

Revised

Pharmacokinetic Variability of Mycophenolic Acid in Pediatric and Adult Patients with Hematopoietic Stem Cell Transplantation

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Abstract

The aim of this study was to evaluate the pharmacokinetic variations of mycophenolic acid (MPA), the active metabolite of mycophenolate mofetil (MMF), in both pediatric and adult patients following hematopoietic stem cell transplantation (HSCT). Twenty pediatric patients with a median age of 3 years (range, 0.2-12 years) and thirteen adult patients with a median age of 54 years (range, 18-63 years) were enrolled. Blood samples were collected on days 0, 7, 14, 21 and 30 after allogeneic HSCT. Total and free (unbound) MPA, as well as MPAG were quantified using a validated LC-MS/MS assay. The plasma protein binding of MPA and MPAG did not change significantly in pediatric patients over the one month sampling period post HSCT. However, it increased in adult patients from day 7 to day 30 post HSCT, from $97.3\pm0.8\%$ to $98.3\pm0.6\%$ for MPA ($P <0.05$), and $74.6\pm9.4\%$ to $82.9\pm8.1\%$ for MPAG ($P <0.05$). The plasma protein binding of MPA was significantly higher in males compared to females in both pediatric (98.3 ± 1.1 vs $97.4\pm1.1\%$) and adult (98.1 ± 0.7 vs $97.4\pm1.2\%$) patients ($P <0.05$). The MPAG/MPA ratios on an mg/kg dose basis in adult patients were significantly higher than those in pediatric patients (4.3 ± 3.4 vs 2.4 ± 2.6 ; $P <0.05$). Time-dependent plasma protein binding and age-related differences in MPA metabolism, at least in part, impact the reported large inter- and intra-individual variability in MPA pharmacokinetics. These patient and pharmacologic factors, if incorporating into MMF regimen design and modification, may contribute to the rational dose selection of MMF in HSCT patients.

Keywords

Mycophenolic acid; Mycophenolate Mofetil; protein binding; metabolism; hematopoietic stem cell transplantation, pediatric and adult patients

Introduction

Mycophenolate mofetil (MMF) is an immunosuppressive drug approved by the FDA in 1995 to prevent acute rejection in renal allograft recipients. Besides in solid organ transplantation, MMF is increasingly used in the prevention and treatment of acute and chronic graft-versus-host disease (GVHD) post allogeneic hematopoietic stem cell transplantation (HSCT). ¹ MMF itself is biologically inactive and must be metabolized by carboxylesterases to mycophenolic acid (MPA), which is a potent, reversible, uncompetitive inhibitor of the rate-limiting enzyme inosine monophosphate dehydrogenase (IMPDH) in the *de novo* purine biosynthesis. Inhibition of IMPDH blocks T- and B-lymphocyte proliferations, and reduces antibody production and the generation of cytotoxic T lymphocytes, consequently contributing to the prevention of allograft rejection and treatment of ongoing rejection.^{2,3}

MPA metabolism occurs primarily in the liver but also to some extent in the intestine and kidney.⁴ A major fraction is converted to the inactive 7-O-glucuronide (MPAG) and a minor fraction is converted to the active acyl glucuronide (AcMPAG). UGT1A9, 1A8, 1A1, 1A7 and 1A10 produce MPAG in significant amounts, with UGT 1A9 being the most active isoform. UGT 2B7 is the only isoform producing AcMPAG in a significant amount.^{5,6} UGT1A8 expressed in the kidney and throughout the GI tract, and UGT1A9 expressed in the liver, intestine and kidney, are believed to be the major isoforms involved in MPA glucuronidation.^{7,8} MPAG is mainly excreted in urine via active tubular secretion and glomerular filtration. It could be partly excreted into the bile by Mrp2 (multidrug resistance-associated protein), de-conjugated back to MPA by the gut microflora β -glucuronidases, and then reabsorbed into the portal circulation, characterized as enterohepatic circulation (EHC). In humans, the mean contribution of EHC to the overall AUC of MPA is 37% (ranging from 10 to 61%).⁹

MPA extensively binds to human serum albumin and has a free fraction of <3% in patients with normal renal and liver function. Only unbound MPA is capable of inhibiting IMPDH in vitro and in vivo. Changes in albumin levels may potentially change activity or toxicity. MPAG also displays

high serum albumin binding (82%) in stable patients. Therefore, competition for albumin binding between MPA and MPAG may exist. AcMPAG forms an irreversible covalent bound with albumin, which makes the measurement of the free fraction technically challenging.¹⁰

