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ORIGINAL ARTICLE

High tacrolimus clearance – a risk factor for development of interstitial fibrosis and tubular atrophy in the transplanted kidney: a retrospective single-center cohort study

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SUMMARY

Patients with high tacrolimus clearance are more likely to experience transient under-immunosuppression in case of a missed or delayed dose. We wanted to investigate the association between estimated tacrolimus clearance and development of graft interstitial fibrosis and tubular atrophy (IFTA) in kidney transplant recipients. Associations between estimated tacrolimus clearance [daily tacrolimus dose (mg)/trough concentration (μg/l)] and changes in IFTA biopsy scores from week 7 to 1-year post-transplantation were investigated. Data from 504 patients transplanted between 2009 and 2013 with paired protocol biopsies (7 weeks + 1-year post-transplant) were included. There were no differences in baseline biopsy scores (7 weeks) in patients with different estimated tacrolimus clearance. Increasing tacrolimus clearance was significantly associated with increased ci + ct score of ≥2 at 1 year, odds ratio of 1.67 (95% CI; 1.11–2.51). In patients without fibrosis (ci + ct ≤ 1) at 7 weeks (n = 233), increasing tacrolimus clearance was associated with development of *de novo* IFTA (i + t ≤ 1 and ci + ct ≥ 2) at 1 year, odds ratio of 2.01 (95% CI; 1.18–3.50) after adjusting for confounders. High tacrolimus clearance was significantly associated with development of IFTA the first year following renal transplantation.

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Introduction

Tacrolimus is characterised by a narrow therapeutic range and high inter- and intraindividual pharmacokinetic variability, and therefore is therapeutic drug monitoring (TDM) mandatory [1]. If tacrolimus is dosed too high following renal transplantation the treatment is hampered by the development of drug-induced histological lesions in the renal graft [2,3]. Ideally, TDM should be able to discriminate efficacy from toxicity in all individuals but so far no clear association between whole-blood exposure (C_0 or AUC_{0-12h}) and the development of chronic nephrotoxicity has been established [4,5]. An easy assessable biomarker to identify recipients

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at risk of developing tacrolimus induced nephrotoxicity is clinically warranted.

Recipients with high tacrolimus clearance have an increased risk of under-immunosuppression in case of a delayed or skipped dose [6]. Estimated tacrolimus clearance has also been linked to reduced glomerular filtration rate [7]. In a previous study from our transplant centre it was shown that high tacrolimus clearance was a risk factor for development of biopsy-proven acute rejection (BPAR) during the first 90 days post-engraftment [8].

The main aim of the present analysis was to investigate the association between estimated tacrolimus clearance and development of interstitial fibrosis and tubular atrophy (IFTA) evaluated in paired renal protocol biopsies available from 7 weeks to 1-year post-transplantation.

Materials and methods

Study population

In this retrospective, single-centre study, data from all patients receiving a renal allograft in Norway between 2009 and 2013 were included if they were treated with tacrolimus and had paired protocol biopsies obtained 7 ± 1 weeks and 1 year ± 2 months post-transplantation with a ci + ct score at 7 weeks ≤4 (n = 504). Figure 1 outline the patient flow, including reasons of exclusion.

Oral tacrolimus was initiated at the day of transplantation, starting with 0.04 mg/kg twice daily in standard risk patients and 0.05 mg/kg twice daily in high-risk patients. TDM was applied and doses were adjusted to reach target whole-blood trough concentrations of 3–7 μg/l in standard immunological risk patients, and 8–12 μg/l (days 0–30) followed by 6–10 μg/l (after day 30) in high immunological risk patients. High immunological risk was defined as presence of donor specific antibodies, panel reactive antibodies (PRA) >20% at transplantation or ABO incompatibility between donor and recipient.

Induction therapy consisted of 20 mg intravenous basiliximab at day 0 and 4 after transplantation and 250 mg (standard risk) and 500 mg (high risk) intravenous methylprednisolone on day 0. A single dose of 375 mg/m^2 rituximab was given to DSA-positive and ABO-incompatible patients 4 weeks before transplantation (living donor) or at transplantation (dead donor). From day 0 to 4, 400 mg/kg IVIg were given to DSA-positive patients, whereas ABO-incompatible patients were given 500 mg/kg IVIg at transplantation. Patients with PRA greater than 20%, which were DSA-negative, were given anti-thymocyte globulin induction (Genzyme®).

As maintenance immunosuppression in addition to tacrolimus, all patients received 750 mg mycophenolate mofetil twice daily, and prednisolone once daily, initiated at 20 mg (80 mg in high-risk patients), and tapered to 10 mg at 4 weeks in standard risk recipients and 8 weeks in immunologic high-risk patients.

In the early period after transplantation, patients had their clinical follow-up at the transplant centre. Patients were scheduled for a protocol biopsy 7 ± 1 week after transplantation, followed by a thorough clinical investigation at 8 weeks. Patients were then transferred to their local hospitals for the clinical follow-up. Local nephrologists targeted the same tacrolimus trough concentrations as described above. At 1-year post-engraftment, patients were scheduled for an additional thorough clinical investigation at the transplant centre, including protocol biopsies.

Tacrolimus clearance estimation

Tacrolimus "clearance" was estimated by dividing the total daily dose by morning trough concentration [dose (mg)/trough (μg/l)], as previously described [8], and for simplicity called "clearance" in this manuscript. In short, the mean of all whole-blood tacrolimus concentrations obtained from 7 days prior to and 2 days after an in-depth investigation day performed 8 weeks after transplantation were used. For patients experiencing BPAR during the first 90 days, post-transplant clearance was also estimated from three tacrolimus dose and trough concentration pairs prior to the day of initiation of BPAR treatment. This was done to avoid the potential cytochrome P450 (CYP)-enzyme induction by the intravenous methylprednisolone therapy (treatment for BPAR) which could overestimate the determined tacrolimus clearance [9]. The patients were divided into four groups according to the quartiles of their estimated tacrolimus clearance; low, below average, above average and high clearance groups. The effect of donor age on histological scores was assessed by dividing the patients into three groups according to donor age; under 55, between 55 and 65, and over 65 years. The effect of living versus deceased donor was investigated by comparing biopsy scores between the two groups.

