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Pharmacokinetic and Pharmacodynamic Markers of Mycophenolic Acid Associated with Effective Prophylaxis for Acute Graft-versus-Host Disease and Neutrophil Engraftment in Cord Blood Transplant Patients

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Short title: PK and PD markers of MPA in CBT patients

ABSTRACT (294)

Mycophenolate mofetil (MMF) is a pro-drug of mycophenolic acid (MPA) and is frequently used to prevent acute graft-versus-host disease (aGVHD) in patients receiving hematopoietic stem cell transplants (HCT). However, optimal doses of MMF and target MPA concentrations in HCT patients have not been defined. In this study, relationships between pharmacokinetic or pharmacodynamic markers of MPA and successful aGVHD prevention and neutrophil engraftment were evaluated to inform individualized MPA treatments in HCT patients.

We recruited 35 patients undergoing cord blood transplantation (CBT) who were treated with MMF. Area under the concentration–time curves from 0 to 24 h (AUC_{0-24}) for free MPA and MPA acyl glucuronide (AcMPAG) at one week after the start of MMF treatments were significantly higher in patients with gastrointestinal (GI) aGVHD at stage ≥ 1 than those at stage 0. Patients with faster neutrophil engraftment had higher free MPA AUC_{0-24} at one week after the start of MMF treatments compared with those with slower neutrophil engraftment. Inosine-5'-monophosphate dehydrogenase activity in peripheral blood mononuclear cells and single nucleotide polymorphisms in genes that were previously associated with MPA pharmacokinetics and pharmacodynamics were not an independent predictor for the clinical outcome. Receiver operating characteristic model analyses showed

that cut-off values of AUC_{0-24} for successful GI aGVHD prevention were 0.689 and 15.6 $\mu g \cdot h \cdot mL^{-1}$ for free MPA and AcMPAG, respectively. In addition, the cut-off value of free MPA AUC_{0-24} for neutrophil engraftment by day 25 was 0.405 $\mu g \cdot h \cdot mL^{-1}$.

In conclusion, free MPA AUC_{0-24} may be a better predictor of the prevention of GI aGVHD and neutrophil engraftment compared with that of total MPA in patients receiving CBT. Hence, monitoring of the free MPA AUC_{0-24} between 0.405 and 0.689 $\mu g \cdot h \cdot mL^{-1}$ could be considered informative of individualized MPA treatments in CBT patients.

Keywords: mycophenolic acid; pharmacokinetics; pharmacodynamics; cord blood transplantation

INTRODUCTION

Patients with hematopoietic stem cell transplantation (HCT) require immunosuppressive treatments for 6 to 12 months after transplantation to prevent acute graft-versus-host disease (aGVHD) [1,2]. Methotrexate (MTX) has been used for decades as a combination treatment with calcineurin inhibitors such as tacrolimus and cyclosporine [3]. In a study by Bolwell *et al.* [4], regimens of a calcineurin inhibitor and mycophenolate mofetil (MMF) led to faster hematopoietic engraftment, decreased incidence of mucositis, and similar incidence of aGVHD compared with those of a calcineurin inhibitor and MTX. Accordingly, MMF is increasingly used as an alternative to MTX in HCT recipients [5].

Mycophenolic acid (MPA) is the active and hydrolyzed form of MMF and is metabolized by glucuronosyltransferases in the liver. MPA glucuronide (MPAG) and MPA acyl glucuronide (AcMPAG) are primarily produced by UDP-glucuronosyltransferases (UGT) 1A9 and 2B7, respectively [6], and the *in vitro* pharmacological activity of AcMPAG indicates roles in the gastrointestinal toxicity of MPA [7]. MPA metabolites are eliminated by multidrug resistance associated protein 2 (MRP2) and whereas most are excreted via the urine, some are found in bile[8]. Due to the complicated pharmacokinetics of MPA, area under the concentration–time curves (AUC) for MPA are routinely monitored for individualized treatments in solid organ transplant recipients [9], and the therapeutic 0–12 h AUC range of

MPA was shown to be 30–60 μg·h·mL^{-1} in renal transplant patients [10]. Recently, Arai *et al.* [11] suggested that monitoring of MPA plasma concentrations is necessary for effective prophylaxis against aGVHD in cord blood transplant (CBT) patients. However, optimal doses of MMF and target concentration ranges of MPA remain undefined in CBT patients [12].

MPA selectively inhibits inosine-5'-monophosphate dehydrogenase (IMPDH) and suppresses the proliferation of B and T lymphocytes [13], and different genes encode the isoforms IMPDH1 and 2 [14,15]. In addition, recombinant IMPDH2 was shown to be 4.8-fold more sensitive to MPA than IMPDH1 [16], and area under the effect-time curve (AUEC) values of IMPDH activity on day 21 after HCT were previously associated with non-relapse and overall mortality [17]. Therefore, measurements of IMPDH activity in peripheral blood mononuclear cells (PBMCs) and AUC values of MPA are considered as effective predictors of clinical outcomes for MPA treatments. In addition, single nucleotide polymorphisms (SNPs) in genes were previously associated with MPA pharmacokinetics and pharmacodynamics. In particular, the *UGT2B7* SNP *−842G>A* resulted in significantly higher AcMPAG AUC values at one and three months after renal transplantation [18], and the *MRP2* SNP *−24C>T* was associated with a significantly higher dose-corrected MPA trough levels at later time points after transplantation [19]. In addition, the *IMPDH2* SNP *3757 T>C* was associated with significantly higher IMPDH activity following MMF treatments, but had

no effects on MPA exposures [20]. Finally, the incidence of acute rejection during the first year after transplantation was significantly lower in carriers of *IMPDH1 −106 G>A* and *125 G>A* compared with the respective wild-type individuals [21]. Hence, multiple polymorphisms influence the pharmacodynamics of MPA in HCT patients. Thus, to inform individualized MPA treatments, we associated pharmacokinetic and pharmacodynamic markers of MPA, including previously reported SNPs, with successive aGVHD prevention and neutrophil engraftment in CBT patients.

METHODS

Patients and Study Design

We recruited adult Japanese patients undergoing CBT at the Department of Hematology and Oncology in Kyoto University Hospital between March 2013 and August 2016, and included those who received MMF and tacrolimus for GVHD prophylaxis [22]. In these patients, continuous intravenous administration of tacrolimus was initiated at 0.02 mg/kg/day on day −1 of transplantation, and trough blood concentrations of tacrolimus were monitored to adjust doses to 12–15 ng/mL. Oral administration of MMF was initiated on day −1 of CBT at 10 mg/kg body weight every 8 h (30 mg/kg/day) and was stopped approximately within 1 month, although one patient received MMF at 15 mg/kg body weight every 12 h. Blood

samples were collected immediately before, and at 1, 2, 4, and 8 h after the morning dose in the first and third weeks after the start of MMF treatments. For the patient who was administered MMF every 12 h, additional blood samples were taken at 12 h after the morning dose in the first and third weeks of MMF treatments. This clinical study was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine and Kyoto University Hospital.

