Mycophenolic Acid Exposure Optimization Based on Vitamin D Status in Children with Systemic Lupus Erythematosus: A Single-Center Retrospective Study

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ABSTRACT

Introduction: Systemic lupus erythematosus (SLE) can affect bone metabolism and homeostasis of serum electrolytes that are associated with abnormal levels of vitamin D. Mycophenolate mofetil (MMF) is a commonly used immunosuppressant with the active metabolite mycophenolic acid (MPA). The area under the plasma concentration–time curve (AUC) of MPA is often monitored during the treatment to assess the exposure levels. This study aims to explore the association between exposure levels of MPA and 25-hydroxyvitamin D [25(OH)D] levels in children with SLE.

Methods: Repeated measured data of children with SLE who were treated with MMF and under therapeutic drug monitoring (TDM) were retrospectively collected from the electronic medical records. MPA exposure levels were reflected by the area under the concentration–time curve over 24 h (AUC_{0-24h}). Univariate and multivariate linear regression models were employed to analyze factors associated with 25(OH)D levels. Hierarchical linear models were developed to analyze the intra- and inter-individual effects of AUC_{0-24h} on the variance of 25(OH)D levels.

Results: Data from 184 children with SLE (142 female and 42 male) with 518 follow-ups were collected. The median age was 14 years (range 3–18 years) at TDM. Children with normal 25(OH)D levels had significantly higher AUC_{0-24h} than children with low 25(OH)D levels (98.71 vs. 84.05 mg·h/L, $P = 0.004$). Intra- and inter-individual effects of AUC_{0-24h} on 25(OH)D levels were similar ($\gamma_{10} = 0.034$ vs. $\gamma_{01} = 0.037$) but only the intra-individual effect was significant ($P = 0.001$) in hierarchical models. Other associated factors include age, sex, season at measurement, glucocorticoid daily dose, and external vitamin D_3 supplements.

Conclusion: 25(OH)D levels are associated with MPA exposure levels, and may serve as a potential indicator to optimize the exposure level of MPA during treatment. AUC_{0-24h} of 98.71 mg·h/L or AUC_{0-12h} of 49.36 mg·h/L could be the targeted exposure level for children with SLE.

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Key Summary Points
Why carry out this study?
Systemic lupus erythematosus (SLE) can affect bone metabolism and homeostasis of serum electrolytes that are associated with abnormal levels of vitamin D.
Mycophenolate mofetil (MMF) is a commonly used immunosuppressant with the active metabolite mycophenolic acid (MPA). The optimal exposure level of MPA has not yet been settled.
This study aims to explore the association between the exposure levels of MPA, as indicated by the area under the plasma concentration–time curve (AUC), and the 25-hydroxyvitamin D [25(OH)D] levels in children with SLE, and to optimize MPA exposure levels.
What was learned from the study?
25(OH)D levels are associated with MPA exposure levels in children with SLE.
25(OH)D may serve as a potential indicator to optimize the exposure level of MPA during treatment.
AUC_{0-24h} of 98.71 mg·h/L could be the targeted exposure level for children with SLE.

DIGITAL FEATURES

This article is published with digital features, including a summary slide to facilitate understanding of the article. To view digital features for this article go to https://doi.org/10.6084/m9.figshare.14597163.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with systemic manifestations and multi-organ involvement, which has a more severe phenotype in children than in adults [1–3]. SLE can affect bone metabolism and serum electrolyte homeostasis through renal function impairment and endocrinological disorder [4–6]. Vitamin D is an important modulator of bone metabolism and calcium and phosphorus balance [5]. 25-Hydroxyvitamin D [25(OH)D] is recognized as the main indicator of vitamin D levels of the body [7, 8]. External vitamin D supplements (i.e., vitamin D_2 or vitamin D_3) or vitamin D receptor activators (VDRAs, i.e., calcitriol or alfacalcidol) are used for either prevention or treatment purpose in patients with SLE given the concerns for abnormal renal function and chronic administration of glucocorticoids [5, 9, 10]. Other bone turnover markers include β-Cross-Laps (β-CTx) (a marker of bone resorption) and osteocalcin (a predictor of osteoporosis), which are also closely monitored in patients at a risk of osteoporosis or decreased bone density [11–13].