Many centers use standard MMF dose (1,000 mg, q12h) for adult HSCT patients and 15 mg/kg q8h for pediatric HSCT patients. However, the pharmacokinetics (PK) of MPA and the relationships between dose, plasma concentration and exposure are poorly understood in HSCT patients, especially in pediatric HSCT patients.¹¹ Standard doses (2 g/day) in adult HSCT patients achieve significantly lower MPA exposure compared with renal transplant patients.¹² Increased doses to 3 g/day with cyclosporine still fail to achieve therapeutic plasma exposure in many adult HSCT patients.¹³ The physiologic differences between the kidney and HSCT recipients including renal function, chemotherapy effects, prophylactic antibiotic use and higher severity of illness, may affect MPA disposition. In pediatric HSCT patients, 15 mg/kg q12h intravenously with cyclosporine have a significantly lower total and unbound MPA exposure than pediatric renal transplant recipients receiving 600 mg/m² q12h. Although q8h dosing improves exposure, it does not consistently obtain MPA plasma exposure similar to adults.¹⁴ Another study demonstrates that MMF administration of 900 mg/m² q6h in combination with tacrolimus achieves MPA plasma exposures similar to those of adults.¹⁵ Therefore, despite the increased use of MMF, the optimal dose is unknown in both pediatric and adult HSCT patients.

A number of variables affect MPA pharmacokinetics, including renal and hepatic function, albumin concentration, magnitude of EHC, concomitant immunosuppressive therapy, and genetic polymorphisms in drug metabolizing enzymes and transporters. Because of the complex pharmacokinetics of MPA, high inter- and intra-patient pharmacokinetic variability of MPA is observed in organ transplant patients, childhood-onset systemic lupus erythematosus patients and HSCT patients. MPA exposure could vary more than 10-fold between patients, leading to a significant therapeutic challenge.^{12, 16-21} This study was conducted to gain insights into the pharmacokinetic variability of MPA, from plasma protein binding and metabolic perspectives, in

both pediatric and adult HSCT patients. Identifying the patient and pharmacologic characteristics that significantly affect MPA pharmacokinetics would allow for more rational decisions on MMF dosing in both pediatric and adult HSCT patients.

Methods

Study subjects

This study was conducted as an open-label and inpatient/outpatient clinical study in HSCT patients. The main objective was to evaluate the inter- and intra-patient variability of MPA in pediatric and adult patients post HSCT. Twenty pediatric patients with a median age of 3 years (range: 0.2 to 12 years) and thirteen adult patients with a median age of 54 years (range: 18-63 years), undergoing HSCT from both related and unrelated donors, were enrolled at Indiana University Hospital and Riley Hospital for Children (Table 1). All adult patients and nearly half of pediatric patients (9/20) were diagnosed with malignancies. The study was approved by the Institutional Review Boards of participating centers (IRB # 1111007321). Informed consent was obtained from each patient (or parent/guardian for pediatric patients) and assent was obtained from children who are at least 7 years of age before enrollment.

Study protocol

MMF (CellCept®, Roche) was initiated by a 2-hour intravenous infusion at 15 mg/kg every 8 hours for pediatric patients, and at an oral fixed dose of 1,000 mg twice daily for adult patients prior to transplantation. In this study, 17 pediatric patients were co-administered with cyclosporine, and others with tacrolimus as a concomitant immunosuppressive therapy. Nine adult patients were co-administered with cyclosporine, and others with tacrolimus. The sparse PK sampling design was employed. One blood sample was collected from each patient on day 0 of transplant, and days 7, 14, 21 and 30 post transplant. The sampling time fell into one of the flowing three time ranges: 2-4 h, 4-6 h or 6-8 h. After centrifugation, plasma samples were collected and kept at – 80 °C until analysis. Pre- and post-operative biochemical parameters indicative of liver and renal function (albumin, serum creatinine, total bilirubin, blood urea nitrogen [BUN], aspartate

aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP]) were measured in all patients.

Assessment of GVHD

In this study, clinical staging for each organ/system involved in acute GVHD and overall grading were based on a modified Keystone grading schema. Chronic GVHD was defined by the technical manual of procedures edited by the Blood and Marrow Transplant (BMT) Clinical Trials Network (CTN).²² Symptoms of chronic GVHD if present was reported using the GVHD symptom record.

Total and free MPA and MPAG analysis

MPA and MPAG concentrations were measured by a validated liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method. Briefly, the chromatographic separation was achieved on a C₁₈ column with a gradient elution, and the detection was performed by a triple quadrupole mass spectrometer in the positive electrospray ionization (ESI) and multiple reaction monitoring (MRM) mode. Linearity of the assay was demonstrated over the range of 0.02-10 µg/ml for MPA and MPAG in human plasma. The lower limit of quantification (LLOQ) for this method was 0.02 µg/ml for both MPA and MPAG. The assay was accurate and precise with bias and %CV less than < 15%.

