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A retrospective cohort study found that lipids and albumin had only a little impact on the apparent mycophenolic acid clearance in pediatric transplant recipients.

Mark-Christoph Müller1, Florian Reitz2 · Nicolas Roy3
Department of Pharmacological Research

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Background

Therapeutic drug monitoring (TDM) is necessary for critical dosage medications, such as calcineurin inhibitors and inhibitors of the mammalian target of rapamycin (MTOR), in the context of juvenile kidney transplantation [1]. Mycophenolate mofetil's (MMF) TDM has been required since discussed [2]. There is mounting evidence that TDM of mycophenolic acid (MPA), the active ingredient of MMF, may assist prevent underexposure and enable maintenance of the target exposure area-under-the-time concentration curve (AUC) of 30–60 mg*h/L, which may lessen rejection in the early post-renal transplant period [3-5]. The benefits of long-term MPA monitoring following transplantation are less well-established, despite When considering a fixed combination of tacrolimus and MMF, the trough level might be a viable biomarker of exposure [6]. Long-term MPA underexposure may be linked to the development of de novo donor-specific antibodies (DSA) [7, 9], which are linked to poorer graft outcomes [10, 11], according to some recent data. It's possible that MPA exposure varies more than calcineurin inhibitor exposure [2]. Pediatric kidney transplant recipients' pharmacokinetics are obviously impacted by age and medication interactions [12, 13]. Nevertheless, many facilities do not regularly monitor MPA levels, and TDM of MPA is not generally accessible. Hemoglobin, albumin, and GFR may have an impact on MPA clearance, according to a few early adult articles on the pharmacokinetics of MMF [14–16]. Studies examining the effects of hemoglobin, albumin, or GFR alterations on MPA exposure in pediatric renal transplant recipients are unknown to us. In order to better understand our long-term pediatric renal transplant group that solely received concurrent tacrolimus, we conducted a retrospective study of all available MPA trough levels. Using an estimated AUC derived from published literature, we computed the apparent clearance (CL/F) [6]. The variability of CL/F was predicted to be influenced by serum creatinine, hemoglobin, albumin, cystatin C, and eGFR. Additionally, we added triglycerides and cholesterol. Understanding these interactions may help doctors who do not have access to MPA TDM modify MMF dosage in addition to testing for UGT1A9 gene polymorphisms. In order to investigate the effect, we employed correlation analysis between the suggested factors and the apparent MPA CL/F. Our hypothesis was that MPA CL/F might be enhanced by greater triglycerides, higher cholesterol, and lower albumin.

Materials and Procedures

The Western University Research Ethics Board gave their approval to the project (HSREB File Number 105148). [6, 7, 12, 13] We conducted a retrospective analysis of all available data on 35 children who received kidney transplants and were monitored from January 1, 2004, to June 30, 2018. All pediatric patients under the age of eighteen who had received a kidney transplant, including re-transplants (n=2), had a functional graft, were monitored at the London Health Sciences Center's Children's Hospital, and received MMF plus tacrolimus were included in this study cohort. Patients who were not receiving concurrent sirolimus or cyclosporine medication or MMF therapy were not included. A divisional database was used to identify the patients. Because the follow-up was too brief and the MPA measures were not yet in a steady condition, one recent transplant patient with only three measurements was excluded. (Figure 1) We only took into account the MPA measurements made using HPLC/MS/MS, which led to the exclusion of one patient who only had EMIT assay MPA values. This patient got a combination liver kidney transplant for hyperoxaluria type 1 without any indication of liver disease or

cholestasis. EMIT is known to cause MPA levels to be overestimated [17].