Mycophenolate mofetil (MMF) is an immunosuppressant that is metabolized into the active form called mycophenolic acid (MPA) after oral administration [14]. The mechanism of immunosuppressive action of MPA is inhibiting inosine monophosphate dehydrogenase (IMPDH), which is the rate-limiting enzyme in the de novo synthesis pathway of guanine in T and B lymphocytes [14]. MMF is part of the treatment strategy of SLE in helping with disease control and glucocorticoid tapering [1, 15]. Exposure levels of MPA indicated by area under the concentration–time curve (AUC) were reported to be associated with SLE disease activity, where higher AUC is associated with lower SLE disease activity index (SLEDAI) score [16, 17]. Therapeutic drug monitoring (TDM) is often conducted to help achieve exposure target by measuring the plasma concentration of MPA and estimating the AUC with pharmacokinetic models [18]. There are still controversies about the optimal exposure level of MPA in the treatment of SLE. Some studies recommended

$AUC_{0-12h} > 30$ mg·h/L [19–21], while others reported $AUC_{0-12h} > 45$ mg·h/L was associated with better clinical outcome [22, 23]. Therefore, it is of clinical importance to find more evidence to help decide the treatment target.

Vitamin D status of the body could be a potential indicator to help guide the treatment with MMF, which is based on the rationale that bone metabolism is one of the main concerns during the treatment of SLE and the use of immunosuppressants should be appropriate to avoid the negative effect on homeostasis of other systems [24–27]. However, limited research has looked into the relationship between MMF treatment effect and vitamin D levels in patients with SLE. Therefore, this study is the first one that aims to explore the association between exposure levels of MPA and 25(OH)D levels in children with SLE and recommend the optimal exposure levels of MPA based on the normal range of serum 25(OH)D.

METHODS

Patients and Data Collection

From November 2015 to March 2021, data were retrospectively collected from pediatric patients who were diagnosed with SLE and treated with MMF (dispersible tablets, Saikeping, Huangzhou Zhongmei Huadong Pharmaceutical Co. Ltd.; OR capsules, Cellcept, Shanghai Roche Pharmaceuticals Co. Ltd.) in the Children's Hospital of Fudan University, National Children's Medical Center in Shanghai. This study only included patients with follow-up data when TDM of MPA was conducted. The daily dosing amount of methylprednisolone was converted to the equivalent amount of prednisolone by multiplying it by 1.25 [28].

This study was conducted according to the ethical guidelines of the Declaration of Helsinki, the protocol of which was approved by the Ethics Committee of the Children's Hospital of Fudan University [No. (2020) 490]. Written informed consent was waived because of the retrospective nature of this study.

Mycophenolic Acid Concentration Measurement and Area Under the Curve Calculation

The routine TDM protocol of MPA includes collecting 2 mL of blood samples at 30 min prior to the administration of MMF, and 20 min, 60 min, and 180 min post dose. Blood samples were centrifuged at $3000 \times g$ for 5–10 min and plasma was isolated for MPA concentration measurement. The enzyme-multiplied immunoassay technique (EMIT) was used to measure the MPA plasma concentration on the Viva System (Siemens Healthcare Diagnostics, Eschborn, Germany) at room temperature. The calibration range was 0.10–15.00 mg/L with the lower limit of quantification of 0.10 mg/L. The AUC over 24 h (AUC_{0-24h}) was estimated with the four-point MPA concentrations using Bayesian methods [18] in the MwPharm++ software (Version 1.6.1.128, Mediware, Prague, Czech Republic).

Statistical Analysis

The age at SLE diagnosis was obtained by calculating the time difference between the date of SLE diagnosis and the birth date of each patient. Likewise, the age at TDM was obtained by calculating the time difference between the date of TDM and the birth date. The SLE duration was from the time difference between the date of TDM and the date of SLE diagnosis. Values of both age and SLE duration were rounded to years. Descriptive analyses included summarizing the frequency of each category for nominal or ordinal variables, and the center and variability for variables measured on a ratio scale. β-CTx, osteocalcin, 25(OH)D, calcium, and phosphorus were treated both as continuous variables and categorical variables. These originally continuous variables were categorized into three levels according to the normal reference range on the biochemical report. The normal reference ranges for the biochemical indices are β-CTx 0.30–0.60 ng/mL, osteocalcin 24.00–70.00 ng/mL, 25(OH)D 15.00–35.00 ng/mL, calcium 2.20–2.65 mmol/L, phosphorus 1.29–2.26 mmol/L (age ≤ 14 years), 0.81–1.45

(age > 14 years). Bartlett test was used to test the hypothesis of homoscedasticity of AUC_{0-24h} across different levels of categorical variables. For variables that conformed to the assumption of homoscedasticity, analysis of variance (ANOVA) was conducted to test the statistical difference across different groups. Tukey's honestly significant difference (HSD) test was employed as post hoc statistical test for two of the three groups.