The study was approved by the Regional Committee for Medical Research Ethics and was performed in

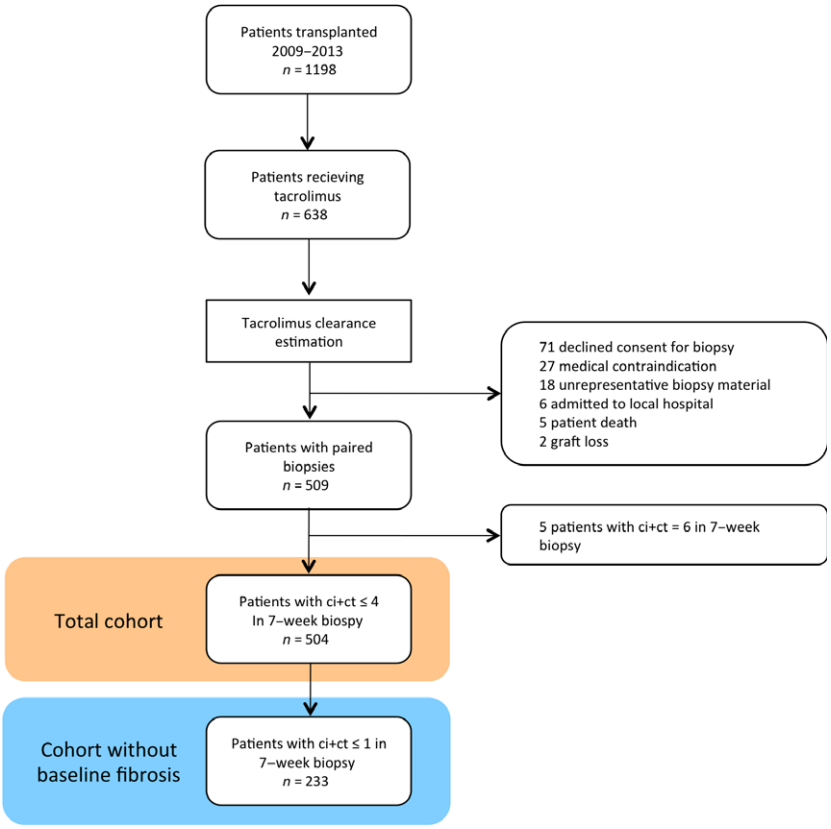

Figure 1 Patient flowchart.

accordance with the declarations of Helsinki and Istanbul. All patients signed a written informed consent.

Biopsies

Protocol biopsies were obtained at 7 ± 1 week and additionally 1 year ± 2 months post-transplantation during a period of stable graft function and without any recent rejections. Core biopsies were obtained with ultrasound guidance using an 18-gauge spring-loaded biopsy gun. Biopsies containing at least seven glomeruli, one artery and sufficient tubulointerstitial tissue to grade interstitial inflammation (i), tubulitis (t), interstitial fibrosis (ci), tubular atrophy (ct) and arteriolar hyalinosis (ah) were included in the analysis.

Histological classification

All biopsies were prospectively scored by three experienced nephropathologists at our transplant centre. Renal lesions were graded according to the Banff criteria [10]. Biopsies were classified into four groups according to previous investigations; (i) normal histology ($i + t \leq 1$ and $ci + ct \leq 1$), (ii) inflammation ($i + t \geq 2$ and $ci + ct \leq 1$), (iii) IFTA ($i + t \leq 1$ and $ci + ct \geq 2$) and (iv) IFTA with inflammation (IFTA + i) ($i + t \geq 2$ and $ci + ct \geq 2$) [11]. Arteriolar hyalinosis (Ah)-score was also included as a potential marker for tacrolimus nephrotoxicity [12]. Areas with fibrotic scars were excluded from evaluation.

Analysis of evolution of histological lesions

Evolution of IFTA was done in two different cohorts of patients; (i) the total cohort ($n = 504$), which excluded the five patients with ci + ct score of 6 at 7 weeks, and in (ii) the cohort without fibrosis at baseline ($n = 233$) showing $ci + ct \leq 1$ in the 7-week biopsy. In the total cohort change in ci + ct score of ≥2 was assessed as a proof of concept analysis of IFTA development, while *de novo* IFTA ($i + t \leq 1$ and $ci + ct \geq 2$) and IFTA + i ($i + t \geq 2$ and $ci + ct \geq 2$) were assessed in the cohort without baseline fibrosis. This strategy was used since the biopsy scoring system is not linear, i.e. any increase in ci + ct, in these patients, was considered as a worsening in IFTA. Tacrolimus nephrotoxicity was in addition assessed as an increase in ah-score of one step or more (≥1) in the patients from the total cohort with baseline ah-scores ≤2 in the 7-week biopsy ($n = 493$).