For total MPA and MPAG analysis, 5 µl of internal standard working solution (mixture of 1 µg/ml MPA-d₃ and 5 µg/ml MPAG-d₃) was added to 50 µl of each calibration standard, QC sample or subject sample. The plasma proteins were precipitated with acetonitrile (ACN) and the supernatant was transferred into pre-labeled tubes and evaporated to dryness after vigorous mixing and centrifugation. Samples were reconstituted with 100 µl of 30 % ACN with 0.1% formic acid, centrifuged at 18,000 × g for 15 min, and the supernatant was injected into the UPLC-MS/MS system. The proportions of MPA and MPAG bound to plasma proteins in clinical samples were evaluated after 30 min of incubation at 37 °C. One hundred and fifty (150) µl of plasma sample was filtered with a Centrifree® ultrafiltration device (Millipore, Bedford, MA) assembled with a regenerated cellulose membrane (molecular weight cut-off, 30 kDa) under centrifugation (2,000

× g, Eppendorf centrifuge 5810 R equipped with a swing-bucket rotor A-4-62) for 15 min. The plasma ultrafiltrates were diluted with 30 % ACN with 0.1% formic acid and then directly injected into the UPLC-MS/MS system. Samples with concentrations above the upper limit of linearity were diluted and reanalyzed.

Statistical methods

Non-normally distributed variables were expressed as median and range, and normally distributed variables as mean and SD. All statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA). The normality of the distribution was checked with the Kolmogorov-Smirnoff test. For 2-group comparisons, continuous variables were analyzed by Student's t-test or Mann-Whitney U test, if applicable. For multi-group comparisons, continuous variables were analyzed by ANOVA or Kruskal-Wallis test and post hoc comparisons, if applicable. A p-value of 0.05 was considered statistically significant. Probability of acute and chronic GVHD was estimated with the Kaplan-Meier method, performed with GraphPad Prism 5.0.

Results

Patients

A total of 20 pediatric patients and 13 adult patients received allogeneic HSCT were included in the current analysis. The age range was 0.2-12 years (median, 3 years) and 18-63 years (median, 54 years) for pediatric and adult patients, respectively. The sex distribution was 12/8 and 7/6 males/females for pediatric and adult patients, respectively. A total of 84 blood samples were collected from pediatric patients, and 45 were from adult patients. At the time of pharmacokinetic sampling, 17 pediatric patients were co-administered with cyclosporine and 3 with tacrolimus as a concomitant immunosuppressive therapy. For adult patients, 9 were co-administered with cyclosporine, and 4 with tacrolimus. Demographics and transplant characteristics of the study population are summarized in Table 1.

Plasma protein binding

During the 1-month sampling period post HSCT, a large variation in serum albumin levels was observed and the free fraction (% unbound) of MPA and MPAG did not change significantly in pediatric patients (Figures 1 and 2). For adult patients, a temporary drop in serum albumin levels was observed in the early period post HSCT and the serum albumin levels reached to normal range (3.5–5.0 g/dL) after Day 21 post HSCT (Figure 1). A significant increase of protein binding was observed in adult patients from day 7 to day 30 post HSCT, from 97.3±0.8% to 98.3±0.6% for MPA ($P <0.05$), and 74.6±9.4% to 82.9±8.1% for MPAG ($P <0.05$), resulting in a significantly decreased percentage of unbound MPA and MPAG (Figure 2).

Sex-related differences in serum albumin levels and plasma protein binding of MPA and MPAG were observed. Significantly higher serum albumin levels were observed in pediatric males than those in pediatric females (3.6±0.5 vs 3.1±0.5; Figure 3). However, similar serum albumin levels were observed between adult males and adult females (3.5±0.4 vs 3.5±0.4; Figure 3). In pediatric patients, males displayed significantly higher plasma protein binding of MPA and MPAG compared to females (98.3±1.1 vs 97.4±1.1% for MPA and 78.7±8.7 vs 73.3±9.4% for MPAG), resulting in lower percentage unbound in males than females (1.7±1.1 vs 2.6±1.1% for MPA and 21.3±8.7 vs 26.7±9.4% for MPAG; Figure 4). In adult patients, the plasma protein binding of MPA and MPAG was also significantly higher in males compared to females (98.1±0.7 vs 97.4±1.2% for MPA and 81.5±8.3 vs 73.8±10.1% for MPAG), resulting in lower percentage unbound in males than females (1.9±0.7 vs 2.6±1.2% for MPA and 18.5±8.3 vs 26.2±10.1% for MPAG; Figure 4).

MPAG/MPA ratios

Very high plasma concentrations of MPAG (1.3-168 μ g/ml) in comparison to MPA (0.04-23.5 μ g/ml) were observed in all the patients studied. The MPAG/MPA ratios were similar between males and females in pediatric (30.1±30.9 vs 39.9±38.0) and adult (58.6±35.0 vs 40.4±29.3%) patients (Figure 5). The MPAG/MPA ratios on an mg/kg dose basis were significantly higher in adult patients than those in pediatric patients (4.3±3.4 vs 2.4±2.6; $P <0.05$; Figure 6).

Acute and chronic GVHD

Acute GVHD was observed in 4 pediatric patients (grade II) and 7 adult patients (Grade I, n=1; Grade II, n=3; Grade III, n=3). During the study period, the incidence of grade I to IV acute GVHD was 20% (4/20) and 54% (7/13), in pediatric and adult patients, respectively (Figure 7). One pediatric patient (1 cord) and five adult patient (5 PBSC) developed chronic GVHD.

