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Title: A multiple methods approach to determine adherence with prescribed mycophenolate in children with kidney transplant

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[Mycophenolic acid](#)

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Running head: Adherence to mycophenolate in children with kidney transplant

What is already known on the subject: There is evidence that adherence to prescribed treatment in children is a multi-factorial issue that is poorly understood. Moreover, little is known about the factors that affect adherence in children with kidney transplant.

What this paper adds: This study utilised a multiple method approach to assess the prevalence of adherence to mycophenolate in children with kidney transplant and identified factors that influence their adherence to medication.

The clinical responsibility for patients throughout the study lay with their hospital doctors and their individual general medical practitioners.

Abstract

Aims: The aim of the present study was, to use a multiple methods approach, including, for the first time, dried blood spot (DBS) sampling with PopPK interpretation, to assess adherence to mycophenolate in children with kidney transplant. A second aim was to identify patient/parental factors that influenced adherence and to link adherence behaviour to clinical outcomes.

Methods: A convenience sample of 33 children with kidney transplant (≤ 18 years old) who had been prescribed mycophenolate for at least 3 months were recruited from participating outpatient clinics in the UK and Jordan. Medication adherence was determined via self-report questionnaires, medication refill data from dispensing records, and via mycophenolic acid concentrations in plasma and DBS samples obtained from children during a clinic visit.

Results: Through triangulation of results from the different methodological approaches a total of 12 children (36.4%) were deemed to be non-adherent with their prescribed mycophenolate treatment. Logistic regression analysis indicated that non-adherence was significantly associated with the presence of mycophenolate side effects. Poor adherence was positively linked to measures of poor clinical outcomes (hospitalisation and the need for kidney biopsy).

Conclusions: Despite the imperative regarding medication adherence to help prevent organ rejection, a significant proportion of children are not fully adherent with their therapy. Side-effects appear to be an important factor leading to non-adherence. Measurement of mycophenolate in DBS, coupled with the use of PopPK modelling, was a convenient direct approach to assessing adherence in children with kidney transplant and has the potential to be introduced into routine practice.

Keywords: Adherence; dried blood spot; mycophenolic acid; kidney transplant

Introduction

Paediatric kidney transplant is a complex condition which presents both the children (patients) and their parents with significant treatment management challenges. In children with kidney transplant, the most important treatment involves multidrug immunosuppression in order to avoid transplant rejection [1], with adherence to the prescribed therapeutic regimen being a very important driver of successful clinical outcomes.

There are a limited number of studies which have specifically examined adherence to treatment in children with kidney transplant with the results to date indicating considerable variation. Studies to date [2-8] have varied in the mode of adherence assessment, the patient population studied and the duration of follow-up. The studies have often focused on adherence in adolescent patients [2-6]. The adherence assessment methods used have included self-report [2,4-6,8], measurement of immunosuppressant plasma/serum levels [2,6], medication possession ratios (via prescription refill data) [3], and variations in immunosuppressant blood levels over time [7]. Sample sizes within the studies ranged from 27 [5] to 175 [8] while the rates of non-adherence reported have ranged from 10% [4] to 61% [6].

Patient self-report (the approach most frequently used in the above studies) is a simple, inexpensive, subjective approach to adherence assessment, however, it is prone to underestimation of non-adherence, since patients (or parents) often wish to provide 'socially desirable' answers to questions about their medicine taking behaviour [9-11]. On

the other hand, measurement of the concentration of a medicine (or metabolite) in blood is an objective method, but can be expensive and requires blood sampling [10,11]. Furthermore longitudinal blood sampling e.g. using home dried blood spot sampling [12] is necessary if longitudinal adherence data are required. Review of prescription refill records is an objective approach but is subject to bias (as the patient may fill the prescription but not take the medication) and this methodology requires access to prescriber, pharmacy or third party payer records [10,11]. Other approaches, including special electronic caps on medication containers which record time and date of opening [10,11,13] are generally not practical within a busy clinical environment and in addition, opening the container does not guarantee that the patient has ingested the medication. Direct observation of therapy (DOT) is an excellent but very expensive approach, however, more recently the use of mobile phone technology to directly observe therapy is being used [14] but is at a relatively early stage of development.

The extent of variation in results noted above for non-adherence to immunosuppressant medications in children and young adults is common in adherence research. As a result a conservative approach of using a combination of measurement techniques, with triangulation of the results, is generally recommended to increase the accuracy of detecting non-adherence and indeed several papers, involving a range of illnesses, have recommended the use of a multi-method approach for assessing adherence [12,15-17].

