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# Inflammation Decreases Ciclosporin Metabolism in Allogeneic Hematopoietic Stem Cell Transplantation Recipients

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## Abstract

Graft-versus-host disease (GVHD) remains a significant challenge following allogeneic hematopoietic stem cell transplantation (HSCT). Prevention of GVHD relies mainly on the use of calcineurin inhibitors, notably ciclosporin that exhibits complex pharmacokinetics influenced by many factors including drug-drug interactions (DDIs). Due to the downregulation of drug metabolizing enzymes and transporters, it has been postulated that inflammation may be a contributing factor to the variability observed in ciclosporin pharmacokinetics.

This study aimed to assess the impact of inflammation, as indicated by C-reactive protein (CRP) levels, on the metabolism of ciclosporin in adult allogeneic HSCT recipients. A retrospective observational study was conducted at Rennes University Hospital involving 71 adult HSCT patients. The relationship between the intensity of inflammation (no-to-mild, moderate, and severe), and the metabolism of ciclosporin (estimated by the concentration/dose ratio) was assessed. Severe inflammation significantly decreased the metabolism of ciclosporin, as evidenced by higher concentration/dose ratios. Thanks to the daily dose adjustment, inflammation did not influence the blood levels of ciclosporin. Interestingly, DDIs did not emerge as a significant covariate in influencing ciclosporin metabolism. This is likely because the CYP3A4 inhibitory potential of interacting drugs may be masked in HSCT patients where metabolism is already upstream downregulated by inflammation.

The study highlights the intricate relationship between inflammation and ciclosporin pharmacokinetics in HSCT patients. This underscores the necessity for therapeutic monitoring and the potential adjustment of dosage strategies based on the inflammatory status. These insights could contribute to the development of more personalized, optimized, and effective management strategies for HSCT recipients.

## Keywords

allogeneic hematopoietic stem cell transplantation, ciclosporin, C-reactive protein, drug–drug interactions, inflammation, metabolism

## Introduction

Graft-versus-host disease (GVHD) is a frequent immunologic complication after allogeneic hematopoietic stem cell transplantation (HSCT) and a major cause of morbimortality. Calcineurin inhibitors, ciclosporin or tacrolimus, in combination with other agents represent widely accepted standards of care as immunosuppressive regimens after HSCT. According to consensus recommendations, GVHD prophylaxis with ciclosporin or tacrolimus is roughly equivalent,<sup>1</sup> with the former being more commonly used in Europe. Ciclosporin is administered twice daily with a target level of 200–300 µg/L within the first 4 weeks after transplantation. Careful therapeutic drug monitoring is needed to maintain the ciclosporin concentration within the target range with most patients requiring multiple dose adjustments in the early post-transplant period.

In addition to its narrow therapeutic window, ciclosporin shows poor water solubility with a high intestinal permeability (biopharmaceutical drug disposition and classification system (BDDCS) class 2 drug<sup>2</sup>). Hence, ciclosporin pharmacokinetics displays signif-

icant inter-individual and intra-individual variability. This results from a highly variable absorption and extensive intestinal and liver metabolism by CYP3A4, and to a lesser extent to CYP3A5.<sup>3</sup> Less than 1% of the

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parent drug excreted unchanged in either the urine or feces after either intravenous or oral administration with the vast majority of the metabolites (>95%) appearing in the feces.<sup>4</sup> The oral bioavailability and the systemic clearance of ciclosporin is influenced by CYP3A4/5 and by the efflux pump P-glycoprotein (P-gp) that are both expressed in the gastrointestinal tract (affecting oral bioavailability) and in the liver (affecting both oral bioavailability and systemic clearance, CL).<sup>4</sup> Intestinal metabolism appears to account for as much as 50% of oral ciclosporin metabolism in humans which may be in part explained by the contribution of P-gp that transports ciclosporin back into the intestinal lumen, increasing ciclosporin exposure to intestinal CYP3A4.<sup>3</sup>