Linear regression models with 25(OH)D as the dependent variable were used to identify factors associated with the 25(OH)D level. Repeated measures hierarchical linear models (RM-HLM) were developed to analyze the intra- and inter-individual effect of AUC_{0-24h} on the variance of 25(OH)D levels. The AUC_{0-24h} was separated into two variables, i.e., subject-centered AUC_{0-24h} (AUC_SC) and subject mean of AUC_{0-24h} (AUC_SM) by the following equations:

$$AUC\_SM_j = \frac{\sum_{i=1}^{m_j} AUC_{ij}}{m_j}$$

$$AUC\_SC_{ij} = AUC_{ij} - AUC\_SM_j$$

In the above equations, AUC_SM_j stands for the subject mean of AUC_{0-24h} of the jth individual. m_j means the jth individual having data from m_j follow-ups. AUC_{ij} is the AUC_{0-24h} for the jth individual at the ith follow-up. AUC_SC_{ij} stands for the subject-centered AUC_{0-24h} for the jth individual at the ith follow-up.

The unadjusted RM-HLM was as follows:
Level-1 model:

$$25(OH)D_{ij} = \beta_{0j} + \beta_{1j}AUC\_SC_{ij} + \varepsilon_{ij}$$

$$E(\varepsilon_{ij}) = 0; var(\varepsilon_{ij}) = \sigma_\varepsilon^2$$

Level-2 model:

$$\beta_{0j} = \gamma_{00} + \gamma_{01}AUC\_SM_j + \upsilon_{0j}$$

$$\beta_{1j} = \gamma_{10}$$

$$E(\upsilon_{0j}) = 0; var(\upsilon_{0j}) = \sigma_{\upsilon0}^2$$

Overall model:

$$25(OH)D_{ij} = \gamma_{00} + \gamma_{10}AUC\_SC_{ij} + \gamma_{01}AUC\_SM_j + \upsilon_{0j} + \varepsilon_{ij}$$

where:

β_{0j} = intercept for the jth individual;

β_{1j} = regression coefficient associated with AUC_SC_{ij} for the jth individual;

ϵ_{ij} = random error for the level-1 model;

γ_{00} = overall mean intercept adjusted for AUC_SM_j for the level-1 slope;

γ_{10} = overall mean intercept for the level-1 slope;

γ_{01} = regression coefficient associated with AUC_SM_j relative to level-1 intercept;

υ_{0j} = random effects of the level-2 model on the intercept;

σ_ϵ^2 = variance of the random error for the level-1 model;

$\sigma_{\upsilon0}^2$ = variance of the random error for the level-2 model.

Further, the relation of AUC_{0-24h} and 25(OH)D with SLEDAI score was analyzed with a mediation model in subjects with complete observations of the three variables. SLEDAI score was tested as the mediator with 25(OH)D as the dependent variable.

All the statistical analyses were conducted in R (version 4.0.4, the R foundation for Statistical Computing). RM-HLM was developed with the "nlme" package. Mediation effect was analyzed with the "mediation" package. Two-sided $P < 0.05$ was considered as statistically significant.

RESULTS

Patients

Data were available from 184 pediatric patients (142 female, 42 male) with a total of 518 MPA TDM follow-ups. The median age at MPA TDM was 14 years with a range of 3–18 years. The median AUC_{0-24h} was 88.88 mg·h/L, ranging from 6.00 to 294.98 mg·h/L. The descriptive statistics of patients' demographic and

biochemical data are presented in Table 1. The use of medications of the patients is shown in Table 2. Most of the patients were using gluco-corticoids at follow-up (98.84%), with a median daily dose of 17.50 mg. Patients were also using oral vitamin D_3 supplements, VDRAs, and hydroxychloroquine (HCQ) as prescribed.

Comparisons Across Different Levels of Bone Metabolism Markers and Electrolytes

The comparisons of AUC_{0-24h} across different levels of β-CTx, osteocalcin, 25(OH)D, calcium, and phosphorus are shown in Figs. 1 and 2. There was no significant difference in AUC_{0-24h} between patients with normal and abnormal levels of β-CTx, osteocalcin, or serum phosphorus. However, patients with normal 25(OH)D levels had significantly higher AUC_{0-24h} than patients with low levels of 25(OH)D (mean AUC_{0-24h}, 98.71 vs. 84.05 mg·h/L, $P = 0.004$). There was also significant difference in AUC_{0-24h} across patients with different levels of serum calcium. Patients with normal levels of calcium had significantly higher AUC_{0-24h} than patients in the low-level group.

Linear Association Between Area Under the Curve and 25-Hydroxyvitamin D

The results of linear regression analyses are presented in Table 3. The concentration of 25(OH)D is significantly associated with AUC_{0-24h} in both univariate ($B = 0.040$, $P < 0.001$) and multivariate regression models ($B = 0.034$, $P < 0.001$). Other factors including age, sex, season, glucocorticoid daily dose, vitamin D_3 supplements, and VDRAs were also associated with 25(OH)D levels.

