3 and 12 months, suggesting that the predictive value of a biopsy at 3 months is not comparable to later biopsies. Seron *et al.* [13] reported similar results suggesting that predicting the progression of CAN in sequential protocol biopsies may be inaccurate because of sampling error. Of notice, we found regression of chronic changes probably as a marker of sampling error 22% of patients between 3 and 12 months. On the other hand, similar sampling error probably occurs also in the case of donor biopsies, and therefore we included also negative CADI values in the statistical analyses.

The possibility of detecting subclinical rejection is probably the most common indication for taking protocol biopsies. Including borderline changes, evidence shows subclinical rejection being present in 24–60% of protocol biopsies at 1 month [3,18], 13–48% at 3 months [4,19], 34% at 6 months [4], and 25% of biopsies at 12 months [2,13] . Probably the lowest prevalence has been reported by Gloor *et al.* [19] in mostly living-donor kidney allograft recipients receiving tacrolimus-based immunosuppression, with only 2.6% recipients showing signs of subclinical acute cellular rejection and 11% patients showing borderline changes at 3 months. Only three patients in our study population suffered from subclinical acute rejection and borderline changes were seen in 8 patients; the prevalence of borderline and subclinical acute rejection in our material was 9.8% at 3 months, 8.9% at 6 months, and 7.3% at 12 months. To our knowledge, these figures are probably the lowest reported so far in cadaveric kidney transplantation. One explanation to the low prevalence may be the isolated, genetically and relatively homogenous population in Finland, which makes it possible to get a good HLA-match for almost all transplantations without heavy immunosuppression.

A protocol biopsy provided new information not evident from any symptoms or laboratory values that had direct impact on the treatment of sixteen patients in our retrospective analysis. Subclinical rejections were mostly reacted to, but chronic changes mostly gave information about long-term prognosis, as currently no evidence-based therapy for CAN exists. Reduction of calcineurin inhibitor exposure may be beneficial [20], and a recent study reported benefit of sirolimus in treatment of CAN [21]. Antifibrogenic and renoprotective therapy with ACEI or ARBs also may prevent the progression of CAN [22]. Currently, no data exist about the usefulness and impact of protocol biopsies to the actual treatment of the patients, except in the case of subclinical rejections. The transplant recipient might benefit even more from protocol biopsies, if a systematic approach could be applied to react to changes seen in the biopsy. Before this is possible, however, more data are required for the therapeutic options of CAN. Steroid treatment is associated with several side-effects to the metabolic status of the transplant recipient. Some studies have reported an increased incidence of late acute rejections after steroid withdrawal [23]. Our results suggest that late protocol biopsies seem to be a promising method to evaluate the safety of steroid withdrawal, as only one late acute rejection was recorded after the cessation of steroids. However, the true safety and benefit of steroid withdrawal can only be assessed in a study with a longer follow-up. Furthermore, the retrospective nature and lack of systematic approach to reacting to histologic findings in the biopsies limit the possibility of this study to compare the impact of protocol biopsies at different time-points on the effectiveness of therapeutic interventions to CAN or subclinical rejection.

This study is also limited by the fact that it compared the biopsy results between two different patient groups and time periods. However, major changes other than the biopsy policy were not made during these periods in our treatment protocols concerning immunosuppression or follow-up, which could have influenced our results, and the groups were very comparable in all the parameters analyzed. Although some biopsies in our material were only marginally representative, the predictive value of chronic changes in marginal biopsies may be similar to representative biopsies [13]. Biopsy adequacy in Banff 1997 criteria is designed for the diagnosis of acute rejection, and the diagnosis of CAN, thought to be more equally distributed in the graft, may be reliable with also a less adequate sample. Partly because of this relatively large number of inadequate samples in our present study,

we have now changed our biopsy core needle size to 16 G instead of 18 G. Furthermore, the small number of patients and low prevalence of subclinical rejection made it impossible to find differences between the biopsy time-points in this regard, although other studies have shown that the prevalence decreases after the first weeks and months after transplantation [1].

The optimal timing of protocol biopsies depends naturally on the purpose and on the expected finding from the biopsies, and this study certainly does not give conclusive answer to this question. Early biopsies help reveal possible subclinical rejection, while later biopsies show more chronic changes and seem to predict later graft function better. As expected, our study showed that the later the biopsy, the better is its predictive value. On the other hand, hopefully molecular diagnostics and gene expression profiles may help to predict allograft failure at early time-points before any histopathologic damage is recorded, when intervention still may prevent the development of pathologic response [24].

In conclusion, our findings in this study comparing the different time-points for protocol biopsies show late protocol biopsies provide important information about the status of the allograft, not achievable with any other methods, and help physicians in tailoring immunosuppressive treatment and predicting the long-term prognosis of the graft. Similarly, the progression of histopathologic changes compared with baseline biopsy gives reliable data on long-term prognosis. Chronic changes were mild at 3 months, and the progression of histopathologic changes between baseline and 3 months, and between three and 12 months did not correlate to later graft function. Our data indicate that the predictive value of a biopsy at 3 months is not comparable to later biopsies, and given the low incidence of subclinical rejection in our well-matched kidney transplant recipients, the usefulness of a protocol biopsy in the Finnish kidney transplant recipient at 3 months may be discussed. Biopsies at 6 and 12 months, on the other hand, were equally effective predicting long-term function.

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Authorship

IH: study design, clinical data collection, and manuscript preparation; FO: histopathologic analyses, clinical data collection, and manuscript preparation; HH: histopathologic analyses; AR-S: histopathologic analyses; EH: study design, data analysis; PK: study design, data analysis, head of the project. In addition, each co-author participated in the interpretation of the data and revision of the article.

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