Statistical analyses

Results are expressed as frequencies for categorical variables or as the mean ± standard deviation for continuous variables. Categorical data were analysed with χ^2-test. Continuous data were analysed with Student's *t*-test for comparisons between two groups, and one-way ANOVA for comparisons between more than two groups. Two multivariable binomial logistic regression models were created using augmented backward elimination to study independent associations with outcome variables [13,14]. The outcome variable which was investigated in model 1 was increase of ci + ct score by two or more from 7 weeks to 1-year post-transplant in the total cohort of patients. In model 2 development of IFTA at 1 year, defined as $i + t \leq 1$ and $ci + ct \geq 2$, was the outcome variable in the cohort without baseline fibrosis of patients with $ci + ct \leq 1$ at 7 weeks. During the model building, covariates which had *P*-values above 0.2 or changed the other odds ratio estimates more than 0.05 were eliminated from the model in a stepwise fashion as described by Dunkler *et al.* [13]. The same covariates were included in initial model 1 and 2: Continuous estimated clearance was included as a passive variable (i.e. not excluded in the case of a *P*-value above 0.2 or change-in-estimate larger than 0.05 in the model building) as it is the explanatory variable of interest in the analyses. Donor age [15], number of HLA-DR mismatches [11] and BPAR first 90 days or between day 90 and 400 post-transplantation [16] and diabetes status at 8 weeks [17] were included due to *a priori* knowledge of association with IFTA development. High immunological risk was included since these patients have higher target tacrolimus trough targets than patients with standard immunological risk that may be associated with IFTA [3]. Deceased donor status, recipient age, recipient gender cytomegalovirus infection the first 7 weeks and delayed graft function (DGF, need of dialysis the first 7 days after transplantation) were included due to their significant differences between the clearance groups (Tables 1 and 2) which could be possible confounders in the final models. Patients lacking biopsies at 1 year did not meet the inclusion criteria. These patients were however included in separate sensitivity analyses and preformed for both model 1 and model 2. In one analysis the patients lacking 1-year biopsies were classified as events (i.e. increase of $ci + ct \geq 2$ in Model 1 and incidence of IFTA in Model 2), and in the other analysis the patients were classified as non-events. All *P*-values were 2-tailed and *P*-values below 0.05 were considered statistical significant. All analyses were done in R version 3.4.3 [18].

Results

A total of 1198 adult patients received a renal transplant from January 2009 to December 2013 at our centre. In total, 638 patients received tacrolimus as part of their initial immunosuppressive therapy and were grouped according to their estimated tacrolimus clearance. Of these patients, 509 had paired protocol biopsies of sufficient quality for histological scoring obtained at both 7 weeks and 1 year after transplantation. The 134 patients receiving tacrolimus treatment without paired biopsies or having ci + ct score of 6 at 7 weeks were evenly distributed between the clearance groups. Exclusion rates from the low, below average, above average and high clearance groups were 20%, 20%, 24% and 21% respectively ($P = 0.77$). Patient flowchart with reasons for patient exclusion is shown in Fig. 1.

Baseline demographics of all patients ($n = 504$) included in the analyses are shown in Table 1A. The high clearance group was younger, had more patients with DGF and had more women compared to the other groups. The results were similar in the cohort without baseline fibrosis of patients with $ci + ct \leq 1$ at 7 weeks except for the insignificant difference in the gender distribution (Table 1B). In the high clearance group, tacrolimus doses were higher and trough concentrations were lower in both low and high immunological risk patients (Table 2). All trough concentrations were within target ranges for both low and high immunological risk patients. The tacrolimus trough concentrations in high immunological risk patients without baseline fibrosis were not significantly different (Table 2B). Significantly more patients in the above average clearance group experienced CMV infection the first 7 weeks (Table 2).

Histological diagnosis at 7 weeks

In the 7-week biopsies there were no significant differences in histological scores between patients in the four clearance groups (Fig. 2). Recipients with deceased donors were less likely to have normal histology and showed significantly more IFTA and arteriolar hyalinosis (ah ≥1) compared to recipients with living donors ($P < 0.001$ for all comparisons; Table S1). Donor age below 55 years was associated with a higher rate of normal histology, less IFTA and less arteriolar hyalinosis ($ah \geq 1$) compared to the other donor age groups (55–

Table 1. Patient characteristics at time of transplantation according to tacrolimus clearance groups.

	Low clearance	Below average	Above average	High clearance	*P*-value
A: Total cohort, *n* = 504	*n* = 129	*n* = 128	*n* = 121	*n* = 127	
Immunologic high risk*	14 (11)	24 (19)	15 (12)	23 (18)	0.18†
DSA at transplantation	10 (8)	17 (14)	12 (10)	16 (13)	0.44†
Recipient age, years	51.7 ± 14.7	51.5 ± 15.4	48.2 ± 14.3	47.7 ± 13.5	0.008‡
Recipient male gender	99 (77)	90 (71)	74 (61)	73 (57)	0.004†
Recipient weight, kg	74.6 ± 15.3	75.1 ± 14.1	77.5 ± 17.5	75.0 ± 14.3	0.54‡
Living donor	44 (34)	50 (39)	47 (39)	44 (35)	0.75†
Cold ischemia time, h	10.2 ± 6.2	9.6 ± 6.4	9.5 ± 6.4	9.9 ± 6.6	0.73‡
Delayed graft function	6 (5)	2 (2)	7 (6)	16 (13)	0.003†
Donor age, years	49.6 ± 15.8	50.2 ± 14.5	48.3 ± 16.3	49.7 ± 13.9	0.82‡
Donor male gender	70 (54)	69 (55)	65 (54)	64 (51)	0.93†
B: Cohort without fibrosis, ci + ct ≤ 1 at 7-weeks, *n* = 233	*n* = 68	*n* = 60	*n* = 53	*n* = 52	0.27†
Immunologic high risk*	8 (12)	15 (25)	5 (9)	10 (19)	0.090†
DSA at transplantation	6 (9)	10 (17)	3 (6)	6 (12)	0.28†
Recipient age, years	51.0 ± 13.3	48.9 ± 15.0	46.7 ± 14.7	46.4 ± 12.8	0.048‡
Recipient male gender	48 (71)	35 (58)	32 (60)	32 (62)	0.49†
Recipient weight, kg	73.0 ± 15.1	74.1 ± 13.8	78.1 ± 19.0	76.6 ± 14.6	0.10‡
Living donor	31 (46)	32 (53)	25 (47)	26 (50)	0.84†
Cold ischemia time, h	8.7 ± 6.4	7.9 ± 5.9	8.5 ± 6.1	8.0 ± 5.8	0.66‡
Delayed graft function	1 (1)	1 (2)	1 (2)	6 (12)	0.014†
Donor age, years	42.8 ± 14.1	46.0 ± 12.7	43.5 ± 15.7	43.6 ± 12.4	0.93‡
Donor male gender	34 (50)	31 (52)	23 (44)	21 (40)	0.61†

DSA, donor specific antibodies.