In RM-HLMs, intra- and inter-individual effects of AUC_{0-24h} were analyzed. The intra-individual effect of AUC_{0-24h} had statistical significance in both unadjusted models ($\gamma_{10} = 0.034$, $P = 0.001$) and adjusted models ($\gamma_{10} = 0.028$, $P < 0.001$), while the inter-individual effect was not significant. The coefficient

Table 1 Demographic and biochemical data of children with SLE

Variables	No. of observations	Values
No. of patients	–	184
Sex (F/M)	184	142/42
Age at SLE diagnosis (years)	184	10.82 (2.79), 11 [12–17]
MPA TDM times	518	2 [1–18]
SLE duration (years)	518	2.87 (2.64), 2 [0–12]
Age at MPA TDM (years)	518	13.45 (2.79), 14 [3–18]
Season at MPA TDM	518	
Spring (March to May)		110 (21.24%)
Summer (June to August)		175 (33.78%)
Autumn (September to November)		107 (20.66%)
Winter (December to February)		126 (24.32%)
MPA concentrations (mg/L)		
30 min pre-dose/ trough	509	2.31 (1.75), 1.94 [0.12–15.45]
20 min post-dose	515	8.43 (9.46), 4.91 [0.13–71.40]
60 min post-dose	514	10.65 (9.14), 8.39 [0.34–79.80]
180 min post-dose	514	4.48 (3.12), 3.66 [0.30–25.10]
MPA AUC over 24 h (mg·h/L)	515	95.05 (45.40), 88.88 [6.00–294.98]
SLEDAI	380	5.96 (6.73), 4 [0–35]

Table 1 continued

Variables	No. of observations	Values
β-CTx (ng/mL)	376	0.88 (0.82), 0.66 [0.03–5.85]
< 0.30 ng/mL (low)		61 (16.22%)
0.30–0.60 ng/mL (normal)		110 (29.26%)
> 0.60 ng/mL (high)		205 (54.52%)
Osteocalcin (ng/mL)	378	37.21 (42.55), 24.63 [0.50–256.00]
< 24.00 ng/mL (low)		184 (48.68%)
24.00–70.00 ng/mL (normal)		149 (39.42%)
> 70.00 ng/mL (high)		45 (11.90%)
25(OH) vitamin D (ng/mL)	378	19.81 (8.80), 18.43 [3.00–67.64]
< 15.00 ng/mL (low)		109 (28.84%)
15.00–35.00 ng/mL (normal)		248 (65.61%)
> 35.00 ng/mL (high)		21 (5.56%)
Serum calcium (mmol/L)	482	2.31 (0.17), 2.33 [1.68–2.89]
< 2.20 mmol/L (low)		96 (19.92%)
2.20–2.65 mmol/L (normal)		380 (78.84%)
> 2.65 mmol/L (high)		6 (1.24%)
Serum phosphorus (mmol/L)	480	1.33 (0.31), 1.33 [0.31–3.08]
Low		113 (23.54%)

Table 1 continued

Variables	No. of observations	Values
Normal*		321 (66.88%)
High		46 (9.58%)

Data are presented as mean (standard deviation), median [range], or frequency (percentage)

SLE systemic lupus erythematosus, *MPA* mycophenolic acid, *TDM* therapeutic drug monitoring, *AUC* area under the MPA concentration–time curve, *SLEDAI* SLE disease activity index, *β-CTx* beta-CrossLaps, *No.* Number

*Normal serum phosphorus range 1.29–2.26 mmol/L for children ≤ 14 years old; 0.81–1.45 for children > 14 years old

Table 2 Use of medications in children with SLE

Medication	Frequency	Daily dosing amount (mg)
MMF	518 (100%)	958 (338), 1000 [125–2000]
Glucocorticoids	512 (98.84%)	22.19 (26.04), 17.50 [0–468.75]
Oral vitamin D$_3$ supplements	457 (88.22%)	–
Vitamin D receptor activators (calcitriol or alfacalcidol)	296 (57.14%)	–
HCQ	428 (82.63%)	–