Clinical Outcomes

Diagnoses and classifications of aGVHD were performed by attending physicians using traditional criteria [23]. Dysfunction of each organ (skin, gastrointestinal (GI), and liver) was evaluated from stage 0 to 4 using organ symptoms as well as biopsy for skin and GI. Each patient was assigned to the maximum aGVHD grade based on the stage classification of each organ occurred within day 100 after transplantation. Patients were defined as aGVHD development in the case of grade II or greater. Grade II aGVHD includes some one of skin dysfunction at stage 3, GI dysfunction at stage 1, or hepatic dysfunction at stage 1. In this study, no patients showed liver dysfunction at stage 1 or greater. Therefore, we classified each patient into three groups according to each organ dysfunction: skin G(+), skin aGVHD at stage ≥ 3; GI G(+), gastrointestinal aGVHD at stage ≥ 1; G(-), aGVHD grade 0 or I.

Definitions of myeloablative conditioning (MAC) and reduced-intensity conditioning (RIC) were consistent with those established in the RIC regimen workshop [24]. Disparities in human leukocyte antigens (HLA) A, B, and C, and DR antigens were determined at serology examinations. Neutrophil engraftment was defined by absolute neutrophil counts of more than 0.5×10^9/L for 3 consecutive days [25].

Experimental Methods

Blood samples were collected in 3-mL heparinized collection tubes and were centrifuged at 15,000 *g* for 10 min. Subsequently, 20-μL aliquots of 10% acetic acid were added to 1-mL separated plasma samples to stabilize AcMPAG. Unbound MPA was then separated by ultrafiltration of 500-μL plasma samples using Amicon Ultra 30 K centrifugal filter devices (Merck Millipore Ltd., Carrigtwohill, Ireland) at 14,000 *g* for 10 min. Concentrations of MPA, MPAG, and AcMPAG were analyzed using liquid chromatography - tandem mass spectrometry (LC-MS/MS) as previously reported [26], with lower limits of quantification of 0.05, 0.2, and 0.02 μg/mL, respectively. PBMCs were isolated from the remaining blood samples using density gradient centrifugation with Ficoll-Paque PLUS (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and were then stored at −20°C until analysis. IMPDH activity in PBMC samples was measured according to previously reported methods [26], and

was calculated based on xanthosine-5′-monophosphate (XMP) production normalized to intracellular adenosine-5′-monophosphate (AMP) levels. The lower limit of quantification for XMP and AMP was 50 nM, and samples with lower levels of AMP were excluded from pharmacodynamic analyses due to extremely small numbers of PBMCs.

Genomic DNA was isolated from whole blood samples that were collected from pre-transplant recipients and from recipients at approximately five weeks after CBT (post-engraftment). Subsequently, the SNPs *IMPDH1 −106G>A* (rs2278294), *IMPDH1 125G>A* (rs2278293), and *IMPDH2 3757T>C* (rs11706052) were identified using TaqMan probes that were designed by Applied Biosystems (Foster City, CA). In addition, pre-transplant DNA was also used to identify *UGT2B7 −842C>T* (rs7439366) and *MRP2 −24C>T* (rs717620) genotypes.

Statistical Analysis

AUC values of MPA from 0 to 8 or 12 h (AUC_{0-8} or AUC_{0-12}, respectively) and AUEC values of IMPDH from 0 to 8 or 12 h ($AUEC_{0-8}$ or $AUEC_{0-12}$, respectively) were calculated from measurements using the linear trapezoidal method. AUC_{0-24} was calculated as AUC_{0-8} or AUC_{0-12} multiplied by daily dose divided by morning dose of MMF. Analyses of neutrophil engraftment were performed in patients that were grouped according to median

days of neutrophil engraftment (day 25). Pharmacokinetic or pharmacodynamic parameters of MPA were compared among three groups using Kruskall-Wallis test followed by Dunn's multiple comparison test. Differences between mean values of the two groups were identified using Student's *t*-test when variances were similar, and were identified using Mann-Whitney *U*- test in the presence of unequal variances.

Factors associated with aGVHD development or neutrophil engraftment were identified using Cox proportional hazard models. Covariates included ABO compatibility, age (≥ 50 or ≥ 60), gender (female for a donor and male for a recipient versus other combinations), HLA mismatches ≥ 3, conditioning regimen, genetic polymorphisms at pre- and post-engraftment (heterozygote + homozygote type versus wild type), and pharmacokinetic and pharmacodynamic parameters of MPA ($AUC_{0\text{-}24}$ or $AUEC_{0\text{-}8}$) at 1 and 3 weeks. Additional covariates for aGVHD development included the engraftment day and cytomegalovirus (CMV) relapse, and for neutrophil engraftment included total nucleated cell (TNC) and CD34+ cell counts and cell counts per body weight. Factors were initially assessed in univariate Cox proportional hazard analyses, and independent covariates with *p* values of less than 0.1 were included in multivariate Cox proportional hazard analyses. The final model comprised covariates with *p* values of less than 0.05 and results are expressed as hazard ratios with 95% confidence intervals. Receiver operating characteristic (ROC) model analyses were

performed for cut-off values of aGVHD development for the GI tract and neutrophil engraftment (day < 25) using the Youden index. All statistical analyses were performed using Prism version 6 (GraphPad Software, Inc.) or IBM SPSS Statistics for Macintosh version 22.0 (Armonk, NY). $P < 0.05$ was considered statistically significant.

RESULTS

Patient Characteristics

A total of 40 patients provided consent and the treatment plan was feasible for 35 patients (87.5%). The other five patients were excluded from clinical outcome analyses due to difficulty of continuation with MMF treatment (n = 3), non-compliance (n = 1), and severe GI dysfunction before the first therapeutic assessment of MPA effects, but not due to hyper-acute GVHD (n = 1). Clinical characteristics of all included patients are listed in Table 1. Among these, three patients received HCT twice in the study period, and two of three patients were excluded from evaluations of aGVHD due to engraftment failure. The incidence of aGVHD of > grade II was 30.3% (10/33 patients), and included seven patients with skin aGVHD and three patients with GI aGVHD. No patients were diagnosed with hepatic aGVHD. The ratio of neutrophil engraftment was 94.3% (33/35 patients) and the median engraftment time was 25 days. The onset of skin and GI aGVHD were detected

between day 23 and 67 (median, day 32) and between day 48 and 50 (median, day 49), respectively. Eighteen and one AUEC of IMPDH at one and three weeks, respectively, were not included in analyses, because their AMP levels were below the limits of quantitation reflecting small numbers of PBMCs. Data of $AUEC_{0-12}$ from one patient receiving MMF every 12 h were not included in the analysis. The genotypes of two patients were unknown before transplantation due to a second HCT in one and defective DNA isolation from the other. The genotypes of two other patients were unknown after transplantation due to engraftment failure. All observed genotype distributions were consistent with the Hardy-Weinberg equilibrium.