Discussion

Extensive plasma protein binding is an important pharmacokinetic property of MPA. The inhibition of IMPDH depends on the free MPA. Renal function, albumin level and MPAG concentration competing for the binding may all affect the protein binding of MPA, leading to considerable alterations of free MPA concentration in vivo.^{23, 24} In renal transplant recipients, MPA protein binding negatively correlates with urea and creatinine concentration and positively correlates with albumin concentration. MPA free fraction was highly affected by free and total MPAG AUC₀₋₆.²⁵ Li et al. found total MPA clearance increased with decreased serum albumin concentration in HSCT patients, most likely due to increased unbound MPA fraction.²⁶ Impaired renal function can lead to an accumulation of MPAG, which may displace MPA from its protein binding sites or increase EHC of MPAG, consequently resulting in an increase in total MPA concentration, observed in liver transplant recipients with mild to moderate renal dysfunction.²⁷ In vitro data have shown that MPA plasma protein binding is not affected by other common immunosuppressant medications (cyclosporine, tacrolimus and prednisone).²³

In the early period post HSCT in adult patients, conditioning therapy including chemotherapy with or without radiation might lead to a temporary drop in serum albumin level, resulting in a temporary decrease in protein binding (increase in % unbound). After 1 month post HSCT, the decreased MPA free fraction in adult patients might be due to the increased albumin concentration that increases binding capacity for MPA and decreased competition of MPAG from albumin binding sites. Kuypers et al. also reported serum albumin levels initially decreased and recovered by week 6 in renal transplant recipients.²⁸ Assuming that the liver is the major organ involved in MPA elimination, the hepatic extraction ratio of MPA (the fraction of MPA that is metabolized during a

single pass through the liver) is approximately 0.3 to 0.7, indicating that MPA can be either restrictive or nonrestrictive ⁹. Therefore, its hepatic clearance will be affected by free fraction, intrinsic enzymatic activity of the liver, and the blood flow to the liver. The decrease in % unbound MPA one month post HSCT in adult patients may lead to a decrease in glucuronidation rate, resulting in an decrease in MPA clearance and an increase in total MPA exposure in the patients. Higher serum albumin levels might result in higher plasma protein binding of MPA in pediatric males than in pediatric females. In adult patients, the plasma protein binding of MPA was also significantly higher in males compared to females, although similar serum albumin levels were observed between males and females. Therefore, the plasma protein binding of MPA was not only affected by the serum albumin level, but also by some other factors. The percentage of free MPA correlates with red blood cell and leucocyte counts in renal transplant recipients.²⁹ Increasing hemoglobin causes a decrease in MPA clearance in renal transplant patients found by van HEST et al., indicating that MPA binds not only to albumin but also to hemoglobin or red blood cells. ²⁴ Therefore, sex-related differences on the hematologic parameters may also affect the unbound fraction. This speculation certainly warrants further evaluations to characterize the potential impact of these factors.

Glucuronidation is the major elimination pathway for MPA. Studies evaluating the effect of sex on MPA pharmacokinetics give conflicting results. Morissette et al. reported sex related differences in MPAG/MPA ratio. It was significantly higher in males than in females of kidney transplant patients co-administered with tacrolimus. ³⁰ The effect of sex on MPA clearance has been described by developing a population PK model in renal transplant patients following oral administration of MMF. Based on the final population PK model, it appears that males have an 11% higher MPA clearance than females. ³¹ Tornatore et al. has reported rapid apparent MPA clearance in males than in females in African Americans (13.8 ± 6.27 vs 8.70 ± 3.33 L/h) and Caucasians (10.2 ± 3.73 vs 9.71 ± 3.94 L/h) post renal transplantation. ³² A possible effect has been suggested that the lower metabolism of MPA in females may be due to the competition of estrogen

metabolism with UGTs. The sex related difference in clearance, with males exhibiting a more rapid clearance, could contribute to the large inter-individual pharmacokinetic variability. Other studies found no effect of sex on MPA clearance. The dose-adjusted AUC in females was slightly higher than in females, but this difference did not reach statistical difference in renal transplant patients.³³ In a population pharmacokinetic meta-analysis containing 13,346 MPA concentration-time data points from 468 renal transplant patients, no significant relationship was found between sex and MPA exposure.²⁴ In this study, the MPAG/MPA ratios were similar between males and females in pediatric and adult patients.

Pediatric patients display different pharmacokinetics from those of adult patients. Different MMF deposition rates are expected in pediatric patients compared to adult patients, based on the ontogeny of human hepatic UGTs. Higher MPAG/MPA ratios on an mg/kg dose basis were observed in adult patients than those in pediatric patients in this study. Gajarski et al. also found that MPAG/MPA ratios were higher for adults compared with children in heart transplant recipients.³⁴ This could be due to the higher amount of glucuronide-conjugating enzymes in the liver of adult patients than that of pediatric patients. Further studies are still needed to better understand the underlying developmental changes of hepatic UGTs activity.