Continuous data are presented as mean ± SD, categorical data as *n* (% of group total).

*Defined as presence of donor specific antibodies and/or PRAs >20% and/or ABO incompatibility at transplantation.

†χ^2-test.

‡One-way ANOVA.

Table 2. Immunosuppressive therapy and selected clinical events the first year post-transplant in the total cohort (A) and in the cohort without fibrosis (B).

	Low clearance	Below average clearance	Above average clearance	High clearance	*P*-value
A: Total cohort, *n* = 504	*n* = 129	*n* = 128	*n* = 121	*n* = 127	
Tac clearance, mg/mg/l	0.38 ± 0.10	0.62 ± 0.06	0.85 ± 0.08	1.50 ± 0.55	<0.001*
Tac dose standard risk patients, mg	2.9 ± 0.8	4.1 ± 0.9	5.4 ± 1.2	8.3 ± 2.4	<0.001*
Tac C_0 standard risk patients, mg/l	7.7 ± 2.1	6.6 ± 1.4	6.4 ± 1.4	5.7 ± 1.3	<0.001*
Tac dose high-risk patients†, mg	3.8 ± 1.1	5.6 ± 1.1	6.7 ± 1.6	10.3 ± 2.9	<0.001*
Tac C_0 high-risk patients†, mg/l	9.6 ± 1.7	9.0 ± 1.7	7.8 ± 1.6	7.5 ± 1.6	<0.001*
Prednisolone dose 7 weeks, mg	10.1 ± 2.8	10.5 ± 3.8	10.7 ± 3.1	10.8 ± 3.5	0.12*
Prednisolone dose 1 year, mg	5.3 ± 2.1	5.5 ± 1.7	5.3 ± 1.0	5.4 ± 1.4	0.95*
Mycophenolate dose 7 weeks, mg	1444 ± 155	1459 ± 167	1456 ± 146	1447 ± 176	0.93*
Mycophenolate dose 1 year, mg	1293 ± 337	1299 ± 341	1234 ± 366	1291 ± 318	0.64*
BPAR first 90 days	11 (9)	12 (9)	17 (14)	28 (22)	0.006‡
BPAR day 90–400	3 (2)	8 (6)	6 (5)	1 (1)	0.077‡
CMV infection first 7 weeks	7 (5)	3 (2)	11 (9)	3 (2)	0.039‡
dnDSA at 1 year	5 (4)	6 (5)	6 (5)	2 (2)	0.74‡
B: Cohort without fibrosis, ci + ct ≤ 1 at 7-weeks, *n* = 233	*n* = 68	*n* = 60	*n* = 53	*n* = 52	
Tac clearance, mg/mg/l	0.39 ± 0.10	0.62 ± 0.06	0.85 ± 0.07	1.55 ± 0.69	<0.001*
Tac dose standard risk patients, mg	2.8 ± 0.8	4.1 ± 1.0	5.3 ± 1.0	8.0 ± 2.1	<0.001*
Tac C_0 standard risk patients, mg/l	7.5 ± 2.0	6.6 ± 1.4	6.3 ± 1.1	5.5 ± 1.4	<0.001*
Tac dose high-risk patients†, mg	3.4 ± 0.9	5.6 ± 1.3	6.8 ± 1.8	11.0 ± 3.7	<0.001*
Tac C_0 high-risk patients†, mg/l	9.0 ± 1.7	9.0 ± 1.9	7.5 ± 1.8	8.2 ± 1.3	0.14*
Prednisolone dose 7 weeks, mg	10.2 ± 3.4	10.0 ± 3.7	11.1 ± 3.8	10.8 ± 4.0	0.20*
Prednisolone dose 1 year, mg	5.2 ± 2.8	5.5 ± 1.3	5.4 ± 1.2	5.4 ± 1.2	0.70*
Mycophenolate dose 7 weeks, mg	1443 ± 159	1439 ± 186	1441 ± 154	1453 ± 189	0.82*
Mycophenolate dose 1 year, mg	1359 ± 321	1325 ± 288	1270 ± 340	1347 ± 274	0.55*
BPAR first 90 days	5 (7)	3 (5)	9 (17)	9 (17)	0.069‡
BPAR day 90–400	0 (0)	1 (2)	2 (4)	0 (0)	0.24‡
CMV infection first 7 weeks	3 (4)	0 (0)	6 (11)	1 (2)	0.021‡
dnDSA at 1-year	3 (4)	2 (3)	4 (8)	1 (2)	0.57‡

BPAR, biopsy-proven acute rejection; CMV, cytomegalovirus; dnDSA, *de novo* donor specific antibodies; Tac, tacrolimus.

Continuous data are presented as mean ± SD, categorical data as *n* (% of group total).

*One-way ANOVA.

†Defined as presence of donor specific antibodies and/or PRAs >20% and/or ABO incompatibility at transplantation.

‡χ^2-test.