Relationships between Pharmacokinetic and Pharmacodynamic Markers of MPA and Clinical Outcomes

At one week after starting MMF treatments, AUC_{0-24} values for total MPA, free MPA, MPAG, and AcMPAG, and $AUEC_{0-8}$ values for IMPDH, did not differ significantly between skin G(+) and G(−) groups (Fig. 1). In contrast, AUC_{0-24} values for free MPA and AcMPAG at one week after starting MMF treatments were significantly higher in patients of the GI G(+) group than in those of the G(−) group. AUC_{0-24} values for total MPA, free MPA, MPAG, and AcMPAG, and $AUEC_{0-8}$ for IMPDH, did not differ significantly among G(−), skin G(+), and GI G(+) groups at 3 weeks after the start of MMF treatments.

Patients with faster neutrophil engraftment (< 25 days) had significantly higher free MPA $AUC_{0\text{-}24}$ values at one week after starting MMF treatments (Fig. 2). Moreover, the $AUEC_{0\text{-}8}$ value for IMPDH tended to be lower in patients with faster neutrophil engraftment, although this difference was not significant. At 3 weeks after starting MMF treatments, all $AUC_{0\text{-}24}$ values for total MPA, free MPA, MPAG, and AcMPAG, and $AUEC_{0\text{-}8}$ values for IMPDH, were similar between patients with neutrophil engraftment times of < 25 and ≥ 25 days.

Risk Factors for aGVHD development and Neutrophil Engraftment

Risk factors associated with skin G(+) or GI G(+) in patients receiving CBT were examined in the univariate analysis (Table 2), and risk factors for skin G(+) significantly associated were major ABO compatibility (p = 0.007), age ≥ 50 (p = 0.096), age ≥ 60 (p = 0.093), *MRP2 −24C>T* heterozygote + homozygote type pre-transplants compared with wild type (p = 0.036), *IMPDH2 3757T>C* heterozygote + homozygote type post-transplants compared with wild type (p = 0.021), and $AUC_{0\text{-}24}$ values for total MPA. However, no factors were significantly associated with skin G(+) in the multivariate analysis (Table 3).

In the univariate analysis of GI G(+), significant risk factors included $AUC_{0\text{-}24}$ values for free MPA, MPAG, and AcMPAG at one week after starting MMF treatments, and $AUC_{0\text{-}24}$ values for total MPA and AcMPAG at three weeks after starting MMF treatments

(Table 2). The AUC value for AcMPAG at one week after starting MMF treatments was the most significant risk factor for GI G(+) (p = 0.008). Associations of AUEC values for IMPDH at one week after starting MMF treatments were not assessed using univariate analyses because too few patients were included in the GI G(+) group. Moreover, further multivariate analyses were discarded because all significant factors were highly correlated pharmacokinetic parameters.

As shown in Table 4, significant risk factors for neutrophil engraftment in the univariate analyses included HLA mismatches of ≥ 3 (p = 0.026), CD34+ cell count per body or body weight (p = 0.091 and 0.012, respectively), and $AUC_{0\text{-}24}$ values for total or free MPA at one week after the start of MMF treatments (p = 0.042 and 0.024, respectively). In the multivariate analyses, HLA mismatches of ≥ 3, CD34+ cell count per body weight, and $AUC_{0\text{-}24}$ value for free MPA were significantly associated with neutrophil engraftment.

ROC Analyses of GI aGVHD and Neutrophil Engraftment

Significant pharmacokinetic markers that were identified were further assessed in ROC analyses (Table 5). ROC curve analyses defined the cut-off values for free MPA and AcMPAG $AUC_{0\text{-}24}$ of 0.689 and 15.6 $\mu g \cdot h \cdot mL^{-1}$, respectively, as predictors of GI aGVHD development. Moreover, $AUC_{0\text{-}24}$ values from ROC curves were 0.967 (p = 0.009) for free

MPA and 0.956 (p = 0.010) for AcMPAG, and sensitivity and specificity were 100% and 96.7% for free MPA, and were 100% and 90% for AcMPAG, respectively. The cut-off values of free MPA AUC value for the neutrophil engraftment by day 25 were 0.405 $\mu g \cdot h \cdot mL^{-1}$ in ROC curve analyses, with an AUC value for the ROC of 0.766 (p = 0.007). Sensitivity and specificity were 79% and 75%, respectively. The AUC value for the ROC of total MPA was 0.648 (p = 0.136) and was not significantly predictive.

DISCUSSION

Although MMF is often used to prevent aGVHD in patients receiving HCT [27-29], the therapeutic range of MPA remains undefined in these patients. In this study, higher AUC values for AcMPAG as well as free MPA were associated with aGVHD development of the GI tract, and higher AUC values for free MPA were significantly associated with faster neutrophil engraftment in CBT patients. The therapeutic range of free MPA AUC_{0-24} values was indicated as 0.405–0.689 $\mu g \cdot h \cdot mL^{-1}$. No SNPs that were previously associated with pharmacokinetic and pharmacodynamic parameters of MPA were associated with aGVHD development or neutrophil engraftment.

In analyses of the relationship between pharmacokinetic and pharmacodynamic markers and site-specific aGVHD, the AUC value of free MPA and AcMPAG at one week

after the start of MMF treatments was significantly higher in patients with aGVHD of the GI tract than in those without aGVHD. In addition, the AUC of AcMPAG at one week after the start of MMF treatments was the most significant risk factor for aGVHD of the GI tract in Cox proportional regression analyses. However, since pharmacokinetics of MPA had a large intra-individual variability as shown in our previous report [22], there were no significant relationships between MPA pharmacokinetic parameters at three weeks after the start of MMF treatments and aGVHD. Patients who suffer from aGVHD after HCT do so as a result of excessive production of the inflammatory cytokines interleukin (IL)-1, IL-6, and tumor necrosis factor alpha (TNF-α), during the early stages after HCT [30,31]. Higher free MPA concentration brings to increase AcMPAG concentrations, because free MPA is metabolized to AcMPAG in the liver, and AcMPAG is excreted in bile. AcMPAG concentrations in intestine are greater than those in plasma, reflecting its bile excretory pathway. Due to its unstable structure, AcMPAG was reportedly bound to plasma proteins and other macromolecules such as several proteins and DNA [32]. Furthermore, AcMPAG reportedly induces mRNA and protein expression of the pro-inflammatory cytokines TNF-α and IL-6 [11]. In addition, conditioning regimens cause direct mucosal damage in HCT patients [33], suggesting that increases in AcMPAG plasma concentrations during the early stages after transplantation leads to leukocyte activation and/or direct impairment of the GI tract and

mediates inflammatory GI symptoms. However, only three patients showed aGVHD of the GI tract in this study, and AUC values of AcMPAG from patients receiving CBT were lower than those reported in patients with organ transplants [34]. Other previous studies show that the AcMPAG concentrations required to induce TNF-α and IL-6 *in vitro* are considerably higher than those in clinical studies [11,35,36]. Therefore, further studies are required to characterize the relationship between aGVHD development in the GI tract and AcMPAG or free MPA plasma concentrations.