Metabolic drug-drug interaction may exist when co-administered with other immunosuppressants including cyclosporine and tacrolimus. Cyclosporine, an Mrp2 inhibitor, can cause a decrease in the biliary secretion of MPAG, resulting in an increase in MPAG exposure and a decrease in MPA exposure.³⁵ Tacrolimus, though mainly metabolized by the cytochrome P 450 (CYP) 3A subfamily, is reportedly a good inhibitor of MPA conjugation both in vitro and in vivo. Co-administration with tacrolimus can decrease the intrinsic UGT enzymatic clearance of MPA and consequently augment the bioavailability of MMF.^{36, 37} On the same MMF dose basis, total and free MPA concentrations are lower when co-administered with cyclosporine, but higher with tacrolimus in organ transplant patients.^{17, 38}

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Following an oral administration of MMF, the average plasma half-life in liver and renal transplant patients is about 6 and 11 h, respectively, and the concentration-time profile of MPA often shows two peaks, the first peak occurring within 2 h post-dose and the second one at 6-12 h due to EHC. In clinical HSCT studies, plasma MPA half-lives ranging from 1 to 4 h are observed. Compared to solid organ recipients, MPA exposure is lower and the EHC is markedly reduced or absent in HSCT patients receiving an equivalent dose of MMF.^{13, 39-41} In our study, no secondary peak was observed on MPA concentration-time profile when co-administered with cyclosporine or tacrolimus. The reasons, however, were still unclear. Physiological changes including gut GVHD and damaged epithelium of the intestine due to high-dose chemotherapy and/or the reduction in bacterial flora in the gastrointestinal tract from broad-spectrum antibiotic use could reduce the contribution of EHC, resulting in a lower MPA exposure. Additional studies are needed to determine the pathophysiological mechanisms responsible for the altered MPA pharmacokinetics in HSCT patients.

The current study certainly has several potential limitations. The relatively small number of clinical evaluable HSCT patients may affect our statistical power. Since measurement of MPA exposure using a full set of samples requires considerable volume of blood, which is not feasible for pediatric patients, the relatively sparse sampling approach used in this study limits our ability to characterize the reabsorption of MPA due to the EHC. Another potential limitation is that no pharmacokinetic parameters were derived for cyclosporine and tacrolimus. The inhibition of Mrp2 and UGT by cyclosporine and tacrolimus, respectively, may vary among patients. Despite these limitations, our findings provided useful findings for pharmacokinetic variability in both pediatric and adult HSCT patients.

Conclusions

Mycophenolic acid is a commonly used immunosuppressant with complex pharmacokinetics and substantial inter- and intra-patient variability. This study provides preliminary data to explain inter- and intra-patient pharmacokinetic variability of MPA in both adult and pediatric HSCT patients.

Revised

We have observed time-dependent changes of protein binding and age-related differences on metabolism of MPA post HSCT. Time-dependent changes in plasma protein binding could contribute to the intra-individual variation in adult patients post HSCT. Age-dependent metabolic ability, as well as sex-related plasma protein binding could contribute to the inter-individual variation. In order to achieve a reliable immunosuppression and less toxic side effects in HSCT patients, we believe that effective drug monitoring for MPA needs to be established for the most optimal use of MPA. Incorporating these patient and pharmacologic factors into MMF regimens may contribute to the individualization of MMF dosing in pediatric and adult HSCT patients.

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Declaration of conflicting interests

None of the authors declare conflicts of interest that could be perceived as influencing this research.

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Figures and tables

Table 1. Patient demographic and clinical characteristics. Data are given as median (range)

| Characteristics | Pediatric patients (n=20) | Adult patients (n=13) |
|------------------------------------|----------------------------------|------------------------------|
| Age at transplant (yrs) | 3 (0.2-12) | 54 (18-63) |
| Body weight (kg) | 13.7 (5.4-33.3) | 91.9(38.4-113.3) |
| Sex (Male/Female) | 12/8 | 7/6 |
| Race | | |
| White | 16 | 13 |
| Asian | 2 | 0 |
| Black | 2 | 0 |
| Ethnicity | | |
| Hispanic | 4 | 0 |
| Non-Hispanic | 14 | 13 |
| MMF Dose | | |
| in mg/kg (2-hr infusion, TID) | 15 | |
| in mg(BID) | | 1000 |
| Transplant source | | |
| Bone marrow | 1 | 0 |
| Cord or double cord | 19 | 2 |
| Peripheral blood stem cells (PBSC) | 0 | 11 |
| Donor type | | |
| Related sibling | 1 | 7 |
| Unrelated donor | 19 | 6 |
| Malignant/Non-malignant | 9/11 | 13/0 |
| Alive/Deceased | 18/2 | 8/5 |
| Blood chemistry | | |
| Albumin (g/dL) | 3.6 (2.4-4.4) | 3.5 (2.3-4.5) |
| Creatinine (mg/dL) | 0.3 (0.2-1.7) | 0.9 (0.4-2.7) |
| Total bilirubin (mg/dL) | 0.6 (0.2-7.7) | 0.6 (0.2-18.5) |
| BUN (mg/dL) | 17.5 (3-42) | 17(3-78) |
| AST (Units/L) | 24 (9-119) | 21 (8-173) |
| ALT (Units/L) | 20.3 (6-220) | 18 (9-343) |
| ALP (Units/L) | 130.3 (63-324) | 84 (34-153) |