Little is known about the factors that affect adherence in children with kidney transplant. One study which investigated barriers to medication adherence in children with kidney transplant (and their parents, when responsible for medication administration to the child) highlighted forgetfulness, medication palatability, side effects and interruptions in routine as important factors [18]. Another study which examined barriers to medication adherence revealed similar factors, including forgetfulness, but also highlighted patient beliefs, such as, having greater concern about medication safety as an important potential driver of poor adherence [19]; this latter study investigated the impact of patient beliefs on medication adherence only in adolescents. In research involving other medications, the health related quality of life of the child [16] and the presence of depressed mood in the parent [16] have been shown to be related to adherence to medication in children.

The aim of the present study was to utilise a multiple methods approach to assess the prevalence of non-adherence to mycophenolate in children ≤ 18 years old with kidney transplant. The focus of the work was on the implementation phase within the adherence taxonomy described by Vrijens *et al.* [20]. The methods used included (i) determination of mycophenolic acid concentration in both plasma and dried blood spot (DBS) samples and linking this with the application of population pharmacokinetics (PopPK) modelling; (ii) older children and parent/guardian self-report; (iii) general practitioner prescribing records and (iv) pharmacy dispensing records. A second aim was to identify factors that influence adherence, including beliefs about medicines, parental depressed mood, health-related quality of life in child, and to link adherence behaviour to outcomes in participating children.

Methods

Study patients and study sites

The study was approved by the Office of Research Ethics Committees in Northern Ireland (ORECNI; 14/NI/1133) and by the Research Committee in the Royal Medical Jordanian Services in Jordan. A convenience sample of 33 children with kidney transplant was recruited into this study from outpatient clinics in two different centres, i.e. the Queen Rania Hospital for Children (QRH), Amman and the Royal Belfast Hospital for Sick Children (RBHSC), Belfast.

Queen Rania Hospital for Children is the main hospital for children in Amman, Jordan. It has specialist centre for kidney transplantation. The hospital was providing care to more than 85 children with kidney transplant at the time of the study. The Royal Belfast Hospital for Sick Children is a tertiary referral centre for Northern Ireland. Belfast has the UK NHS system through which (unlike the situation in Jordan) all medicines for use in children are made available free of charge via the patient's general practitioner.

The parents/guardians of children with kidney transplant who were ≤ 18 years and who had been prescribed mycophenolate for at least 3 months, were invited to have their children participate in the study. Children were enrolled in the study if written informed consent was

obtained from their parents/guardians and assent from children in the age range of 8-16 years or consent from patients 16-18 years old.

Once patients were recruited, demographic data, laboratory data, medical history, and past and current medications were recorded via their medical notes.

Collection and analysis of plasma and dried blood spot (DBS) samples

Finger prick blood samples were taken from each child at the time of a regular outpatient clinic appointment via an automatic disposable lancet. Blood drops were spotted on to Guthrie cards using a 15 μ L plastic capillary. The blood spot samples were dried (overnight in the dark) and then stored at -80°C in a greaseproof paper sleeve, within a polypropylene container (with desiccant), prior to analysis. An aliquot of blood (2ml) was also taken from a routine blood sample collected at the clinic. This was centrifuged and plasma stored at -80°C until the time of analysis.

A sensitive, selective microanalytical method for the determination of mycophenolic acid concentration in DBS samples was developed and validated. The method utilised 8-mm disks (incorporating the full 15 μ L sample) punched from the Guthrie cards. The sample preparation for DBS samples involved extraction via 1 mL of methanol. After centrifugation, plasma sample (300 μ L) preparation involved protein precipitation with 300 μ L acetonitrile and after centrifugation, transferring the supernatant aliquot to a clean tube. Both DBS and plasma sample extracts were then dried under a stream of nitrogen at 40°C and the residue dissolved in 1 mL HPLC grade water prior to solid phase extraction (SPE) using Oasis MCX cartridges (1 ml/30g; Waters, Dublin, Ireland). Mycophenolic acid in the SPE eluent was quantified using high performance liquid chromatography (HPLC) with UV detection. The assay limit of quantification in DBS and plasma samples were 0.25 and 0.1 $\mu\text{g}/\text{mL}$ respectively. The intra-day and inter-day accuracy and precision were within the limits recommended by ICH guidelines ($\pm 15\%$).

Study measures and adherence assessment

A number of questionnaires were administered to parents/guardians and/or their children during the clinic visit. Pre-validated English and Arabic versions of the questionnaires were used as appropriate in Belfast and Amman [21].

Medication adherence report scale (MARS)

Two versions of MARS questionnaire [22] exist depending on the time period covered i.e. general overview of adherence and specific adherence over the last month. The latter was utilised in the present study. The parent version consisted of six questions relating to non-adherence behaviours, each scored on a 5-point Likert scale. The scores were added to produce a cumulative score with a range from 6 to 30, with higher scores indicative of higher levels of adherence. In this study, a cut-off point of 90% was used and the children whose parents had total scores ≥ 27 were considered adherent [12].