Clinical data
Anthropometric information (height, weight, and blood pressure), gender, age at transplant, follow-up period following transplantation, and daily concurrent medication (data not shown) were gathered from the patient's paper and computerized documents. We obtained the following laboratory tests using our electronic health record in addition to all accessible MPA concentrations: creatinine, albumin, hemoglobin, cholesterol, and triglyceride levels, as well as the concentration of cystatin C. There were no increased bilirubin or transaminases in any of the subjects. The Filler formula was used to determine eGFR [18]. The following formula was used to determine the AUC from the trough level:

Using the following formula, CL/F was computed by dividing the dose during a given interval by the AUC: Dose/AUC = CL/F

Measurements and modifications of MPA and tacrolimus concentrations
Every patient received concurrent tacrolimus and MMF treatment. Starting at 0.15 mg/kg/dose, tacrolimus was adjusted based on the patient's trough concentrations. Target tacrolimus concentrations, which were not the focus of this investigation, were 10–20 ng/mL in the first month after transplantation. The trough concentration then gradually decreased to 5–10 ng/mL at one year and then to 4-6 ng/mL after that. Because the MPA pre-dose trough level could be used as a stand-in for the MPA area-under-the-time-concentration curve, patients with concurrent tacrolimus were the only ones employed [6]. Only information up until the age of 18 was included.
In two separate dosages, patients were initially given 1200 mg of MMF per 1 m2 of body surface area [19]. For young children, a greater starting dose was sometimes selected. To make dosing easier in the event that they took the prescription in capsule form, dosages were rounded to the closest 250 mg. MPA trough concentrations, which were recorded during the patients' regular clinic visits, were used to modify the dosage on an individual basis. Although maintaining an MPA trough concentration >1.3 mg/L was generally the goal of physicians [7], patients and physicians had different target exposures. For example, although there was no official procedure to this effect, individuals with BK virus nephropathy or leukopenia were generally targeted for a reduced MPA exposure.
HPLC/MS/MS was used to measure the MPA concentrations. The MPA concentrations have a lower limit of quantification of 0.1 mg/L. We employed the novel Schwartz algorithm to estimate creatinine-based eGFR [20]. The formula suggested by Filler and Lepage [18] was used to compute the cystatin C eGFR. All tests have an overall imprecision of less than 5%.
Analysis of statistics
The D'Agostini Pearson Omnibus test was used to determine whether the data were normal. For a normal distribution, descriptive data are shown as mean ± one standard deviation; for a non-normal distribution, they are shown as median and interquartile range. We employed a univariate method to identify the characteristics that linked with CL/F using Spearman rank correlation tests because the majority of the data were not normally distributed. Missing values were not taken into account. Due to the interdependencies of multiple parameters, mixed effect multivariate regression analysis was performed after this.
Excel (Office for Mac 2011, version 14.3.8), STATA version 11.2 for Mac (StataCorp, College Station, TX, USA), and Graph Pad Prism for Mac OS X version 5 (Graph Pad Software, San Diego, CA, USA) were used for all analyses. P-values less than 0.05 were regarded as statistically significant.
Findings
Table 1 describes the cohort's demographic features.

$$AUC = 6.743 \times Ctrough + 34.8 \quad [6]$$

where Ctrough is the MPA's pre-dose trough level.

Characteristic n = 35	
Demographics	
Age at Transplantation, (years) [median, IQR 25-75]	8.7 [4.3 - 12.6]
Gender Male, n % Female, n %	 15 (43%) 20 (57%)
Follow up (years) [median, IQR 25-75]	5.8 [4.6 – 12.6]
Pharmacological parameters	
MPA levels n [mean, Min - Max]	30 [9 – 149]
MPA AUC (mL*h/L) [median, IQR 25-75]	53.2 [45.6 – 62.7]
CL/F [L/ h] [mdn, IQR 25-75]	8.66 [6.31 – 13.20]
Biochemical Parameters	
Creatinine [umol/L] [median, IQR 25-75]	79 [55 - 106]
Cystatin C [mg/L] [median, IQR 25-75]	1.30 [1.08 -1.64]
Cystatin C eGFR [mL/min/1.73m^2] [median, IQR 25-75]	69 [53 – 84]
Hemoglobin [g/L] [median, IQR 25-75]	108 [63 – 178]
Albumin [g/L] [median, IQR 25-75]	44 [42 -46]
Cholesterol (31) [median, IQR 25-75]	3.83 [3.22– 4.43]
Triglycerides (31) [median, IQR 25-75]	1.41 [0.95 - 1.91]

Apart from the age at transplantation and the number of MPA levels, the values above represent the average of the average for each patient. One patient never received a triglyceride measurement. Table Glossary: MPA=Mycophenolic Acid, CL/F=Apparent Clearance, eGFR=estimated cystatin C GFR.