MMF mycophenolate mofetil, *HCQ* hydroxychloroquine
Data are presented as mean (standard deviation), median [range], or frequency (percentage)

estimates and *P* values of the RM-HLMs are displayed in Table 4.
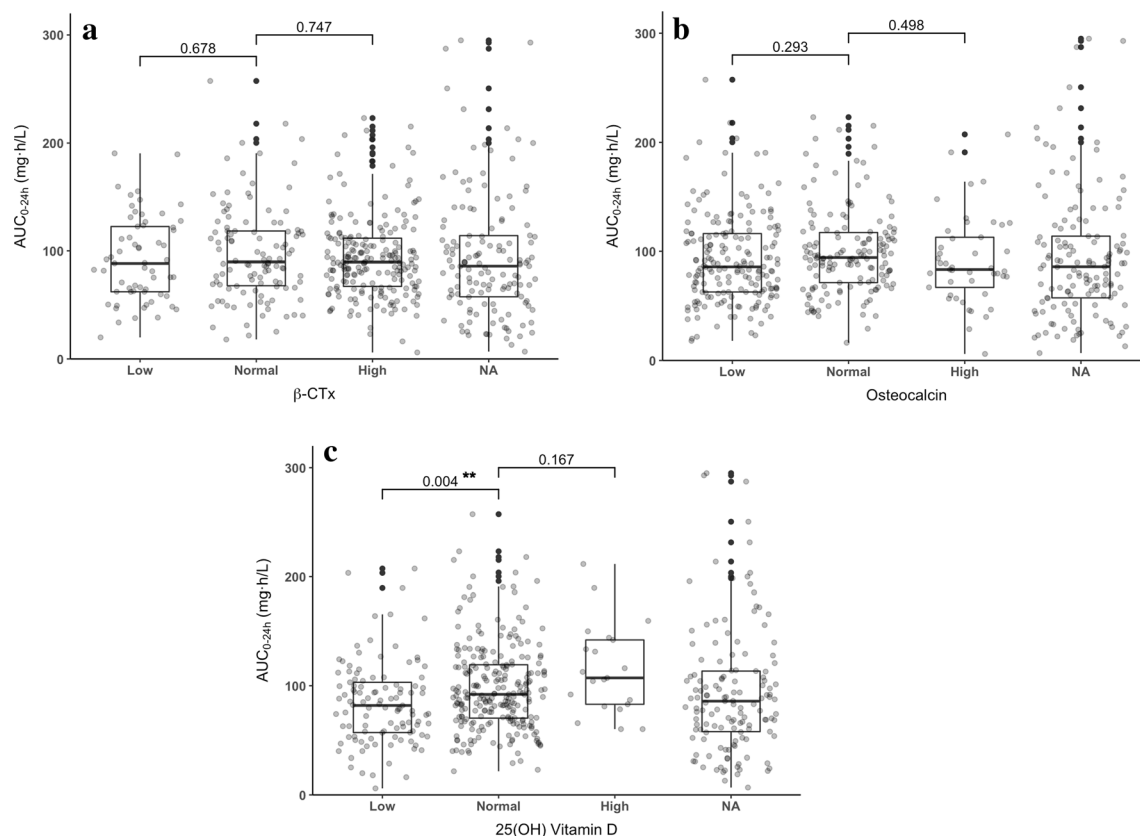
Fig. 1 AUC of MPA by levels of bone turnover markers. **a** β-CTx. **b** Osteocalcin. **c** 25(OH) vitamin D. MPA mycophenolic acid, AUC_{0-24h} area under the MPA concentration–time curve over 24 h, β-CTx beta-Cross-Laps, NA missing data. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$

25-Hydroxyvitamin D Levels Were Associated Calcium and Phosphorus Homeostasis

As is shown in Fig. 3, serum calcium concentrations were positively correlated with 25(OH)D levels ($y = 0.006x + 2.197$, $P < 0.001$). For children over 14 years old, serum phosphorus concentrations were negatively correlated with 25(OH)D levels ($y = -0.012x + 1.517$, $P < 0.001$), while for children 14 years old or younger, the correlation was not significant ($P = 0.912$).

Mediation Effect of Disease Activity Score in Relationship Between 25-Hydroxyvitamin D Levels and Area Under the Curve