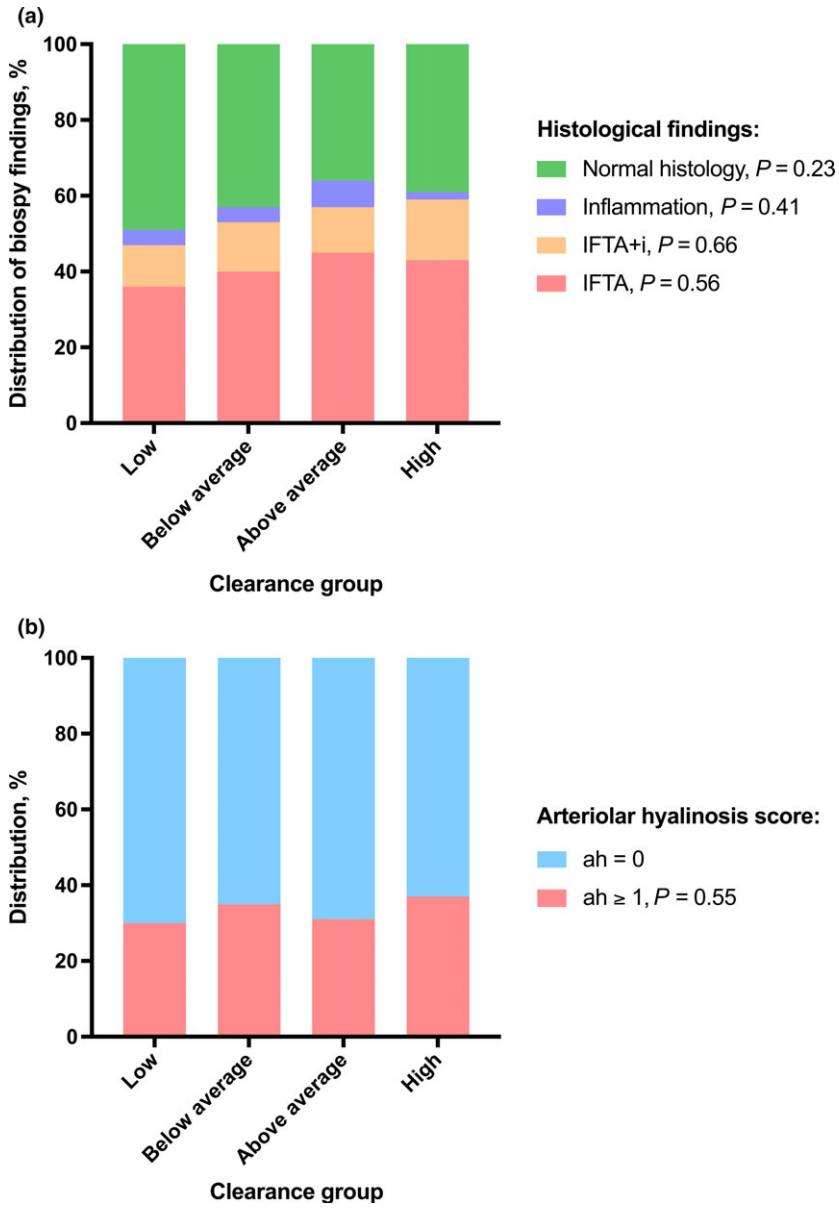

Figure 2 Prevalence of (a) biopsy scores in the total patient cohort (n = 504) and (b) arteriolar hyalinosis scores in the patients with ah-score of ≤2 (n = 493) at 7-weeks post-transplantation.

65 years and above 65 years) ($P < 0.001$ for all comparisons; Table S2).

Evolution of change in histological lesions between 7 weeks and 1 year

In the total cohort, more patients in the high clearance group had a ≥2 increase in ci + ct score from 7 weeks to 1-year post-transplant compared to the other clearance groups (Fig. 3). In the cohort without baseline fibrosis (n = 233) a significantly larger proportion of the patients in the high clearance group of developed *de novo* IFTA (i + t ≤ 1 and ci + ct ≥ 2) from 7 weeks to 1 year compared to the other clearance groups (Fig. 4a). There were subsequently more patients with normal histology in the low clearance group compared with the other groups. There was no difference in the increase in the ah-score with one step or more (≥1) between the clearance groups (Fig. 4b). No other histological findings showed significant changes during the first year post-transplantation between the clearance groups.

Grafts from deceased donors showed significantly less normal histology (i + t ≤ 1 and ci + ct ≤ 1) at 1 year compared to living donors (Table S3). There was however no significant difference in development of IFTA during the first post-transplant year between living and deceased donor patients in the cohort without baseline fibrosis (Table S4).

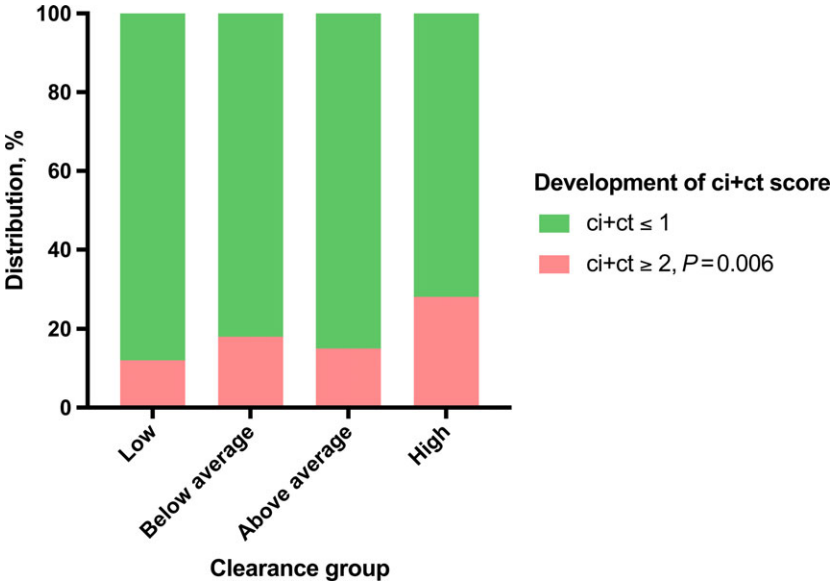

Figure 3 Increase of ci + ct score ≥2 from 7-weeks to 1-year post-transplantation in the total patient cohort (n = 504).