Table 1: Patient characteristics.

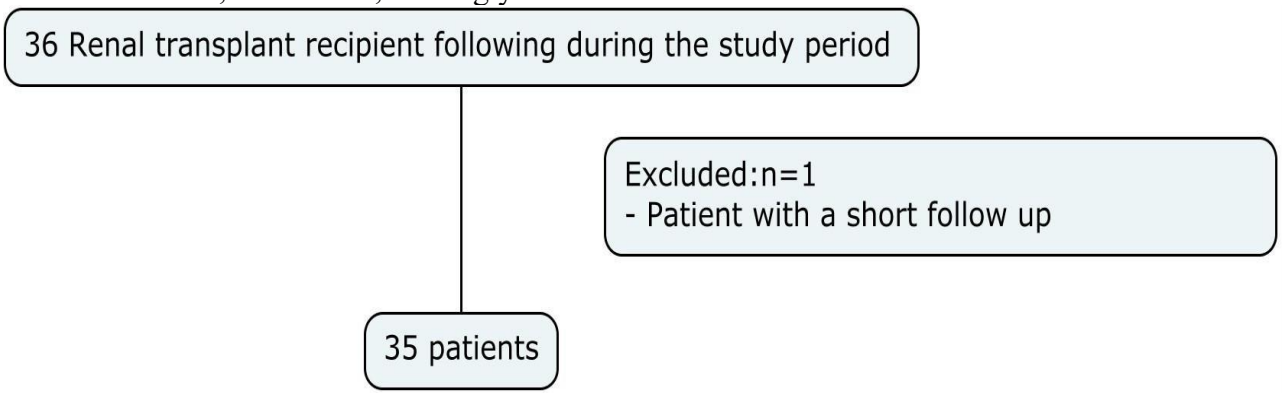
Twenty of the thirty-five patients were female. Renal dysplasia (n = 10), obstructive uropathy (n = 11), FSGS (n = 4), ARPDK (n = 3), glomerulonephritis (n = 2), spina bifida (n = 2), cystinosis (n = 1), HUS (n = 1), and hyperoxaluria type I (n = 1) were the underlying diagnosis. With a mean follow-up of 7.8₱4.8 years (median 5.8 years, 25th percentile 4.6, 75th percentile 12.6 years), the average age at transplant was 8.7₱4.6 years. The median MPA trough concentrations for each subject were 30.(from 9 to 149). We were able to compute 1133 MPA AUCs and 1005 CL/F from the 1138 MPA levels. With a 25th percentile of 45.59 and a 75th percentile of 62.72 mg*h/L, the median AUC was 53.21 mg*h/L. With an interquartile range of 6.31 to 13.20 L/kg, or a 70-fold variation, the median CL/F was 8.66 L/h.

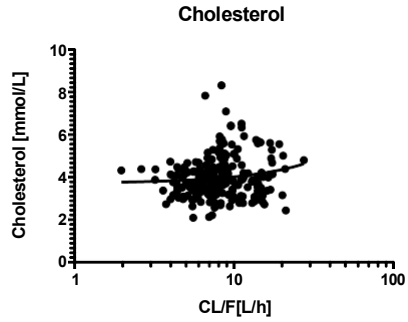
Parameter	Creatinine	Cystatin C	CysC eGFR	Hgb	Albumin	Chol	Trig
Number of XY Pairs	898	334	334	771	657	231	229
Spearman r	0.1624	-0.058	0.058	-0.055	-0.144	0.163	0.189
95% confidence interval	0.096 to 0.227	-0.168 to -0.052	-0.052 to 0.168	-0.128 to -0.017	-0.221 to -0.066	0.031 to 0.290	0.128 to 0.308
P value (two-tailed)	<0.0001	0.289	0.293	0.124	0.0002	0.0132	0.0058