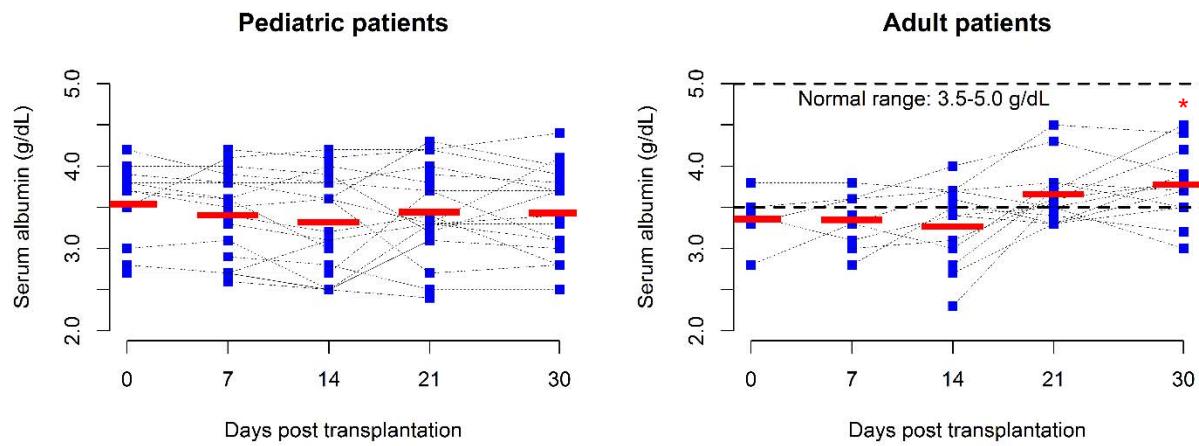


Figure 1. Changes in serum albumin levels over 1-month sampling period in pediatric and adult patients after HSCT. Horizontal solid lines indicate mean values. Reference range of serum albumin concentrations for adult patients: 3.5–5.0 g/dL.

* Kruskal-Wallis test at $p<0.05$.

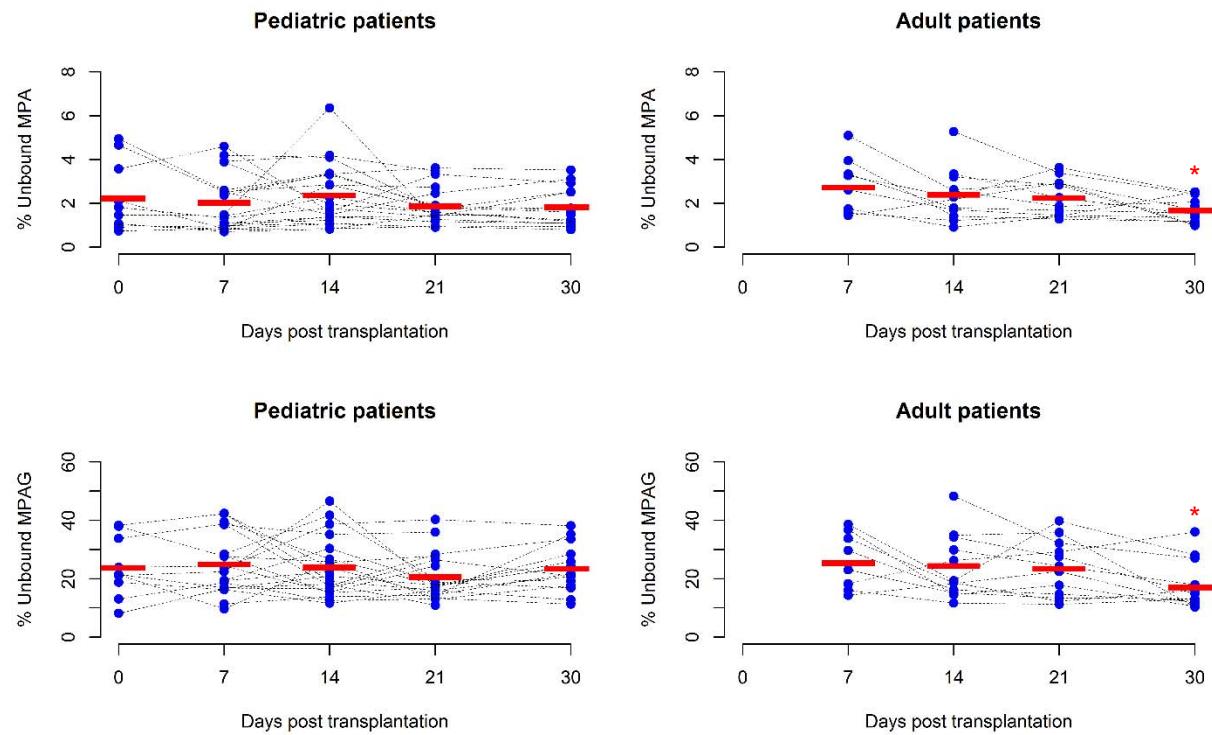


Figure 2. Changes in percent unbound MPA and MPAG over 1-month sampling period in pediatric and adult patients after HSCT. Horizontal solid lines indicate mean values of pharmacokinetic parameters.