In the mediation model, data from 303 follow-ups with complete observations of the three variables were included for the analysis. The mediation relation of SLEDAI with AUC_{0-24h} and 25(OH)D is illustrated in Fig. 4. Both the direct ($B = 0.040$, $P = 0.004$) and indirect effects ($B = 0.014$, $P < 0.001$) of AUC_{0-24h} were significant. Of the total effect, 25.4% could be explained by mediation effect of SLEDAI.
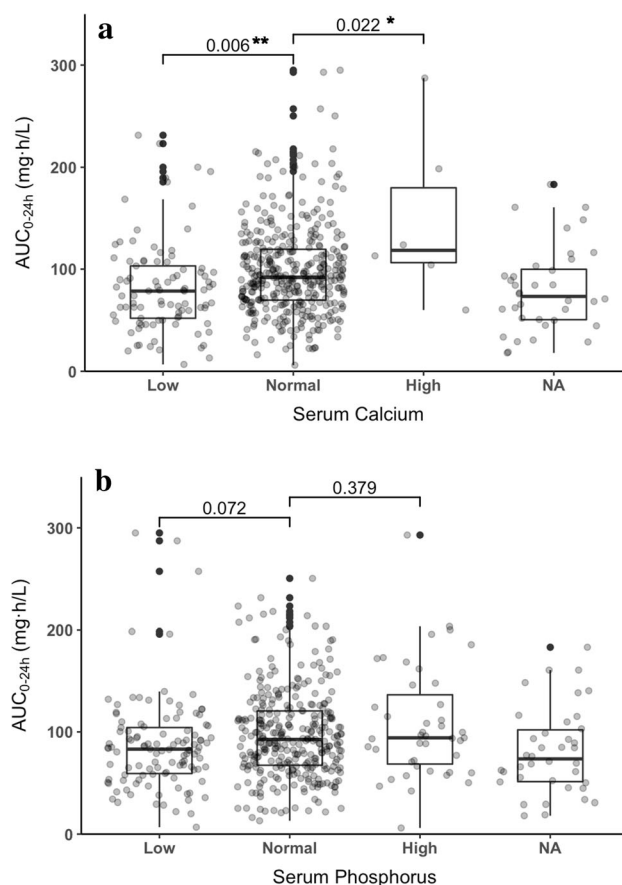
Fig. 2 AUC of MPA by levels of serum calcium (**a**) and phosphorus (**b**). MPA mycophenolic acid, AUC_{0-24h} area under the MPA concentration–time curve over 24 h, NA missing data. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$

DISCUSSION

This is the first study that has explored the association between vitamin D levels and MPA exposure levels in children with SLE. A significantly positive association was found between the two variables. As the main indicators of vitamin D status in the human body, 25(OH)D may also serve as a potential indicator to optimize the exposure level of MPA during treatment. The optimal exposure level of MPA in children with SLE could be 98.71 mg·h/L in AUC_{0-24h} or 49.36 mg·h/L in AUC_{0-12h}, which is consistent with a previous study using SLEDAI score to evaluate the ideal exposure levels of MPA, with AUC_{0-12h} over 50 mg·h/L being recommended as the treatment target [16].

Vitamin D status is important for monitoring patients with SLE and it is closely related to the

bone metabolism [4, 29]. Chronic glucocorticoid use in children with SLE could lead to abnormal bone metabolism and growth [6, 9, 30]. Studies have shown that serum 25(OH)D levels of 15 ng/mL or less are independently associated with a lower bone formation rate and an increased fracture risk [31] in adults as well as in children [32]. In our study, higher 25(OH)D levels were found to be positively associated with higher levels of serum calcium and lower levels of phosphorus, which is related to the role of parathyroid hormone in regulating the levels of calcium under the circumstances of vitamin D deficiency and hypocalcemia [5].

The relationship between blood drug concentrations and vitamin D has also been investigated by other researchers. Lindh et al. [33] observed seasonal variation in plasma

Table 3 Univariate and multivariate linear regression models with 25(OH) vitamin D as the dependent variable

Predictors	Univariate analysis		Multivariate analysis	
	Coefficient	P value	Coefficient	P value
Age at TDM	− 0.959	< 0.001***	− 0.934	< 0.001***
Sex				
Female	Reference		Reference	
Male	5.958	< 0.001***	4.370	< 0.001***
Season				
Spring	0.379	0.785	− 0.219	0.859
Summer	4.213	< 0.001***	3.162	0.002**
Autumn	3.497	0.010*	2.632	0.030*
Winter	Reference		Reference	
MPA $AUC_{0–24h}$	0.040	< 0.001***	0.034	< 0.001***
Glucocorticoid daily dose	− 0.094	< 0.001***	− 0.101	< 0.001***
Vitamin D_3 supplements	4.834	0.002**	4.946	< 0.001***
Vitamin D receptor activators	− 2.768	0.003**	− 1.089	0.206

TDM therapeutic drug monitoring, *MPA* mycophenolic acid, $AUC_{0–24h}$ area under the MPA concentration–time curve over 24 h

***P < 0.001, **P < 0.01, *P < 0.05

concentrations of cytochrome P450 3A4 (CYP3A4) like tacrolimus and sirolimus, which was highly consistent with seasonal changes in vitamin D. A possible explanation could be the stimulatory effect of vitamin D on drug metabolism by inducing the expression of CYP3A4. In their study, MPA was included as a control drug that is independent of CYP3A4 metabolism. Another study by Wang et al. [34] found that the co-administration of 1-alpha,25-dihydroxyvitamin D_3 [$1\alpha,25(OH)_2D_3$] could alter the pharmacokinetics of MPA by regulating the extrahepatic enzymes UGT1A8 and UGT1A10 in renal transplant recipients. However, Wang's study used within-individual repeated-measure comparison to demonstrate the significant change in $AUC_{0–12h}$ of MPA from the preadministration of $1\alpha,25(OH)_2D_3$ (on day 8) to the post-administration (on day 16). One potential problem they might not have considered was that the post-transplantation days

could affect the pharmacokinetics of MPA, thus confounding the effect of $1\alpha,25(OH)_2D_3$ [35].