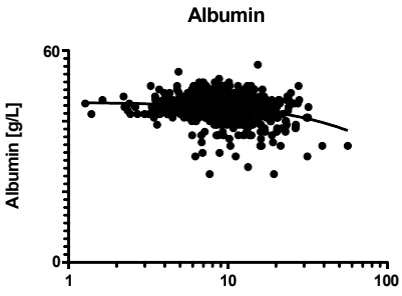
CysC=cystatin C, Hgb=hemoglobin, Chol=cholesterol, Trig=triglycerides

Table 2: Correlation analysis of the main biomarkers for GFR, eGFR, albumin, hemoglobin and lipid parameters with CL/F based on dose/kg. All were significant using univariate analysis, except albumin and cholesterol

After that, we conducted correlation analyses. Table 2 presents the CL/F based on dose/kg findings. Creatine, albumin, cholesterol, and triglycerides all showed significant correlations with CL/F [L/h] in the univariate study (table 2), while cystatin C, Hemoglobin and cystatin C did not eGFR. Figure 1 shows the non-linear regression lines and the relationships between CL/F and albumin, cholesterol, and triglycerides.







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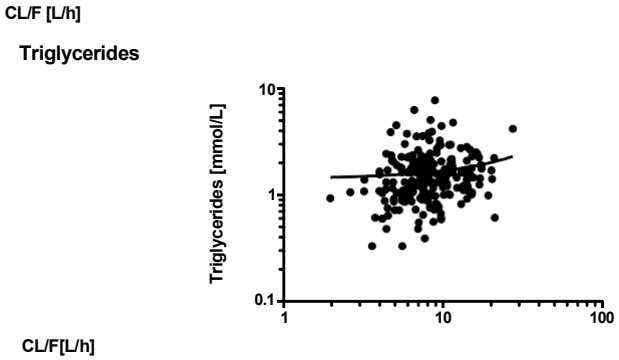

Figure 2: Correlation analysis of cholesterol, albumin and triglycerides. The regression lines were calculated using a non-linear one-phase exponential growth equation.

Lastly, we used mixed effect multivariate regression analysis on all available observations for CL/F [L/h]. Just albumin and creatinine and the multivariate logistic regression analysis showed that cholesterol was still significant (table 3). Triglycerides were no longer important.

Equation	Obs	Parms	RMSE	"R-sq"	F	P
mpaclf	184	5	3.694936	0.1147	5.79847	0.0002

mpaclf	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
creatinine	.0240089	.0070069	3.43	0.001	.010182	.0378357
triglyceri~s	.0573255	.3385712	0.17	0.866	-.6107788	.7254299
albumin	-.2321305	.1065531	-2.18	0.031	-.4423924	-.0218687
cholesterol	.9893421	.3259055	3.04	0.003	.3462309	1.632453
_cons	13.13755	4.521911	2.91	0.004	4.214436	22.06066

Table 3: Mixed model multivariate regression analysis of the main parameters that were significant in the univariate approach affecting apparent MPA Clearance [L/h] (mpaclf): Creatinine, triglycerides, albumin, cholesterol. Only creatinine, albumin and cholesterol remained significant. Lower albumin and higher cholesterol enhances the clearance.

Discussion

We were able to compute CL/F for the 1138 MPA levels of 35 renal transplant recipients in this retrospective cohort research. Our median follow-up was 5.8 years, and our median MPA CL/F values were 30. We discovered that 50% of the predicted AUC in this sizable group fell between 45 and 62 mg*h/L, which is substantially within the anticipated range [2]. Additionally equivalent to that seen in the literature, the median CL/F was 8.66L/h [21]. The mean CL/F in our group was 10.19±5.6 L/h, while the mean in the literature was 11.7±7.0 L/h. There was no discernible difference between this and Jaqcz Aigrain [21]. MPA CL/F was found to be linked with creatinine, albumin, cholesterol, and triglycerides using univariate correlation analysis. It was surprising that there was no association between cystatin C and estimated GFR. The effect of GFR on MPA clearance is likely not very significant because cystatin C is a better indicator of GFR in children than creatinine [22]. Using mixed model regression multivariate analysis, creatinine, albumin, and cholesterol continued to have independent effects on CL/F. It should be noted that these results only showed a weak correlation and might not have significant clinical implications. However, they indicate elevated MPA clearance in the context of hyperlipidemia and hypoalbuminemia, which the doctor should be cautious of, particularly in the absence of MPA monitoring.