In studies of the relationship between pharmacokinetic and pharmacodynamic markers and neutrophil engraftment, patients with faster neutrophil engraftment had significantly higher concentrations of free MPA at one week after the start of MMF treatments than those with slower engraftment. In the multivariate Cox proportional regression analyses, the AUC value for free MPA at one week after the start of MMF treatments was significantly associated with neutrophil engraftment in addition to HLA mismatch ≥ 3 and CD34+ cell count per body weight. Factors that have been shown to affect neutrophil engraftment in CBT are degree of HLA mismatches and total nucleated cell counts per body weight [37]. In this study, the median (range) of TNC per body weight was 2.55 (1.80 – 5.14) x 10^7 cells/kg, and almost all patients had enough counts greater than 2 x 10^7 cells /kg. This can be the reason for no association of TNC with neutrophil engraftment. Patients after CBT suffer from various

symptoms of pre-engraftment immune reactions (PIR), and these are caused by the excessive production of inflammatory cytokines [38]. In particular, such patients suffer from hyperthermia, increased weight, and skin rashes until approximately day 9 after CBT [39]. In addition, hemophagocytic syndrome (HPS) is induced by activated macrophages [40], and PIR and HPS delay engraftment and cause engraftment failure [41, 42]. The incidence of severe pre-engraftment syndrome in patients treated with a calcineurin inhibitor in combination with MMF was significantly lower than in those treated with the calcineurin inhibitor only [8]. Moreover, MMF combination regimens prevented HPS and improved the rate of engraftment in CBT patients, likely reflecting better control of PIR [26]. Therefore, the inhibition of immune cell activation by higher concentrations of free MPA may facilitate neutrophil engraftment by preventing the onset of PIR and HPS.

Based on total MPA concentrations, the therapeutic range of MPA in renal transplant patients was defined as $AUC_{0\text{-}12}$ of 30–60 $\mu g \cdot h \cdot mL^{-1}$ [10]. However, total MPA concentrations were not related to neutrophil engraftment or aGVHD development in this study. Although free MPA concentrations were previously identified as a biomarker of the immunosuppressive effects of MPA compared with total MPA [43], Jacobson *et al.* [44] showed that subjects with free MPA $AUC_{0\text{-}6}$ values of less than 0.15 $\mu g \cdot h \cdot mL^{-1}$ had higher cumulative incidences of aGVHD development than subjects with a greater AUC values, and

free MPA $AUC_{0\text{-}12}$ values of less than 0.3 $\mu g \cdot h \cdot mL^{-1}$ were also associated with more frequent aGVHD. Atcheson *et al*. [34] reported significantly higher free MPA $AUC_{0\text{-}6}$ values in patients with thrombocytopenic, leukopenic, and/or infectious outcomes than in other patients. Therefore, free MPA concentrations are likely associated with the efficacy and/or toxicity of MPA. In the present ROC analyses, free MPA $AUC_{0\text{-}24}$ values of less than 0.689 $\mu g \cdot h \cdot mL^{-1}$ were associated with successive aGVHD prevention for the GI tract and the values of more than 0.405 $\mu g \cdot h \cdot mL^{-1}$ were essential to achieve neutrophil engraftment by day 25, suggesting that the therapeutic range of 0.405–0.689 $\mu g \cdot h \cdot mL^{-1}$ as free MPA $AUC_{0\text{-}24}$ is preventative of aGVHD in the GI tract and concomitantly achieves early neutrophil engraftment in patients receiving CBT.

The present study was limited by relatively small numbers of patients with aGVHD (seven with skin aGVHD and three with GI aGVHD). Thus, further studies are warranted to clarify the importance of therapeutic monitoring of free MPA AUC values in larger numbers of CBT patients. Conditioning intensity has been shown to be a risk factor for aGVHD in HCT [45, 46]. The relationship between the total body radiological dosage and aGVHD development was examined in this study (data not shown), but there were no significant relationship. Since conditioning regimens were variable among patients and the present study included a relatively small number of patients, effects of conditioning regimens on aGVHD

development should be examined in a future study.

In conclusion, serum concentrations of AcMPAG and free MPA were both associated with aGVHD development of the GI tract, and free MPA concentrations were predictive of early neutrophil engraftment in CBT patients. Therefore, free MPA monitoring could be used to inform MMF dose adjustments in patients receiving CBT.

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Reference

1. Gatza E, Choi SW. Approaches for the prevention of graft-versus-host disease following hematopoietic cell transplantation. *Int J Hematol Oncol*. 2015; 4: 113-26.
2. MacMillan ML, Robin M, Harris AC, *et al.* A refined risk score for acute graft-versus-host disease that predicts response to initial therapy, survival, and transplant-related mortality. *Biol Blood Marrow Transplant.* 2015; 21: 761-7.
3. Storb R, Deeg HJ, Whitehead J, *et al*. Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host-disease after marrow transplantation for leukemia. *New Engl J Med.* 1986; 314: 729-35.
4. Bolwell B, Sobecks R, Pohlman B, *et al*. A prospective randomized trial comparing cyclosporine and short course methotrexate with cyclosporine and mycophenolate mofetil for GVHD prophylaxis in myeloablative allogeneic bone marrow transplantation. *Bone Marrow Transplant.* 2004; 34: 621-5.
5. Iida M, Fukuda T, Uchida N, *et al.* Mycophenolate mofetil use after unrelated hematopoietic stem cell transplantation for prophylaxis and treatment of graft-vs.-host disease in adult patients in Japan. *Clin Transplant.* 2014; 28: 980-9.
6. Dupuis R, Yuen A, Innocenti F. The influence of UGT polymorphisms as biomarkers in solid organ transplantation. *Clinica Chimica Acta*. 2012; 413: 1318-25.

7. Wieland E, Shipkova M, Schellhaas U, *et al.* Induction of cytokine release by the acyl glucuronide of mycophenolic acid: a link to side effects? *Clin Biochem.* 2000; 33: 107-13.
8. Patel CG, Ogasawara K, Akhlaghi F. Mycophenolic acid glucuronide is transported by multidrug resistance-associated protein 2 and this transport is not inhibited by cyclosporine, tacrolimus or sirolimus. *Xenobiotica.* 2013; 43: 229-35.
9. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of mycophenolate in solid organ transplant recipients. *Clin Pharmacokinet.* 2007; 46: 13-58.
10. van Gelder T, Hilbrands LB, Vanrenterghem Y, et al. A randomized double-blind, multicenter plasma concentration controlled study of the safety and efficacy of oral mycophenolate mofetil for the prevention of acute rejection after kidney transplantation. *Transplantation.* 1999; 68: 261-6.
11. Arai Y, Kondo T, Kitano T, *et al*. Monitoring mycophenolate mofetil is necessary for the effective prophylaxis of acute GVHD after cord blood transplantation. *Bone Marrow Transplant.* 2015; 50: 312-4.
12. Wakahashi K, Yamamori M, Minagawa K, *et al.* Pharmacokinetics-based optimal dose prediction of donor source-dependent response to mycophenolate mofetil in unrelated hematopoietic cell transplantation. *Int J Hematol.* 2011; 94: 193-202.