The child version (suitable for use in children over 12 years) has similar items to the parent version except for the last question, which is not relevant to the child i.e. 'I don't give it because my child refuses it'. Scores for the five items were summed with a total score of 25 indicating perfect adherence. In the present study, a cut-off point of 90% was again used and the children with a MARS score of ≥ 23 were considered adherent. These conservative cut points were used due to the critical need for effective immunosuppression to prevent the onset of organ rejection. Moreover, based on previous studies a cut-off score ≥ 23 had the best predictive ability of adherence for the 5-item version of the MARS questionnaire. When lowering the non-adherence cut-off stepwise from 23 to 20, MARS did not achieve sufficient sensitivity to detect non-adherers compared with other objective methods.

Beliefs about medicines questionnaire (BMQ) specific

In this questionnaire, beliefs about the specific medication prescribed are grouped under two core themes, i.e. 'Necessities' and 'Concerns'. The beliefs about Medicines Questionnaire (BMQ) specific scale assesses patients' (children over 12 years) and parents' beliefs about the necessity of prescribed medication for controlling illness and also concerns about the potential adverse events [22]. The application of the Necessity Concerns Framework (NCF), which is operationalised using the BMQ, is justified through a range of

systematic reviews and meta-analyses that support the utility of this approach in explaining variation in adherence across countries and healthcare systems [23,24] and through a review of the key theoretical frameworks explaining non-adherence which has identified the NCF as a leading explanatory model [25].

Center of epidemiologic studies depression scale (CES-D)

CES-D contains 20 questions that enable the assessment of depressive symptoms in the general population. Parents/guardians of participating children were asked to complete the questionnaire to allow rating of how often they experienced depressed mood symptoms over the past week. In the questionnaire, each question is scored from 0 to 3 based on the frequency of symptom occurrence. Total scores range from 0 to 60 where higher scores i.e. scores of ≥ 16 are indicative of depressed mood.

Pediatric Quality of Life InventoryTM (PedsQLTM)

The Pediatric Quality of Life InventoryTM 3.0 Transplant Module (PedsQLTM) has been validated to assess health related quality of life in paediatric patients with solid organ transplant [26]. PedsQLTM is composed of 46 items in 8 dimensions covering medical, physical, emotional and social aspects. Scaling of points awarded depends on the participating child, with higher total scores indicating better health related quality of life.

Mycophenolic concentration in blood samples

To determine whether a child was adherent, a computer simulation method was utilized to estimate the 95% interval of predicted plasma concentrations for MPA at the time of sampling relative to time of dose administration ($n = 1,000$ sets of simulations using the non-linear mixed effect modelling software package, NONMEM[®] version 7.3.0, Icon Development Solutions, Ellicott City, MD, U.S.A.). In this approach, literature values of PopPK parameters for MPA were employed [27-31]. In addition, significant covariates reported to influence PK parameters (e.g., age, body weight, dose) were incorporated into the simulation models. For each patient, the measured DBS concentrations were transformed to corresponding plasma concentrations based on each individual patient's haematocrit level using published reports [32,33].

Patients were considered adherent if their measured MPA concentrations were within the calculated 95% prediction intervals taking into consideration individual MPA dosing histories in those patients (more details are provided in Appendix 1).

Patient Medication Records (PMRs) from patient's pharmacy

A patient was judged non-adherent if the proportion of required doses dispensed over the period of up to 12 months prior to enrolment in the study was lower than 0.8 [16]. This was calculated using the Medication Refill Adherence (MRA) formula, which has been used by other authors who have evaluated adherence in children [12,16]:

$$MRA = \frac{\text{Sum of days' supply obtained throughout the timed period}}{\text{Total days from the beginning to end of the timed period}} \times 100$$

General practitioner (GP)/consultant prescribing records

The same approach (see above) was also used for prescribing records.

Data analysis

Statistical analyses were carried out after entering all data into SPSS Version 21.0 (SPSS Inc, USA). Descriptive statistics were used to describe patient demographics. The Kappa coefficient (κ) was used to assess the extent of agreement between the adherence assessment methods. Appropriate parametric and non-parametric tests were conducted based on the normality of the data distribution. Adherent/non-adherent group differences were compared using t-tests for continuous variables and Chi-squared analysis or the Fisher's Exact test for categorical variables. Univariate analysis was utilised to explore the relationship between adherent and non-adherent groups and age, transplant duration, beliefs about medicine, parental depressed mood and patient quality of life. Multivariate analysis (logistic regression) was then used to assess the relative contribution of factors leading to non-adherence.