Although we cannot completely rule out the pharmacokinetic relationship between MPA exposure and vitamin D levels, the significant association found in our study was likely due to the indirect pharmacodynamic effect. A possible explanation for the observed association could be that full exposure to MPA is associated with disease control and reduced dose of prescribed glucocorticoids, thus leading to improved profiles of bone turnover markers like 25(OH)D. In our study, the $AUC_{0–24h}$ of MPA was found to have significantly negative association with SLEDAI score, with higher $AUC_{0–24h}$ predicting lower SLEDAI score, thus better disease control (B = − 0.030, P = 0.002). Meanwhile, higher SLEDAI score was significantly associated with lower 25(OH)D levels when $AUC_{0–24h}$ was controlled (B = − 0.456, P < 0.001), indicating the possible mediation

Table 4 Repeated measures hierarchical linear models with 25(OH) vitamin D as the dependent variable

Fixed effects	Unadjusted models		Adjusted models	
	Coefficient	P value	Coefficient	P value
MPA AUC$_{0-24h}$ (subject-centered)	0.034	0.001**	0.028	< 0.001***
MPA AUC$_{0-24h}$ (subject mean)	0.037	0.074	0.028	0.139
Age at TDM	–	–	− 0.793	< 0.001***
Sex				
Female	–	–	Reference	
Male	–	–	3.199	0.018*
Season				
Spring	–	–	0.546	0.560
Summer	–	–	2.970	< 0.001***
Autumn	–	–	1.818	0.054
Winter	–	–	Reference	
Glucocorticoid daily dose	–	–	− 0.108	< 0.001***
Vitamin D$_3$ supplements	–	–	2.190	0.051
Vitamin D receptor activators	–	–	− 0.171	0.822
Random effects				
$\sigma_{\upsilon0}^2$	46.559		35.203	
σ_{ε}^2	25.150		23.062	

MPA mycophenolic acid, *AUC$_{0-24h}$* area under the MPA concentration–time curve over 24 h, *TDM* therapeutic drug monitoring

$\sigma_{\upsilon0}^2$ variance of the random intercept, σ_{ε}^2 variance of the residual

***$P < 0.001$, **$P < 0.01$, *$P < 0.05$

effect by SLEDAI in the relationship between AUC$_{0-24h}$ and 25(OH)D. However, the effect of AUC$_{0-24h}$ on 25(OH)D levels remained significant after controlling for SLEDAI scores in the model ($B = 0.040$, $P = 0.002$). In the mediation analysis, both the direct and indirect effects of AUC$_{0-24h}$ were significant, with 25.4% of the total effect explained by the mediation effect of SLEDAI score.

In addition, the intra- and inter-individual effects of AUC$_{0-24h}$ were analyzed in hierarchical linear models. The effect sizes were similar while only the intra-individual effect was statistically significant, suggesting the importance of maintaining stable exposure levels during treatment.