13. Allison AC, Eugui EM. Mycophenolate mofetil and its mechanisms of action. *Immunopharmacology.* 2000; 47: 85-118.

14. Collart FR, Huberman E. Cloning and sequence-analysis of the human and Chinese-hamster inosine-5'-monophosphate dehydrogenase cdnas. *J Biol Chem.* 1988; 263: 15769-72.

15. Natsumeda Y, Ohno S, Kawasaki H, *et al.* Two distinct cDNAs for human IMP dehydrogenase. *J Biol Chem.* 1990; 265: 5292-5.

16. Carr SF, Papp E, Wu JC, Natsumeda Y. Characterization of human type-I and Type-II IMP dehydrogenases. *J Biol Chem.* 1993; 268: 27286-90.

17. Li H, Mager DE, Sandmaier BM, *et al.* Pharmacokinetic and pharmacodynamic analysis of inosine monophosphate dehydrogenase activity in hematopoietic cell transplantation recipients treated with mycophenolate mofetil. *Biol Blood Marrow Transplant.* 2014; 20: 1121-9.

18. Djebli N, Picard N, Rerolle JP, *et al.* Influence of the UGT2B7 promoter region and exon 2 polymorphisms and comedications on acyl-MPAG production in vitro and in adult renal transplant patients. *Pharmacogenet Genom.* 2007; 17: 321-30.

19. Naesens M, Kuypers DRJ, Verbeke K, *et al.* Multidrug resistance protein 2 genetic polymorphisms influence mycophenolic acid exposure in renal allograft recipients. *Transplantation.* 2006; 82: 1074-84.

20. Sombogaard F, van Schaik RHN, Mathot RA, *et al.* Interpatient variability in IMPDH activity in MMF-treated renal transplant patients is correlated with IMPDH type II 3757T > C polymorphism. *Pharmacogenet Genom.* 2009; 19: 626-34.

21. Wang J, Yang JW, Zeevi A, *et al.* IMPDH1 gene polymorphisms and association with acute rejection in renal transplant patients. *Clin Pharmacol Ther.* 2008; 83: 711-7.

22. Yoshimura K, Yano I, Yamamoto T, *et al*. Population pharmacokinetics and pharmacodynamics of mycophenolic acid using the prospective data in patients undergoing hematopoietic stem cell transplantation. *Bone Marrow Transplant.* 2017, *in press*. doi: 10.1038/bmt.2017.213.

23. Przepiorka D, Weisdorf D, Martin P, *et al.* 1994 consensus conference on acute GVHD grading. *Bone Marrow Transplant.* 1995; 15: 825-8.

24. Giralt S, Ballen K, Rizzo D, Bacigalupo A, *et al.* Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. *Biol Blood Marrow Transplant.* 2009; 15: 367-9.

25. Uchida N, Wake A, Nakano N, *et al.* Mycophenolate and tacrolimus for graft-versus-host disease prophylaxis for elderly after cord blood transplantation: a matched pair comparison with tacrolimus alone. *Transplantation.* 2011; 92: 366-71.

26. Kawanishi M, Yano I, Yoshimura K, *et al.* Sensitive and validated LC-MS/MS methods to evaluate mycophenolic acid pharmacokinetics and pharmacodynamics in hematopoietic stem cell transplant patients. *Biomed Chromatogr.* 2015; 29: 1309-16.

27. Johnston, L, Florek M, Armstrong R, *et al.* Sirolimus and mycophenolate mofetil as GVHD prophylaxis in myeloablative, matched-related donor hematopoietic cell transplantation. *Bone Marrow Transplant* 2012;47: 581-88.

28. Carnevale-Schianca F, Caravelli D, Gallo S, *et al.* Post-transplant cyclophosphamide and tacrolimus-mycophenolate mofetil combination prevents graft-versus-host disease in allogeneic peripheral blood hematopoietic cell transplantation from HLA-identical donors. *Biol Blood Marrow Transplant*. 2016; 23: 459-66.

29. Bejanyan N, Rogosheske J, DeFor T, *et al.* Higher dose of mycophenolate mofetil reduces acute graft-versus-host disease in reduced-intensity conditioning double umbilical cord blood transplantation. *Biol Blood Marrow Transplant.* 2015; 21: 926-33.

30. Ferrara JL, Cooke, KR, Teshima, T. The pathophysiology of acute graft-versus-host disease. *Int J Hematol.* 2003; 78: 181-7.

31. Ferrara JL, Levine JE, Reddy P, *et al*. Graft-versus-host disease. *Lancet*. 2009; 373: 1550-61.

32. Iwamura A, Nakajima M, Oda S, *et al.* Toxicological potential of acyl glucuronides and its assessment. *Drug Metab Pharmacokinet*. 2016; 32: 2-11.

33. Keefe DM, Schubert MM, Elting LS, *et al.* Updated clinical practice guidelines for the prevention and treatment of mucositis. *Cancer.* 2007; 109: 820-31.

34. Atcheson BA, Taylor PJ, Mudge DW, *et al.* Mycophenolic acid pharmacokinetics and related outcomes early after renal transplant. *Br J Clin Pharmacol.* 2005;59: 271-80.

35. Stracke S, Shipkova M, Mayer J, *et al.* Pharmacokinetics and pharmacodynamics of mycophenolate sodium (EC-MPS) co-administered with cyclosporine in the early-phase post-kidney transplantation. *Clin Transplant.* 2012; 26: 57-66.

36. Xia Z. Jun CY, Hao C, *et al.* The occurrence of diarrhea not related to the pharmacokinetics of MPA and its metabolites in liver transplant patients. *Eur J Clin Pharmacol.* 2010; 66: 671-79.

37. Barker JN, Byam C, Scaradavou A. How I treat: the selection and acquisition of unrelated cord blood grafts. *Blood*. 2011;117:2332-9

38. Uchida N, Wake A, Nakano N, *et al.* Mycophenolate and tacrolimus for graft-versus-host disease prophylaxis for elderly after cord blood transplantation: a matched pair

comparison with tacrolimus alone. *Transplantation.* 2011; 92: 366-71. Kishi Y, Kami M, Miyakoshi S, *et al.* Early immune reaction after reduced-intensity cord-blood transplantation for adult patients. *Transplantation.* 2005; 80: 34-40.

39. Nishiwaki S, Nakayama T, Murata M, *et al*. Dexamethasone palmitate successfully attenuates hemophagocytic syndrome after allogeneic stem cell transplantation: macrophage-targeted steroid therapy. *Int J Hematol.* 2012; 95: 428-433.
40. Takagi S, Masuoka K, Uchida N, *et al.* High incidence of haemophagocytic syndrome following umbilical cord blood transplantation for adults. *Br J Haematol.* 2009; 147, 543-53.
41. Tanaka H, Ohwada C, Sakaida E *et al.* Successful engraftment by second cord blood transplantation with reduced-intensity conditioning after graft rejection due to hemophagocytic syndrome following initial CBT. *Bone Marrow Transplant.* 2007; 40: 995-96.
42. Yoshimura K, Yano I, Kawanishi M, *et al*. Pharmacokinetics and pharmacodynamics of mycophenolic acid in Nagase analbuminemic rats: Evaluation of protein binding effects using the modeling and simulation approach. *Drug Metab Pharmacokinet.* 2015; 30: 441-8.