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS

Guide to PHARMACOLOGY [34], and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 [35].

Results

Patient characteristics

Fifty two paediatric patients with their parents at the Royal Belfast Hospital for Sick Children and Queen Rania Hospital for Children were identified as being eligible to take part in the study (Figure 1). Forty two of the eligible parents and their children were approached and invited to participate, while thirty four patients and their parents agreed to participate in the present study. One patient had to be excluded as the GP, pharmacy and some medical data were not complete. Medical characteristics of participating children are shown in Table 1. The gender distribution of the participating children was as follows: 21 males and 12 females. Age range was between 8 to 18 years with a median of 14 years.

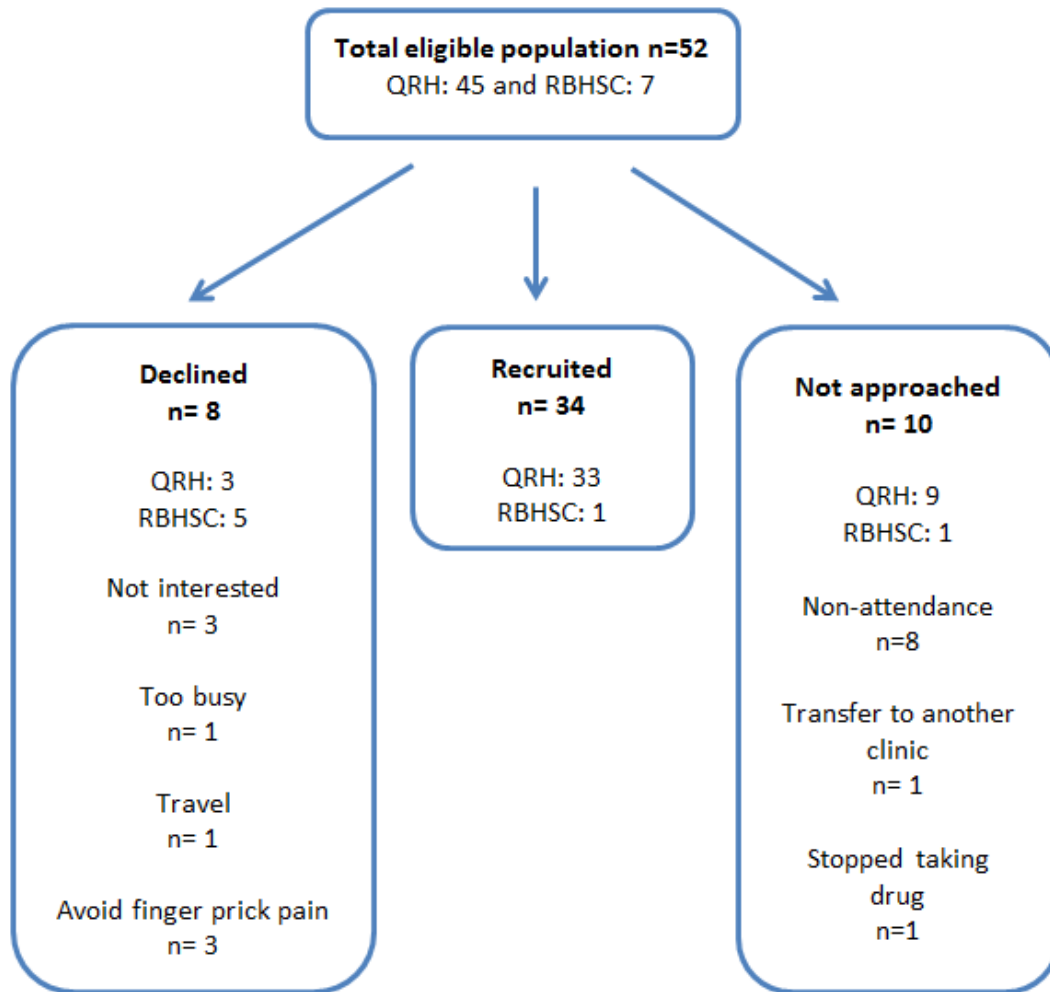


Figure 1 Participation of eligible patients in the study

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Table 1: Medical characteristics of the participating children (n=33)

Child characteristic	Participants
Duration of kidney transplant	
Median (range) in years	4.5 (0.5-8.0)
Type of kidney transplant	
Living donor transplant	27 (82%)
Deceased donor transplant	2 (6%)
	(4: missing data)
Cause of kidney failure	
• Congenital*	15 (45%)
• Non-congenital**	7 (21%)
• Unknown cause	9 (27%)
	(2: missing data)
Previous dialysis	
Yes	16 (48%)
No	17 (52%)
Duration of dialysis	
Median (range) in years	0.34 (0.08-5.0)
Other medical diagnosis	
No	28 (85%)
Yes	5 (15%)
Number of immunosuppressants prescribed	
Median (range)	3 (2-3)
• Two	1 (3%)
• Three	32 (97%)
Number of other concomitant medicines prescribed[§]	
Median (range)	1 (0-5)
• Nil	6 (18%)
• One	12 (36%)
• Two	6 (18%)
• Three	5 (15%)
• More than three	4 (12%)