* Kruskal-Wallis test at $p<0.05$.

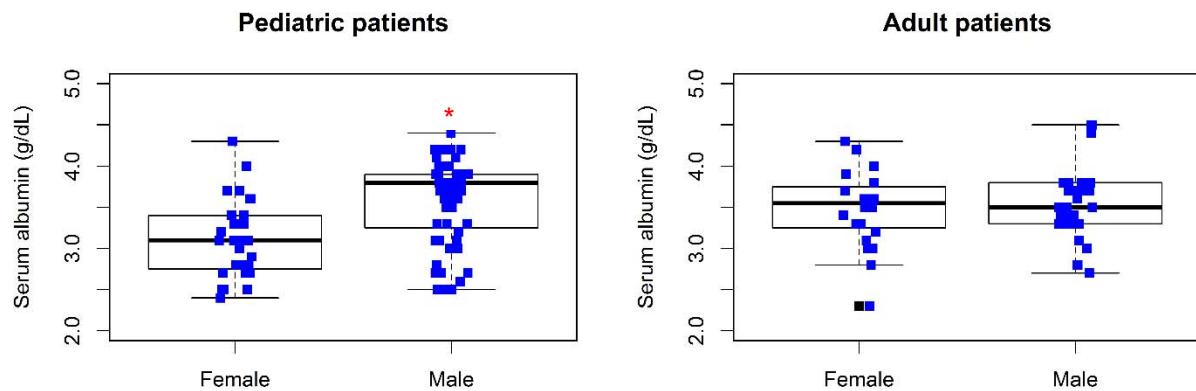


Figure 3. Sex differences in serum albumin levels in pediatric and adult patients after HSCT. *

Unpaired t-test at $p<0.05$.