43. Jacobson P, Rogosheske J, Barker JN, *et al.* Relationship of mycophenolic acid exposure to clinical outcome after hematopoietic cell transplantation. *Clin Pharmacol Ther.* 2005; 78: 486-500.

44. Abdul Wahid SF, Ismail NA, Mohd-Idris MR, *et al.* Comparison of reduced-intensity and myeloablative conditioning regimens for allogeneic hematopoietic stem cell transplantation in patients with acute myeloid leukemia and acute lymphoblastic leukemia: a meta-analysis. *Stem Cells Dev*. 2014; 23: 2535-52.

45. Oran B, Wagner JE, DeFor TE, Weisdorf DJ, Brunstein CG. Effect of conditioning regimen intensity on acute myeloid leukemia outcomes after umbilical cord blood transplantation. *Biol Blood Marrow Transplant.* 2011; 17: 1327-34.

Table 1. Characteristics of cord blood transplant (CBT) patients (n = 35)

Parameter	Number or median (range)	
MAC/RIC	17 / 18	
Male/Female	22 / 13	
Age (years)	43 (21–66)	
Body weight (kg)	58.1 (33.5–76.3)	
Dose of MMF (mg/day)	1750 (1000–2500)	
Dose of MMF/body weight (mg/day/kg)	30.5 (22.1–57.7)	
ABO compatibility (Match/Major/Minor/Bidirectional)	16 / 7 / 8 / 4	
Total nucleated cell count per body ($x10^8$ cells)	15.0 (10.7–26.1)	
Total nucleated cell count per body weight ($x10^7$ cells/kg)	2.55 (1.80–5.14)	
CD34+ cell count ($x10^6$ cells)	3.21 (1.26–9.41)	
CD34+ cell count per body weight ($x10^5$ cells/kg)	0.57 (0.21–1.69)	
CMV relapse (Yes/No)	18/17	
HLA mismatch (0-2/3-8)	20/15	
Acute GVHD grade (0 / I / II / III / IV) [a]	9 / 14 / 8 / 2 / 0	
Skin G(+) / GI G(+) [a,b]	7 / 3	
Day of GVHD development (days) [a]	45 (23–67)	
Day of neutrophil engraftment (days)	25 (14–45)	
Engraftment failure	2	
Genetic polymorphism (wild / heterozygote / homozygote) [c]	Pre	Post
IMPDH1 -106G > A (rs2278294)	10 / 15 / 8	9 / 13 / 11
IMPDH1 125G > A (rs2278293)	15 / 12 / 6	12 / 14 / 7
IMPDH2 3757T > C (rs11706052)	32 / 1 / 0	25 / 8 / 0
MRP2 -24C > T (rs717620)	24 / 9 / 0	-
UGT2B7 −842G>A (rs7439366)	14 / 15 / 4	-

CMV, cytomegalovirus; GI, gastrointestinal; GVHD, graft-versus-host disease; IMPDH, inosine monophosphate dehydrogenase; MAC, myeloablative conditioning; MMF, myophenolate mofetil; MRP, multiple-drug resistance protein; RIC, reduced-intensity conditioning; UGT, UDP-glucuronosyltransferase. [a]Data from two patients were excluded because of engraftment failure. [b] Skin G(+) means stage ≥ 3 skin acute GVHD and GI G(+) means stage ≥ 1 gastrointestinal (GI) acute GVHD corresponding to acute GVHD grade II or greater. [c] Genotypes of two patients were unknown.

Table 2. Univariate Cox proportional hazards regression analyses of risk factors associated with skin or GI aGVHD in CBT patients-

	Skin		GI	
Risk factors	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
ABO compatibility				
Major	8.01 (1.77–36.2)	0.007	0.036 (N.E.)	0.602
Minor	0.645 (0.078–5.36)	0.685	2.30 (0.184–22.4)	0.563
Bidirectional	1.24 (0.149–10.3)	0.843	0.041 (N.E.)	0.674
Age ≥ 50	4.03 (0.779 –20.8)	0.096	3.34 (0.303–36.9)	0.325
Age ≥ 60	3.62 (0.807–16.2)	0.093	1.35 (0.122 –14.9)	0.808
Days of engraftment	0.994 (0.901–1.10)	0.910	1.00 (0.860–1.19)	0.985
CMV relapse	0.581 (0.130 –2.60)	0.478	1.62 (0.147–17.9)	0.693
Gender (donor and recipient combination)				
Female for donor and male for recipient	1.89 (0.365 –9.76)	0.449	0.036 (N.E.)	0.602
HLA mismatch ≥ 3	1.53 (0.340–6.82)	0.580	0.591 (0.054–6.52)	0.668
RIC	1.14 (0.256–5.12)	0.860	0.460 (N.E.)	0.526
Genetic polymorphism				
Pre				
IMPDH1 -106G > A	1.92 (0.224–16.4)	0.552	0.184 (0.017–2.03)	0.166
IMPDH1 125G > A	0.324 (0.059–1.77)	0.194	0.377 (0.034–4.16)	0.426
IMPDH2 3757T > C	0.047 (N.E.)	0.761	0.047 (N.E.)	0.834
MRP2 -24C > T	6.22 (1.13–34.3)	0.036	0.029 (N.E.)	0.509
UGT2B7 −842G>A	0.734 (0.148–3.64)	0.705	0.394 (0.036–4.35)	0.447
Post				
IMPDH1 -106G > A	35.6 (N.E.)	0.303	0.176 (0.016–1.94)	0.156
IMPDH1 125G > A	46.8 (N.E.)	0.228	0.274 (0.025–3.02)	0.291
IMPDH2 3757T > C	5.86 (1.3–26.4)	0.021	0.032 (N.E.)	0.543
PK/PD parameters				
1 week				
MPA AUC_{0-24}	1.04 (1.01–1.06)	0.006	1.02 (0.977–1.06)	0.432
Free MPA AUC_{0-24}	1.00 (0.999 –1.00)	0.271	1.00 (1.00–1.01)	0.021
MPAG AUC_{0-24}	0.999 (0.997–1.00)	0.385	1.00 (1.00–1.00)	0.051
AcMPAG AUC_{0-24}	0.966 (0.847–1.10)	0.612	1.28 (1.07–1.54)	0.008
IMPDH $AUEC_{0-8}$	1.00 (0.999–1.00)	0.761	N.E.	N.E.