* Congenital includes some cases of renal segmental hypoplasia, focal segmental glomerulosclerosis, neurogenic bladder dysfunction and children born with single kidney

** Non-congenital includes some cases of focal segmental glomerulosclerosis, complications of diabetes and hypertension

§ excluding immunosuppressant agents

Analysis of study measures

Adherence using MARS specific

Thirty parents of the 33 participating children completed the MARS questionnaire. The questionnaire revealed that 28 parents (93.3%) scored $\geq 90\%$ i.e. described their child as adherent. On the other hand, 27 children completed the child version of the MARS questionnaire with 14.8% of children classified as non-adherent using this measure.

Adherence using MPA concentrations in plasma and DBS samples

Plasma and DBS samples were obtained from the 33 patients recruited in the study. All DBS samples were of good quality and analysable since careful sampling was performed.

According to the plasma sampling method, 31 children (93.9%) were considered adherent and two (6.1%) were classified as non-adherent. On the other hand, 30 children (90.9%) were classified as adherent based on the analysis of the DBS samples, while three children (9.1%) were classified as non-adherent.

Adherence using the MRA measure

Prescribing records were available for all patients in hard copy. The mean value for the medication refill adherence (MRA) using prescribing records was 0.84 (± 0.04). A total of 42.4% of patients (14 patients from 33) was classified as non-adherent based on the prescribing records (i.e. MRA $< 80\%$), i.e. the overall adherence rate with this assessment measure was 57.6%.

Electronic records of pharmacy prescription refill data were available for all participating children. When the MRA score was calculated for each patient, 75.8% of participants scored $\geq 80\%$ and were classified as adherent, while 24.2% were classified as non-adherent.

Comparison of the different measures of adherence

The Kappa coefficient (κ), a statistical measure of agreement between categorical variables, was used to assess the extent of agreement between the adherence assessment methods.

The agreement coefficient (Kappa) between the assessment methods used in the present study ranged from slight to substantial agreement. The best agreement was between the plasma and the DBS sample method ($\kappa=0.784$). Substantial agreement was also found

between prescribing and dispensing records ($\kappa=0.606$) and fair/moderate agreement between adherence classification using blood levels (i.e. plasma and DBS analysis) and pharmacy dispensing records ($\kappa=0.336$ and 0.476).

Since variation in adherence rates using different assessment tools is well described in the literature, it is recommended that a multi-method approach is utilised. We used the conservative approach of classifying patients as non-adherent if any of the assessment measures indicated non-adherent behaviour [12]. In the present study, 21 patients had the same classification irrespective of the assessment method used (all were adherent). The highest percentages of patients classified as adherence were recorded using the plasma levels of MPA (93.9%) and the parent MARS questionnaire assessment (93.3%). The MRA calculated from the prescribing records displayed the highest percentage of patients as non-adherent (42.4%) when compared with the other methods. Adherence assessment using the prescribing data was omitted from the final overall classification in order to avoid over-estimation of non-adherence due to potential inaccuracy of manual recording. Upon combining all other methods of adherence assessment, 36.4% of all patients recruited (12 children) were classified as non-adherent as shown in Figure 2.