However, we did not find significant differences in AUC$_{0-24h}$ across patient groups with different levels of β-CTx and osteocalcin. A study in female patients with SLE without the influence of glucocorticoids found lower osteocalcin and 25(OH)D levels and higher β-CTx levels compared to healthy controls, indicating that SLE itself is associated with changed bone
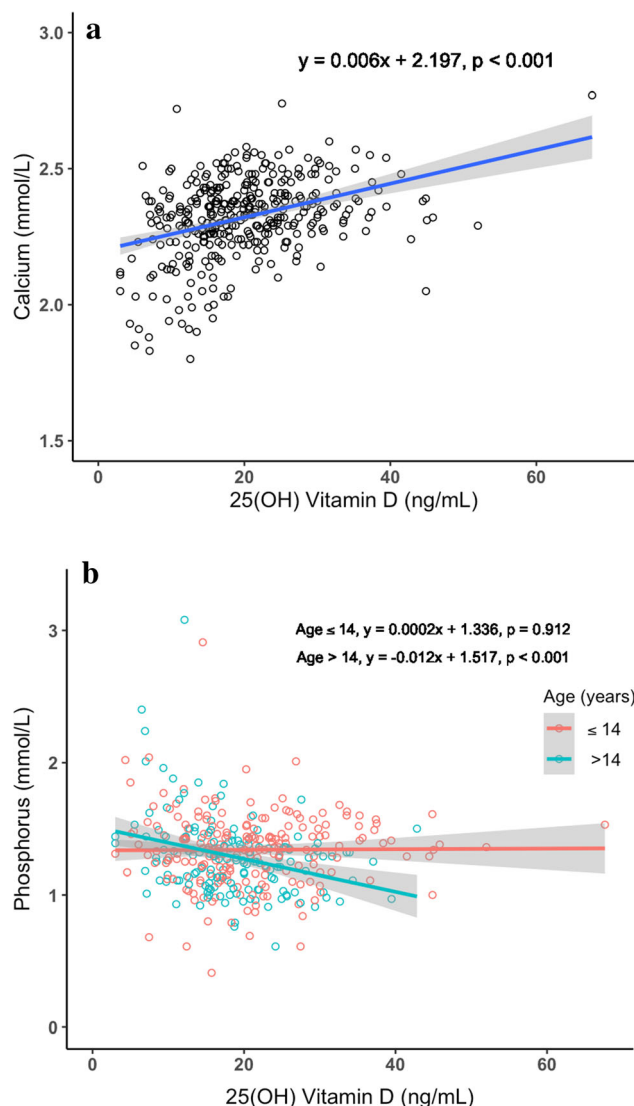
Fig. 3 Relationship between serum electrolyte levels and 25(OH) vitamin D. **a** Calcium. **b** Phosphorus
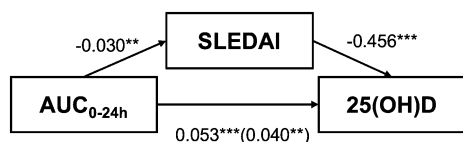
Fig. 4 Mediation effect of SLEDAI score in the relationship between AUC and 25(OH)D. AUC_{0-24h} area under the MPA concentration–time curve over 24 h, *SLEDAI* systemic lupus erythematosus disease activity index, *25(OH)D* 25-hydroxyvitamin D. The number outside the parentheses is the total effect. The number inside the parentheses is the direct effect. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$

metabolism [11]. Our study included both male and female patients, and most patients were put on glucocorticoids at TDM follow-up. Yet, the abnormal levels of β-CTx and osteocalcin were not associated with significant changes in AUC_{0-24h}.

Besides the exposure levels of MPA, other factors were also associated with the variance of 25(OH)D, including age, sex, season at measurement, glucocorticoid daily dose, and use of vitamin D_3 supplements and VDRAs. Age and glucocorticoid daily dose were negatively associated with 25(OH)D, while male sex, summer

season, and external vitamin D_3 supplements were positively associated with 25(OH)D, which are consistent with previous findings from other studies [9, 36–38]. One large population-based multicenter study in Jiangsu Province of China investigated 5289 children and revealed that children of older age and being a girl were at a higher risk of vitamin D deficiency [36]. In our study, boys with SLE had about fivefold higher 25(OH)D levels than girls with SLE ($B = 5.958$, $P < 0.001$) and increased age was associated with decreased 25(OH)D concentrations ($B = -0.959$, $P < 0.001$).

Vitamin D_3 supplements were significantly associated with increased levels of 25(OH)D in our study. However, the use of VDRAs (i.e., calcitriol or alfacalcidol) had a negative effect on 25(OH)D levels ($B = -2.768$, $P = 0.003$), and this negative effect became nonsignificant after adjusting for other variables ($B = -1.089$, $P = 0.206$). This is probably because VDRAs were used when vitamin D was insufficient and their effect on hyperparathyroidism bypassed 25(OH)D, thus their application would not increase the level of 25(OH)D.

There are some limitations in this study. First, this is a retrospective single-centered study. The data collection purely relied on the electronic medical records of the patients. This may lead to inaccuracy in recorded medications. Second, 25(OH)D levels of the patients had large variances, and factors considered in our study only explain a small portion of them. Third, as a result of the cross-sectional nature of this study, the causal relationship between exposure levels of MPA and 25(OH)D cannot be concluded. Therefore, further prospective and multicentered studies are needed to confirm these initial findings.

CONCLUSIONS

MPA exposure levels are positively associated with 25(OH)D independent of age, sex, season, glucocorticoids, and vitamin D_3 supplements, but not significantly associated with β-CTx and osteocalcin levels. The intra-individual effect of MPA AUC_{0-24h} is more significant than the inter-individual effect on 25(OH)D levels. On

the basis of the normal reference range of 25(OH)D at 15.00–35.00 ng/mL, MPA AUC_{0-24h} of 98.71 mg·h/L or AUC_{0-12h} of 49.36 mg·h/L might be the targeted exposure levels for SLE treatment in children.

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