Donor age also influenced histology scores at the 1-year time point, showing significantly less normal histology, more IFTA and ah ≥1 with increasing age (P-value for all comparisons <0.001, Table S5). Twenty-eight percent (52 of 187) of recipients with donor age below 55 years which had ci + ct ≤ 1 at 7 weeks developed *de novo* IFTA at 1 year compared to 33% (10 of 30) of recipients with donors between 55 and 65 years and 63% (10 of 16) of recipients with donors older than 65 years, P = 0.015 (Table S6). Kidney age also affected development of arteriolar hyalinosis during the first year after transplantation. Twenty-six percent (20 of 77) of recipients with donors above 65 years and 25% (26 of 103) of recipients with donors between 55 and 65 years had an increase in ah-score of ≥1 compared to 15% (46 of 313) of recipients with donors younger than 55 years, P = 0.012.

Tacrolimus clearance as a predictor of IFTA

Tacrolimus clearance was applied as an explanatory variable in two separate multivariable binomial logistic regression models presented in Tables 3 and 4. Model 1 was made with the total patient cohort (n = 504). Tacrolimus clearance was significantly associated with an increase in ci + ct score ≥2 from 7 weeks to 1 year (Table 3). The final model 1 was adjusted for high immunological risk and BPAR between day 90 and 400, which also was significantly associated with increase in ci + ct score. All other covariates were backwards eliminated due to P-values above 0.2.

The second multivariable model included the 233 patients without baseline fibrosis, having ci + ct ≤ 1 in their 7-weeks biopsies. The final model 2 showed significant association between tacrolimus clearance and increased odds of developing *de novo* IFTA. The final multivariable model 2 was adjusted for immunological high risk and donor age, which also was significantly associated with *de novo* IFTA development and recipient male gender which showed a protective tendency for development of *de novo* IFTA. The model is presented in Table 3. The overall results from the sensitivity analyses were similar to the results from the final models (Model 1 and 2) (Tables S7–S10).

Discussion

The main finding in this large, longitudinal study was that renal transplant recipients with a phenotype of high tacrolimus clearance showed an increased risk of developing IFTA during the first year post-engraftment. This was shown in two different analyses investigating biopsy changes from 7 weeks to 1-year post-engraftment. The first analysis included all tacrolimus treated patients with paired biopsies from 7 weeks and 1 year where more patients in the high clearance group developed a ci + ct ≥ 2 score in theses biopsies. The other analysis included patients with kidneys not showing any signs of IFTA at 7 weeks. More than twice as many patients in the highest quartile of tacrolimus clearance developed *de novo* IFTA during the first post-transplant year compared to the lowest quartile.

The significance of this finding is further substantiated by the multivariable logistic regression analyses of this easily assessable risk marker of tacrolimus clearance. An increase of 1 "clearance unit" was associated

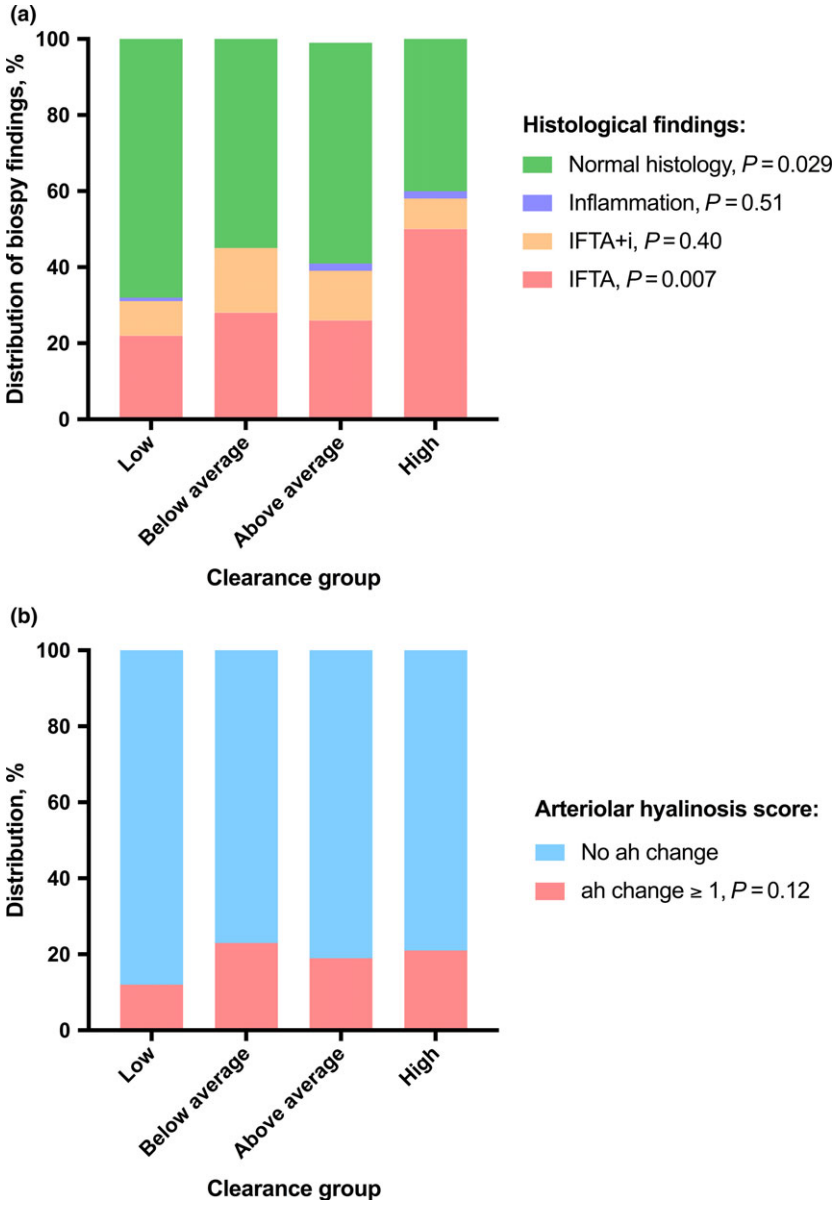

Figure 4 Development of (a) biopsy scores in the (n = 233) patients without baseline fibrosis at 7-weeks and (b) arteriolar hyalinosis in the patients with ah-score ≤2 at 7 weeks (n = 493) from 7-weeks to 1-year post-transplantation.