A review of MMF's pharmacological characteristics and removal is required to explain these results [15]. The liver's first pass metabolism quickly transforms MMF, a prodrug of MPA, into MPA [23, 24]. In patients with normal liver and kidney function, the drug's high binding to serum albumin (99.99%) is primarily responsible for its volume of distribution [15]. Over a broad measurement range, MPA's binding to albumin remains consistent [25]. Although we are unable to evaluate MPA plasma protein binding without measuring free MPA levels, it is possible that albumin availability influences the quantity of unbound MPA and, thus, changes clearance [15]. MPA-G, the primary metabolite, is found in urine in 93% of cases, whereas 6% are found in feces. Active tubular secretion is thought to be the primary method of MPA-G excretion in the urine [26]. We discovered that serum albumin had an impact on the CL/F of MPA, same like in adult patients [26]. While the unbound MPA is unaffected, de Winter et al. reported a correlation between albumin concentrations and total MPA [27]. Although we lack free MPA levels, our results

would be consistent with adult evidence indicating an increase in MPA clearance during hypoalbuminemia [26]. To the best of our knowledge, no prior reports on the effect of cholesterol and triglycerides on MPA clearance in pediatric patients exist. In adults, we only came across one report [28]. The moderately beneficial effect of triglycerides on MPA CL/F that has been revealed here is consistent with a previous study by Vial et al. that detailed how medicines can be removed from plasma protein binding through competition or allosteric regulation [29]. It's possible that secondary hypertriglyceridemia-induced hypoalbuminemia is more responsible for the previously documented effect of extremely low albumin on MPA clearance. When the molar ratio of fatty acid/albumin rises to 5:1, which would mimic substantial hypertriglyceridemia, Vial et al. report a 2.2-fold increase in MPA CL/F [29]. There are various limitations to our study. One major weakness of the study is its retrospective design. However, it would be difficult to collect so many CL/F values in prospective investigations. One drawback is the absence of comprehensive pharmacokinetic profiles. However, when MPA trough levels only have concomitant tacrolimus, as demonstrated in our earlier work, they approximate a good proxy of the AUC [6]. Actually, there is a pretty good comparison between reported AUCs and our estimated AUCs. Only the HPLC/MS/MS measurements were included. EMIT is known to cause MPA levels to be overestimated [17]. The number of studies on albumin, hemoglobin, and lipids in these patients varies greatly because this was not a planned study. This obviously reduces the multivariate analysis's power. particularly because there aren't many lipid studies. It was just realized how important it is to measure the patients' lipids [30]. However, a significant strength is the enormous quantity of CL/F measurements.

In conclusion :

The present study emphasizes the mild effect of MPA CL/F, which is enhanced by hypoalbuminemia and increased cholesterol. In the event of hypoalbuminemia or markedly high cholesterol, transplant doctors caring for young renal transplant recipients should be aware of these events and may think about raising the MMF dosages. It makes sense that juvenile renal transplant recipients would receive reduced MMF dosages in cases of renal failure, but it is believed that dose

adjustments do not take into account the effects of hypercholesterolemia and hypoalbuminemia. TDM is ideal if Physicians should think about the increased clearance for MMF dose adjustment if MPA levels (especially unbound MPA) are not available.

Conflict of Interest

None of the writers had any connections or situations that could create a conflict of interest.

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Recognitions

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Contributions of the Authors

GF came up with the idea for the study, submitted an ethics application, participated in every step of the paper's creation, edited each draft, carried out the statistical analysis, and oversaw the work of all coauthors. The first draft was written by GF and CR, who also carried out the statistical analysis and data interpretation. The data was gathered and analyzed by CR and EY. ACA participated in every stage of the paper writing process and contributed significantly to every facet of the study's planning and implementation. Every author contributed to the manuscript's critical revision for significant intellectual substance and gave their approval for the final draft to be sent to the journal.

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