3 weeks				
MPA AUC_{0-24}	1.00 (0.950–1.05)	0.986	1.10 (0.994–1.22)	0.065
Free MPA AUC_{0-24}	0.999 (0.996–1.00)	0.785	1.00 (0.998–1.01)	0.187
MPAG AUC_{0-24}	0.999 (0.997–1.00)	0.116	1.00 (0.999–1.00)	0.470
AcMPAG AUC_{0-24}	0.966 (0.843–1.11)	0.617	1.28 (1.02 –1.60)	0.031
IMPDH $AUEC_{0-8}$	1.00 (0.997–1.00)	0.809	0.997 (0.991–1.00)	0.333

CMV: cytomegalovirus; GI: gastro-intestine; IMPDH: inosine monophosphate dehydrogenase; HLA: human leukocyte antigen; HR: hazard ratio; MRP: multiple-drug resistance protein; N.E.: not estimated; PK/PD, pharmacokinetic/pharmacodynamic; UGT: UDP- glucuronosyltransferase.

Table 3. Multivariate Cox proportional hazards regression analyses of risk factors associated with skin aGVHD in CBT patients

Factors	Hazard ratio (95% CI)	*p*-value
ABO compatibility		
Major	4.91 (0.739–32.6)	0.100
Age		
≥60	1.82 (0.184–18.0)	0.610
Genetic polymorphism		
Pre		
MRP2 -24C > T	3.02 (0.353–25.9)	0.313
Post		
IMPDH2 3757T > C	4.05 (0.437–37.6)	0.218
MPA $AUC_{0\text{-}24}$ (1 week)	1.00 (0.960–1.05)	0.910

IMPDH: inosine monophosphate dehydrogenase; MRP: multiple-drug resistance protein.

Table 4. Univariate and multivariate Cox proportional hazards regression analyses of risk factors associated with days to neutrophil engraftment following CBT

	Univariate		Multivariate	
Factors	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
ABO compatibility				
Major	1.16 (0.472–2.86)	0.745		
Minor	0.790 (0.341–1.83)	0.582		
Bidirectional	0.992 (0.314–2.71)	0.883		
Age				
≥50	1.04 (0.517–2.10)	0.908		
≥60	1.09 (0.500–2.36)	0.834		
Gender (donor and recipient combination)				
Female for donor and male for recipient	0.947 (0.366–2.31)	0.948		
HLA mismatch ≥ 3	2.30 (1.11–4.79)	0.026	2.34 (1.11–4.93)	0.026
RIC	1.02 (0.515–2.03)	0.860		
Total nucleated cell count per body	1.07 (0.945–1.20)	0.294		
Total nucleated cell per body weight	1.35 (0.818–2.23)	0.438		
CD34+ cell count per body	1.33 (1.02–1.73)	0.091		
CD34+ cell per body weight	4.35 (1.10–17.2)	0.012	4.61 (1.14–18.7)	0.033
Genetic polymorphism				
Pre				
IMPDH1 -106G > A	1.82 (0.816–4.07)	0.143		
IMPDH1 125G > A	1.43 (0.693–2.95)	0.334		
IMPDH2 3757T > C	1.47 (0.194–11.1)	0.710		
MRP2 -24C > T	1.18 (0.529–2.61)	0.694		
UGT2B7 −842G>A	1.05 (0.510–2.14)	0.905		
Post				
IMPDH1 -106G > A	0.866 (0.397–1.89)	0.717		
IMPDH1 125G > A	1.26 (0.614–2.58)	0.531		
IMPDH2 3757T > C	0.722 (0.319–1.64)	0.435		
PK/PD parameters				

1wk				
MPA-AUC_{0-24}	1.02 (1.00–1.04)	0.042		
Free MPA-AUC_{0-24}	1.00 (1.00–1.003)	0.024	1.00 (1.00–1.00)	0.018
MPAG-AUC_{0-24}	1.00 (1.00–1.001)	0.386		
AcMPAG-AUC_{0-24}	1.03 (0.973–1.10)	0.302		
IMPDH-$AUEC_{0-8}$	0.999 (0.997–1.00)	0.160		

IMPDH, inosine monophosphate dehydrogenase; HLA, human leukocyte antigen; HR, hazard ratio; MRP, Multiple-drug resistance protein; PK/PD, pharmacokinetic/pharmacodynamic; RIC, reduced-intensity conditioning; UGT, UDP-glucuronosyltransferase.

Table 5. Receiver operating characteristic curve analyses of MPA pharmacokinetics at 1 week after the start of MMF treatments for aGVHD development for the gastrointestinal (GI) tract and neutrophil engraftment in CBT patients

	Cut-off value ($\mu g \cdot h \cdot mL^{-1}$)	AUC-ROC (95% CI)	*p*-value
aGVHD development for the GI tract			
Free MPA-AUC_{0-24}	0.689	0.967 (0.902–1.03)	0.009
AcMPAG-AUC_{0-24}	15.6	0.956 (0.880–1.03)	0.010
Neutrophil engraftment by day 25			
Free MPA-AUC_{0-24}	0.405	0.766 (0.606–0.927)	0.007
Total MPA-AUC_{0-24}	53.6	0.648 (0.461–0.835)	0.136

Figure legends

Figure 1. Relationship between site-specific acute graft-versus-host disease (aGVHD) and pharmacokinetic parameters of mycophenolic acid (MPA) and inosine-5'-monophosphate dehydrogenase (IMPDH) activity at one week after the start of mycophenolate mophetil (MMF) treatments; (A) 0–24 h area under the concentration–time curve (AUC_{0-24}) values for total MPA, (B) free MPA AUC_{0-24}, (C) MPAG AUC_{0-24}, (D) AcMPAG AUC_{0-24}, (E) 0–8 h area under the effect–time curve ($AUEC_{0-8}$) for IMPDH. G(−), aGVHD grades 0–I; skin G(+), stage ≥ 3 skin aGVHD, GI G(+), stage ≥ 1 gastrointestinal aGVHD; n.d., not detected. Bars show median values; *$p < 0.05$, Kruskall-Wallis test followed by Dunn's multiple comparison test.

Figure 2. Relationship between days to neutrophil engraftment and pharmacokinetic parameters of MPA and IMPDH activity at 1 week after the start of MMF treatments; (A) Total MPA AUC_{0-24}, (B) Free MPA AUC_{0-24}, (C) MPAG AUC_{0-24}, (D) AcMPAG AUC_{0-24}, (E) IMPDH $AUEC_{0-8}$; The median day of engraftment was 25 days after cord blood transplantation (CBT). Bars show median values; *$p < 0.05$, Student *t*-test.