with twice as high odds ratio for development of *de novo* IFTA during the first year. In other words, a patient needing 7.5 mg tacrolimus per day to achieve a trough concentration of 5 µg/l had a twice as high odds ratio for developing *de novo* IFTA compared with a patient taking 2.5 mg tacrolimus per day to achieve the same trough concentration. Similarly do the same patients with a higher clearance have a 64% higher odds for a two or more biopsy verified increase in ci + ct score from 7 weeks to 1 year. The latter analysis was adjusted for immunological high-risk status and BPAR between day 90 and 400 and the interpretation should be done with caution as fewer adjustment variables may lead to larger odds ratios. Of note, there was no association with increasing tacrolimus clearance and the development of ≥1 ci + ct score (data not shown). Implying that this biomarker is not sensitive enough for predicting small changes in IFTA-score in kidneys already exposed to some degree of IFTA.

Tacrolimus trough concentrations were not included in the multivariable analyses since both high- and standard immunological risk patients were included for a more real-world analysis of IFTA development. These patient groups have different target trough concentrations and inclusion of the concentrations could have biased the analyses. However, the final models were adjusted for high immunological risk status and tacrolimus clearance was still significantly associated with both development of *de novo* IFTA in the cohort without

Table 3. Multivariable logistic regression analysing odds ratios for increase in ci + ct score of ≥2 from 7 weeks to 1-year post-transplant (n = 504).

	Initial multivariable model 1			Final model 1		
	Odds ratio	95% CI	*P*-value	Odds ratio	95% CI	*P*-value
Clearance*	1.54	0.98–2.40	0.057	1.67	1.11–2.51	0.013
Immunologic high risk†	2.03	1.12–3.60	0.018	2.18	1.23–3.79	0.006
Donor age, years	1.01	0.99–1.03	0.30			
Recipient male gender	0.93	0.57–1.55	0.79			
Recipient age, years	1.00	0.98–1.01	0.68			
HLA-DR mismatch	0.82	0.56–1.21	0.32			
DGF, yes	1.25	0.48–3.03	0.63			
BPAR first 90 days	1.43	0.70–2.79	0.31			
BPAR 90–400 days	4.06	1.46–10.96	0.006	3.84	1.40–10.20	0.007
Living donor	1.24	0.75–2.04	0.39			
Diabetes at 7 weeks	0.93	0.49–1.67	0.80			
CMV infection first 7 weeks	1.42	0.48–3.67	0.49			

BPAR, biopsy-proven acute rejection; DGF, delayed graft function; HLA, human leukocyte antigen.

The final multivariable model was built by augmented backwards elimination.

*Odds ratio per unit increase in continuous tacrolimus clearance estimated by [daily tacrolimus dose (mg)/trough concentration (μg/l)] at 8 weeks or before BPAR treatment.

†Immunological high risk is defined as presence of donor specific antibodies and/or PRAs >20% and/or ABO incompatibility at transplantation.

Table 4. Multivariable logistic regression analysing odds ratios for developing IFTA at 1-year post-transplantation in patients with ci + ct ≤ 1 at 7 weeks (n = 233).

	Initial multivariable model 2			Final model 2		
	Odds ratio	95% CI	*P*-value	Odds ratio	95% CI	*P*-value
Clearance*	2.19	1.16–4.20	0.016	2.01	1.18–3.50	0.010
Immunologic high risk†	3.32	1.49–7.53	0.003	3.27	1.51–7.19	0.003
Donor age, years	1.06	1.03–1.09	<0.001	1.06	1.03–1.08	<0.001
Recipient male gender	0.65	0.34–1.22	0.18	0.62	0.33–1.14	0.12
Recipient age, years	0.99	0.97–1.01	0.43			
HLA-DR mismatch	0.88	0.52–1.47	0.62			
DGF, yes	0.70	0.11–4.06	0.70			
BPAR first 90 days	0.80	0.24–2.41	0.70			
Living donor	0.92	0.48–1.77	0.81			
Diabetes at 7 weeks	1.20	0.53–2.63	0.66			
CMV infection first 7 weeks	2.21	0.44–10.59	0.32			

BPAR, biopsy-proven acute rejection; DGF, delayed graft function; HLA, human leukocyte antigen.

The final multivariable model was built by augmented backwards elimination.

*Odds ratio per unit increase in continuous tacrolimus clearance estimated by [daily tacrolimus dose (mg)/trough concentration (μg/l)] at 8 weeks or before BPAR treatment.

†Immunological high risk is defined as presence of donor specific antibodies and/or PRAs >20% and/or ABO incompatibility at transplantation IFTA defined as i + t ≤ 1 and ci + ct ≥ 2.

baseline fibrosis and an increase of ci + ct score ≥2 in the total cohort.

As expected, kidneys from both older and deceased donors showed significantly more IFTA and arteriolar hyalinosis at 7 weeks post-engraftment. In the analysis investigating development of *de novo* IFTA from 7 weeks to 1 year, the patients already having IFTA at 7 weeks were not included. Even when removing this

bias there was a significant effect of donor age on the development of IFTA. This probably implies that kidneys from older donors, even if in good condition at the time of transplantation are more vulnerable for development of tacrolimus induced IFTA [15].