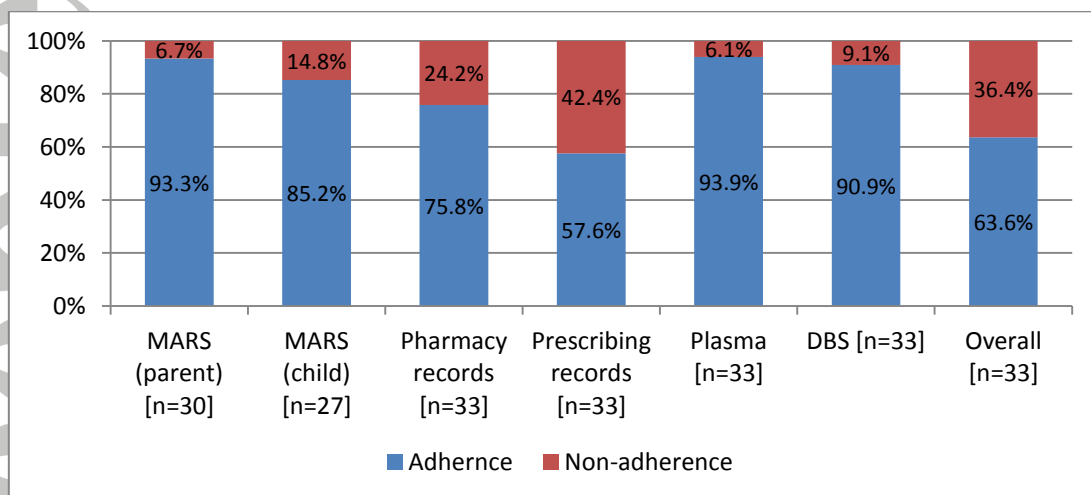


Figure 2 Comparison of results of mycophenolate adherence classification of children with kidney transplant using the different methods of assessment

Patient and parent factors that influence adherence

All parents had necessity scores in the BMQ above the scale midpoint which indicated that they had a strong belief that mycophenolate was necessary after kidney transplant, whereas

about half of the parents had concerns about the potential harmful effects of mycophenolate prescribed for their children. The majority of parents (n= 21; 70%) did not experience depressed mood as measured by the CES-D scale (i.e. total score < 16). However, 9 parents (30%) had a total score of ≥ 16 , indicating depressed mood. Three parents (10%) scored ≥ 27 which is indicative of major depressed mood. Domain scores of the PedsQL™ questionnaire were transformed into percentage scores with higher scores describing higher quality of life. Average scores of the PedsQL™ across all domains were higher than 60% [mean value 82.9 (range 62.5-95.7)] indicating a relatively high health related quality of life in the participating children.

Univariate analysis was performed for all available categorical and continuous variables to compare those who were deemed overall to be adherent and those who categorised as non-adherent to mycophenolate. Two variables were significantly associated with adherence to mycophenolate ($p < 0.05$). These factors were (i) gender (adherence more likely in female patients) and (ii) presence of side effects (non-adherence more likely in patients exhibiting side effects). Other factors were tested and found to be non-significant such as age, previous dialysis, time from the transplantation, presence of comorbidities, total number of medications taken, number of family members, parental education, family income, child and parental BMQ, parental depressed mood and health related quality of life in child.

Logistic regression analysis indicated that the presence of side effects was the only factor which remained in the model as being significantly and independently associated with non-adherence; the presence of side effects increased the probability (Odds ratio) of a child being non-adherent by a multiple of 6.99 ($P = 0.032$).

Linking non-adherence to patient outcomes

Renal function (estimated creatinine clearance CrCL), the need for biopsy procedures to check on status of the transplanted kidney and the number of hospital admissions in the last year were deemed to be clear markers of poor clinical outcomes. Two of these three outcome measures (Table 2), representing poor control, were statistically linked to non-adherence ($p < 0.05$).

Table 2: Linkage between overall adherence classification and clinical outcomes

Outcome	Adherent	Non-adherent	P-value*
Renal function (CrCL; ml/min) (n= 27, 6 missing data)	n= 17	n= 10	0.108
Median (range)	80.5 (29.7-161.7)	62.3 (22.5-91.5)	
Admissions in the last year			
Yes (n= 14)	5 (35.7%)	9 (64.3%)	0.004
No (n= 19)	16 (84.2%)	3 (15.8%)	
Biopsy			
Yes (n= 20)	8 (38.1%)	12 (100%)	0.001
No (n= 13)	13 (61.9%)	0 (0.0%)	

* Significance level at $p < 0.05$

Discussion

Since no single measure of adherence is likely to be accurate, a combination of approaches was employed in the present study. This is the first study that investigated the adherence to mycophenolate in children with kidney transplant using a multi-method approach involving both subjective (i.e. self-report questionnaires) and objective (i.e. PopPK interpretation of MPA in plasma and DBS samples, and dispensing records) methods.

Assessing adherence to mycophenolate after transplant using plasma MPA concentration has been reported in the literature [7], however, in the present study assessment of patient adherence using this approach also involved PopPK modelling, adding to the robustness of the technique. Published research on the PopPK of MPA in children has been reported previously [27-31]. The clearance model developed by Zeng *et al.* (2010) [27] was selected as the most suitable model for the use in the present population of children as the latter model includes concomitant use of cyclosporine or tacrolimus as covariates. The results also illustrated the utility of using the DBS sampling approach to assess adherence to mycophenolate, i.e. strong correlation in adherence classification between venipuncture plasma samples and the finger prick DBS samples. The latter approach is much more convenient for clinicians and patients and introduces the opportunity for home sampling with DBS cards being mailed to the laboratory for analysis [12].