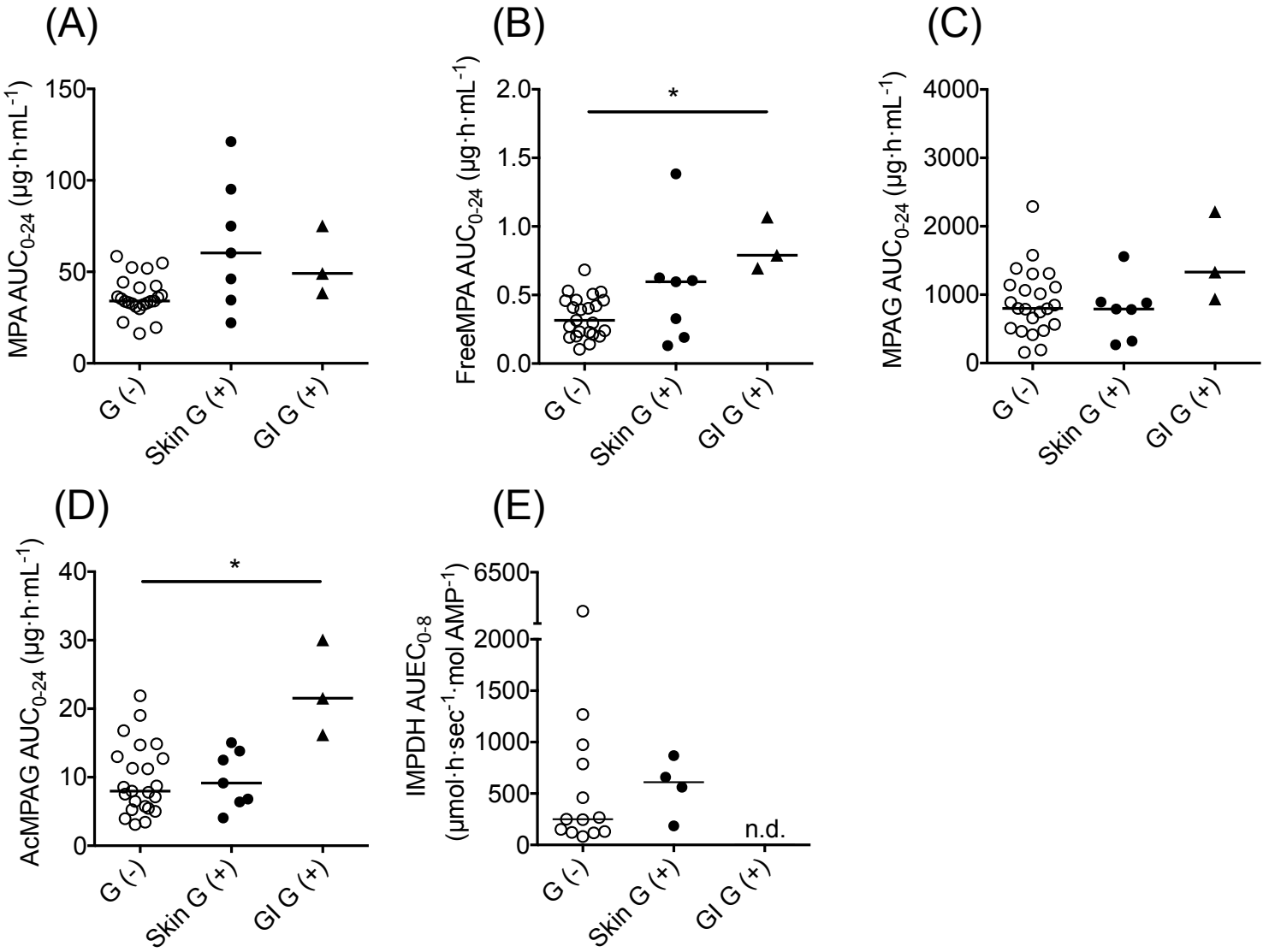

Figure 1 (2-column fitting image)

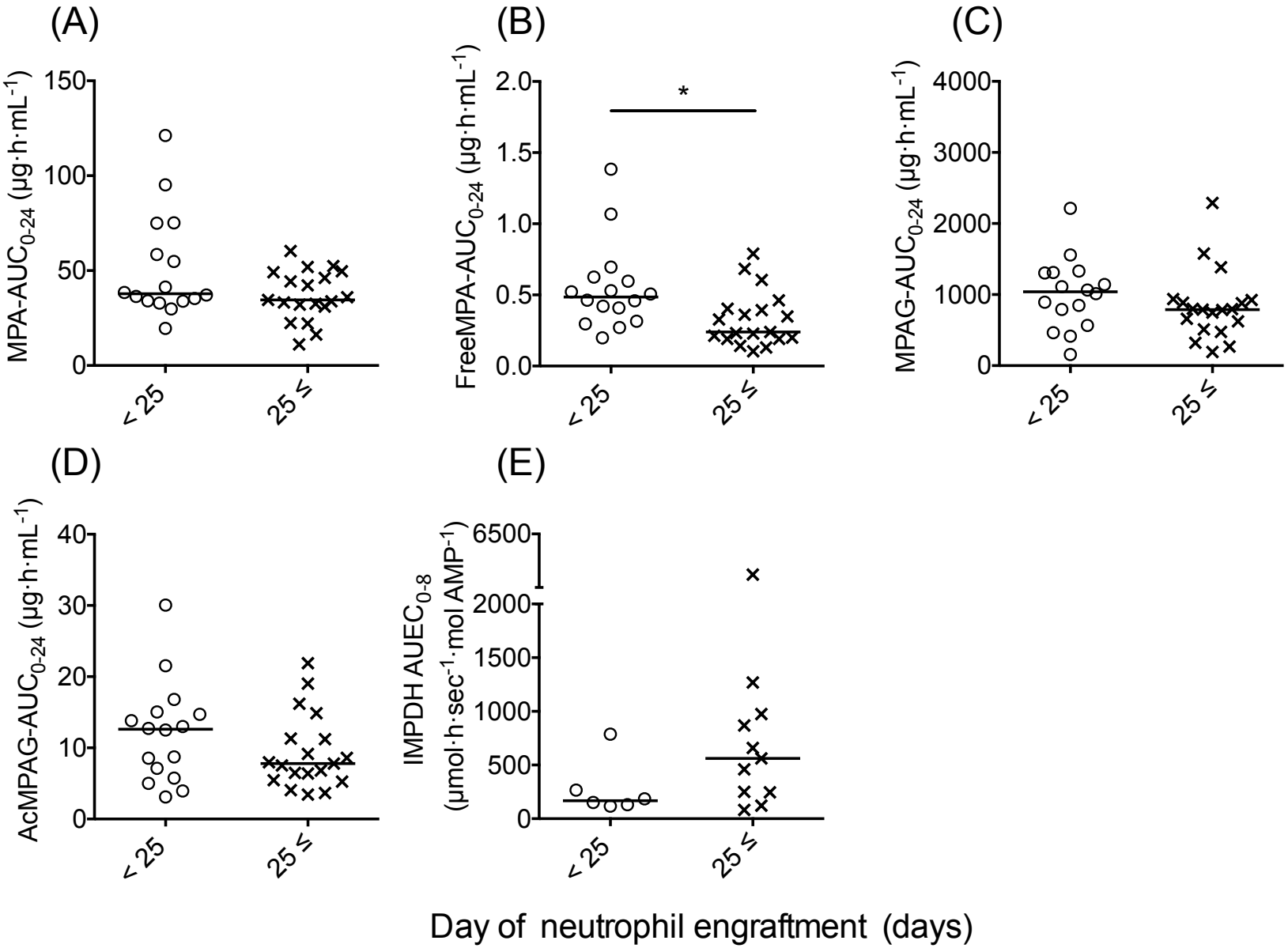

Figure 2 (2-column fitting image)