We have previously found that high tacrolimus clearance was associated with an increased risk of BPAR during the first 3 months post-engraftment [8]. There was no association between earlier BPAR status and development of IFTA in the final multivariable analyses.

In the current study no association was shown between high tacrolimus clearance and development of arteriolar hyalinosis. Historically, arteriolar hyalinosis has been regarded as the hallmark of calcineurin inhibitor nephrotoxicity [12]. Arteriolar hyalinosis has also been associated with aging, diabetes and hypertension [19,20]. The present data support that arteriolar hyalinosis is not a specific marker for tacrolimus nephrotoxicity.

Tacrolimus is extensively metabolised by the CYP3A-family and patients expressing functional CYP3A5 enzymes (i.e. hetero- or homozygote *CYP3A5*1*) have higher tacrolimus clearance [21]. CYP3A5 genotype data were not routinely assessed in this cohort and are a limitation to the present study. There are most likely a higher proportion of CYP3A5 expressers in the group of patients with high clearance phenotype compared with the other phenotypes. But there will be patients in the groups with lower estimated clearance that express CYP3A5 and patients in the high clearance group that do not express CYP3A5 [5]. CYP3A5-status is likely to influence the clearance estimate, but other factors affecting tacrolimus clearance will also be reflected in the estimate. Our clearance estimate phenotypes the high clearance which may come from other factors in addition to CYP3A5. The association between CYP3A5-expression and incidence of various histological lesions has earlier been investigated in renal transplanted patients with inconsistent findings. Some studies have observed a significant association [4,5], while others have not [15,22–24]. One study has even found an association between the non-expressing genotype (*CYP3A5*3*) and nephrotoxicity [25], supporting that other factors most probably also are involved.

The mechanisms of tacrolimus induced nephrotoxicity need elucidation. To achieve similar tacrolimus trough concentrations patients with high clearance need, higher doses and hence higher tacrolimus peak concentrations (C_{max}) are obtained. The more rapid elimination will also result in lower concentrations in case of an offset or skipped dose that may induce transient periods of subtherapeutic tacrolimus concentrations [6]. Such intermittent underexposure may lead to alloimmune activation, which has been shown to drive fibrosis [26,27]. If this is the case then the present findings cannot discriminate if it is the high peak concentrations and/or transient under-immunosuppressive episodes that drives the IFTA development. In high clearance patients using immediate release, twice daily, tacrolimus formulations will risk experiencing both low and high concentration patterns. Switching to a prolonged release, once daily, formulation may benefit these patients [28]. However, a previous study comparing twice daily versus once daily tacrolimus showed however no differences in biopsy-findings obtained 14 days and 6–12 months after transplantation [29].

Another potential explanation for the present findings is higher levels of tacrolimus metabolites in patients with high clearance. To our knowledge, no studies comparing demethylated metabolite concentrations and histological lesions in transplanted kidneys have been conducted. Zegarska *et al.* [30] found a significant negative correlation between the 15-O-demethyl tacrolimus (15-DMT, also named M-III) concentration and estimated glomerular filtration rate, which indirectly may support this hypothesis.

Patients lacking biopsy 1-year post-transplant were not included in the main analyses. This may lead to bias due to conditioning on a future event. The sensitivity analyses did however reveal that there was only minor bias from this conditioning.

The tacrolimus clearance estimate used in this study was calculated from a single period after transplantation for each patient. A limitation to this clearance estimate is that we unfortunately do not have data estimating clearance over time in the weeks and months following transplantation. We have not found any associations between estimated tacrolimus clearance and survival of patients or grafts (data not shown). This may be due to insufficient follow-up time and will be reassessed. The main strength of this retrospective study is the large sample size and the fact that it is a national cohort of patients transplanted at the same centre with a uniform clinical follow-up.

Conclusion

We have found a significant association between high tacrolimus clearance estimated in the early phase post-transplantation and the development of IFTA from 7 weeks to 1 year after transplantation. The causality of the association is not assessable from this study, but larger fluctuations in tacrolimus concentrations with high

peaks and transient under immunosuppression, and increased exposure to metabolites may be a part of the explanation. Switching to extended release tacrolimus may be beneficial for these patients, but this must be further investigated.

Authorship

EJE, AVR and AÅ: designed the study. EJE, AVR, KM, EHS, HH, AH and AÅ: collected the data. EJE and AÅ: did data analysis and statistics. EJE, IR, and AÅ: wrote the article, whereas all authors have been involved in the discussion of results and have contributed to, read, and approved the final article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Prevalence of selected biopsy classifications 7-weeks post-transplantation in recipients with living and deceased donors.

Table S2. Prevalence of selected biopsy classifications 7-weeks post-transplantation with different donor ages.

Table S3. Prevalence of selected biopsy classifications 1-year post-transplantation in recipients with living and deceased donors.

Table S4. Development of selected biopsy-scores by 1-year post-transplant in recipients with living and deceased donors with ci + ct ≤ 1 at 7 weeks.

Table S5. Prevalence of selected biopsy classifications 1-year post-transplantation with different donor ages.

Table S6. Development of selected biopsy-scores by 1-year post-transplant in recipients with different donor ages in patients with ci + ct ≤ 1 at 7 weeks.

Table S7. Multivariable logistic regression analysing odds ratios for increase in ci + ct score of ≥2 from 7 weeks to 1-year post-transplant in patients with 7 week biopsies (n = 573).

Table S8. Multivariable logistic regression analysing odds ratios for increase in ci + ct score of ≥2 from 7 weeks to 1-year post-transplant in patients with 7-week biopsies (n = 573).

Table S9. Multivariable logistic regression analysing odds ratios for developing IFTA at 1-year post-transplantation in patients with ci + ct ≤ 1 at 7 weeks (n = 257).

Table S10. Multivariable logistic regression analysing odds ratios for developing IFTA at 1-year post-transplantation in patients with ci + ct ≤ 1 at 7 weeks (n = 257).

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