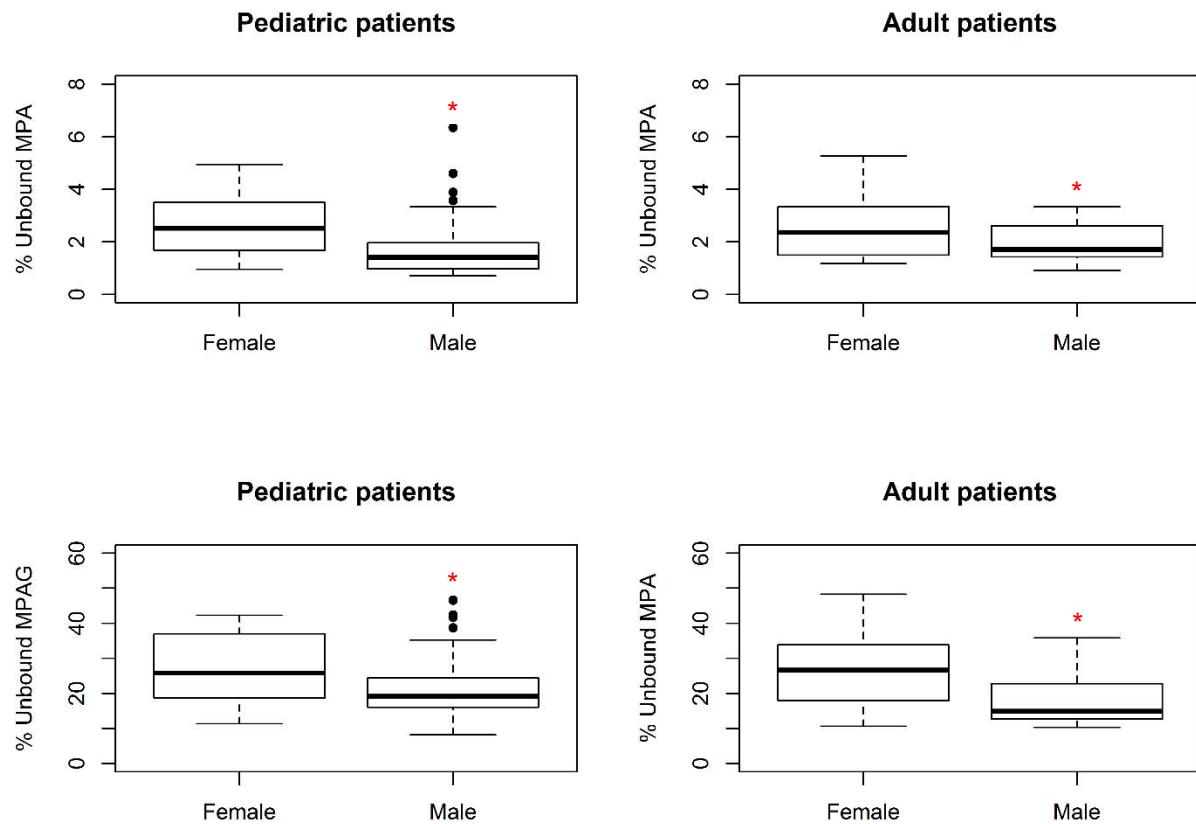


Figure 4. Sex differences in percent unbound MPA and MPAG in pediatric and adult patients after HSCT. * Unpaired t-test at $p<0.05$.

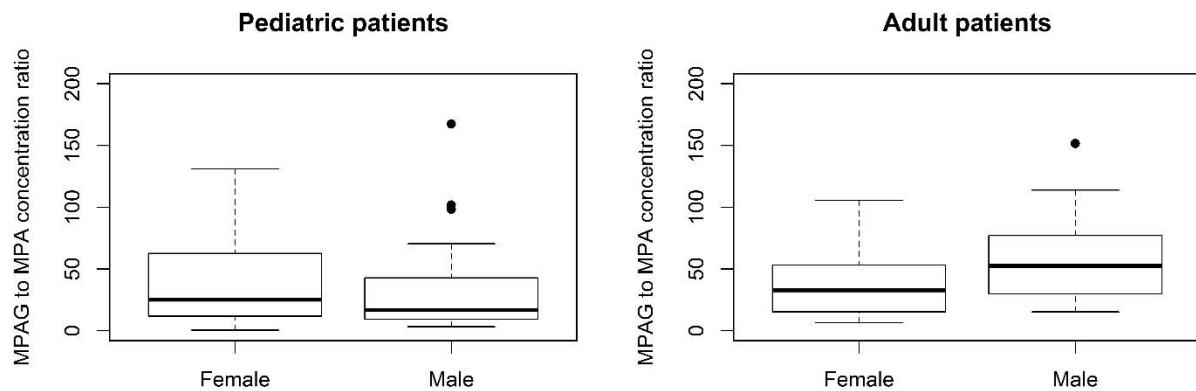


Figure 5. Sex Differences in MPAG to MPA concentration ratios in pediatric and adult patients after HSCT. Unpaired t-test at $p<0.05$.

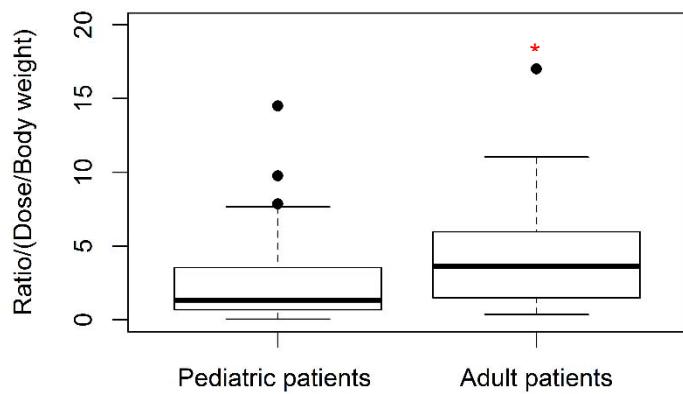


Figure 6. Differences in MPAG to MPA concentration ratios in pediatric and adult patients after HSCT. MPAG to MPA concentration ratios normalized by dose/body weight. * Unpaired t-test at $p < 0.05$.

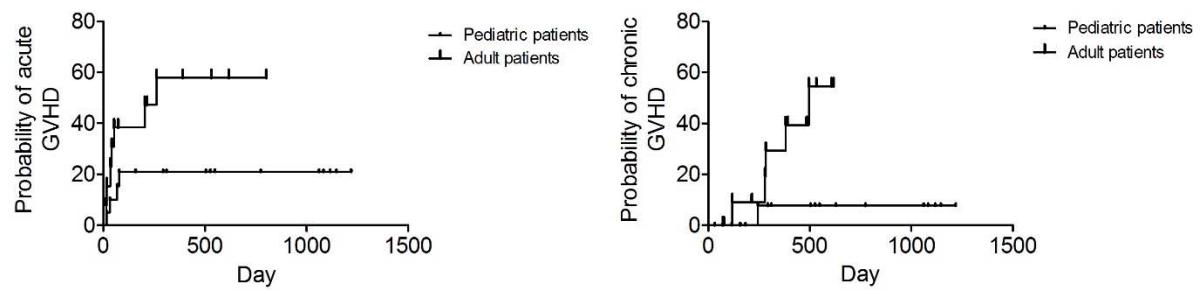


Figure 7. The probability developing grade I to IV acute GVHD or chronic GVHD in pediatric and adult patients.