The results of adherence rates using the MARS questionnaires were higher than the self-reported adherence rates reported in previous studies on immunosuppressants [2-8]. Parent and patient unwillingness to disclose non-adherent behaviour may have resulted in an overestimation of the rate of adherence in the studied population [32]. Children reported a higher non-adherence rate compared to parents, although the difference was not statistically different. This agrees with a previous study that reported a higher level of non-adherence stated by children than their parents, in this latter case in children with epilepsy [12].

Prescribing and dispensing records for a medicine of interest provide information on the adherence level over a period of up to 12 months pre-assessment. The accuracy of this MRA approach depends on completeness of the data and the assumption that the patient actually consumes the medicine prescribed and/or dispensed. Approximately 20% of the recruited patients classified as non-adherent based on the prescribing records were found to be adherent according to the pharmacy dispensing data, suggesting incomplete prescribing data. The prescribing data were therefore not used in the final adherence classification for this reason.

Factors affecting adherence to mycophenolate

The presence of side effects in the child was the only variable that independently ($P < 0.05$) predicted adherence to mycophenolate in the present study (logistic regression modelling). Side effects of medication have been reported as a common barrier to medication adherence in patients with kidney transplant [33]. No other significant associations were seen between patient demographics and transplant characteristic variables with overall adherence. However, univariate analyses indicated a significant association between the child's gender and overall adherence with female children tending to have higher adherence. Published data on child's gender as a predictor of adherence to treatment is conflicting [36], with some studies showing that female children are more adherent to medications than male children while others have found no difference between the sexes [37,39].

The present study was unable to confirm the hypothesis that necessity belief about medication would be positively associated with patient adherence, as there was no

significant association between the BMQ necessity scores for children or parents and overall adherence.

The presence of depressed mood was evaluated in the parents of the participating children.

There is no published information, to our knowledge, linking adherence of children with transplant with parental depressive symptoms. Although, 30% of the parents exhibited depressed mood based on the CES-D scale, there was no significant association with non-adherence in their children. This finding contrasts with studies previously published on some other illnesses. A study by *Shah et al.* (2013), for example, has indicated such a relationship in children with epilepsy [12]. Another recent study carried out by Barker and Quittner [39] found that children with cystic fibrosis whose parents had depressed mood were less adherent to enzymatic therapy. However an earlier study by Goodfellow *et al.* [16] showed no effect of parental depressed mood on adherence in another group of children with cystic fibrosis.

Health-related quality of life in children with kidney transplant has been assessed in relatively few studies to date. Quality of life as reported by children in the present study was shown to be relatively high on average across most domains (mean value 82.9%). The findings were consistent with a previous study carried out by Dobbels and his colleagues [40] where the QOL of adolescent kidney transplant patients was found to be comparable to healthy adolescents. No significant association was found in the present study between child quality of life score as measured by PedsQL™ and overall adherence.

Linking non-adherence to patient outcomes

Significant correlations ($P < 0.05$) were found between hospital admissions and patients requiring biopsies and non-adherence in the present study. This was as predicted, as consistent blood levels of medication, through good adherence, are necessary to maintain the required level of immunosuppression. Similar correlations have been found by a previous group [8]. These latter correlations, despite the low sample size, added clinical validity to the overall adherence assessment used.

Limitations

The sample size of the study was relatively small, although similar in size to a number of the cohorts examining adherence in children published in the scientific literature to date. Despite the study providing statistically significant, clinically validated findings, it is likely to be underpowered given its reliance on a convenience sample. Since paediatric patients with kidney transplant are usually prescribed a combination of immunosuppressant agents, the clinical outcomes addressed in the present study i.e. the need for biopsies and the number of hospital admissions could be related to non-adherence to other immunosuppressants; further investigations on individual immunosuppressants are therefore recommended. In addition, other factors related to medication adherence such as characteristics of the healthcare system, the healthcare organisation, together with cultural differences and family support were not studied in the present research. These factors should be addressed in future studies with inclusion of a larger number of patients from a range of centres internationally.

Conclusions

A multiple-methods approach was utilised to evaluate the adherence to mycophenolate therapy in children with kidney transplant. Measurement of mycophenolate concentrations using DBS sampling, together with the use of a published PopPK model, was shown to be a useful approach for estimating adherence levels in children with kidney transplant.

The overall non-adherence to mycophenolate in children with kidney transplant was estimated to be 36.4% (12 out of 33 children classified as non-adherent), which was within the reported range in the literature. Univariate analyses identified male gender and the presence of side effects as potential predictors of non-adherence to mycophenolate therapy. Only the presence of side effects was found to be independently predictive of non-adherence following logistic regression analysis. Non-adherence to mycophenolate was correlated with poor health outcomes such as need for biopsy and hospitalisation. The development of successful interventions to improve adherence should be a priority within this group of patients whose continued good health is so dependent on effective pharmacotherapy.

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APPENDIX 1.

Adherence evaluation using Population Pharmacokinetic (popPK) approach:

Adherence level to mycophenolate was determined based on both mycophenolic acid concentration in plasma samples and mycophenolic acid concentration in DBS samples.

(a) Mycophenolic acid (MPA) concentration in plasma samples

Plasma concentrations in collected samples were determined using a validated HPLC analytical method in our laboratory. In order to assess patient adherence, MPA concentrations in plasma were compared with MPA predicted concentrations based on a previously published PopPK model as detailed below.

(i) PopPK of MPA in children

Prediction of MPA concentrations was based on a two compartment PopPK model which was developed by Zeng *et al.* (2010). The population model was as follow:

$$Cl(L.hr^{-1}) = 6.42 \times (1 + 1.09 \times \frac{WT(kg)}{27.9}) \times (1 + -0.6 \times CYTA) \times EXP(IIV)$$

$$V1(L) = 7.24 \times EXP(IIV)$$

$$Q(L.hr^{-1}) = 3.74 \times EXP(IIV)$$

$$V2(L) = 16.8 \times EXP(IIV)$$

$$Ka = 0.39(hr^{-1})$$

Where Cl is the total drug clearance, V1 is the volume of distribution in the central compartment, Q is the inter-compartmental clearance, V2 is the volume of distribution of second compartment and Ka is the absorption rate constant for oral administration. The value of CYTA= 0 if the patient is taking ciclosporin and CYTA= 1 if the patient is taking tacrolimus without ciclosporin. The inter-individual error has

an exponential model, with a variance of 31.6. The random residual variability of 0.48 was applied.

(ii) The simulation

The simulation was carried out on NONMEM® software (Version 7.3.0) with a built-in compiler (G95 compiler). The process is summarised in the flow chart (Figure 1).

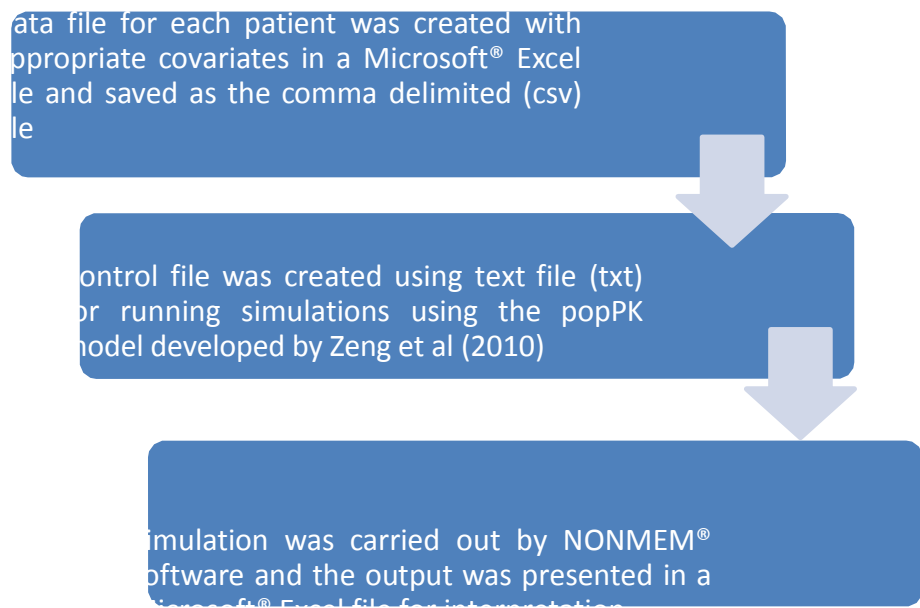


Figure 1 Flowchart on MPA simulation using NONMEM®

In order to determine whether a patient was adherent to mycophenolate, 1000 simulated plasma concentration values were computed via inserting the individual patient characteristics into the MPA population model. A patient with a measured MPA concentration in plasma sample within the simulated concentrations (5th and 95th percentiles) was categorised as adherent.

(b) Mycophenolic acid concentration in DBS samples

The equivalent plasma concentration values for all DBS samples were calculated using the following equation based on published reports (Gummert et al., 1999; Langman et al., 1994).

$$EPC = \frac{DC}{1 - \left(\frac{Hct}{100}\right)}$$

The equation is based on the assumption that the uptake of MPA by erythrocytes is negligible and it is almost exclusively found in the plasma part, where EPC is the expected plasma concentrations from measured DBS sample concentrations (DC) and Hct is the individual patient's haematocrit level. These calculated concentrations were used to categorise patients as adherent or non-adherent as described above.

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