Although extensively metabolized, ciclosporin is a low-extraction ratio drug with an estimated extraction ratio  $\leq 0.30$  based on clearance measurements after IV administration in solid organ transplantation<sup>3,5,6</sup> as well as in HSCT patients.<sup>7-9</sup>

As a low-extraction ratio drug, ciclosporin CL is highly dependent on intrinsic clearance ( $CL_{int}$ ) and fraction unbound ( $f_u$ ). In patients undergoing HSCT, a decrease in  $CL_{int}$  should be considered, as inflammation, which is common in these patients, has the potential to downregulate CYP450 metabolism.<sup>10</sup> Indeed, CYP3A4 is strongly downregulated by inflammatory cytokines IL-6 and IL-1 $\beta$ . Downregulation of transporters has also been described, and in vitro studies have shown that cytokines (IL-6, IL-1 $\beta$ , TNF- $\alpha$  and INF- $\gamma$ ) downregulate hepatic influx transporters (organic anion transporting polypeptide (OATP-1B1) and OATP-1B3, organic cation transporter (OCT1), and OAT2) and hepatic efflux transporters (P-gp, breast cancer resistance protein (BCRP), and multi-drug resistance protein (MRP2)).<sup>10</sup> In an early study conducted on six patients undergoing HSCT and receiving IV ciclosporin, IL-6 peak level was associated to a 3.6-fold increase in ciclosporin levels 4.8 days later<sup>11</sup> suggesting a potential decrease in  $CL_{int}$ .

The aim of the present study was to assess the impact of the severity of the inflammatory state, as indicated by C-reactive protein (CRP) levels, on the metabolism of ciclosporin in adult allogeneic HSCT inpatients following IV and oral administration.

## Methods

### Human Subject Protection

The study received approval from the Institutional Research & Ethics Committee of Rennes University Hospital (agreement n° 24.12). All aspects of the study were conducted in accordance with the ethical standards set forth in the 1964 Declaration of Helsinki and its subsequent amendments, or comparable

ethical standards. Due to the retrospective and non-interventional nature of the study, utilizing data from a database, a consent waiver was granted. The principles of ethical research, such as confidentiality and anonymity, were strictly followed.

### Study Setting

The study was performed as a retrospective (24 months from November 2020 to November 2022), observational, single-center study on 77 adult patients hospitalized for allogeneic transplant in the Department of Clinical Hematology of the Rennes University Hospital.

Data were retrieved from a hospital clinical data warehouse (CDW) developed at the Rennes University Hospital (eHOP). The extracted data did not contain any nominative data, and each data sample was de-identified to ensure confidentiality.

Drug information retrieved were daily dosing of ciclosporin and of drugs potentially leading to severe pharmacokinetic-based DDI with ciclosporin throughout the hospital stay (either by induction and inhibition mechanism). Only drugs leading to potential DDI of highest severity (contra-indication and major severity) according to Micromedex with excellent or good documentation were collected. Regarding dosing regimen, the information retrieved was the dose actually administered.

Laboratory data retrieved were CRP levels as well as the glomerular filtration rate (GFR) calculated according to chronic kidney disease - epidemiology collaboration (CKD-EPI) formula. The inflammatory state of patients was categorized into three classes according to the following ranges<sup>12</sup>: no-to-mild (CRP <40 mg /L), moderate (CRP 41 to 200 mg/L) and severe (CRP >200 mg/L).

Drug administrations (date of administration and ATC classification), laboratory data and information on patient stay (date of admission, date of discharge, length of stay, and ICD-10 diagnosis codes) were collected for each patient.

Trough blood ciclosporin levels at steady state were retrieved throughout the hospitalization. Since clinicians actively tune ciclosporin doses according to trough blood concentrations in order to keep them within the therapeutic window, blood ciclosporin level is not a relevant indicator of metabolism. Indeed, in terms of pharmacokinetics, it reflects both the input function (related to dose) and the output function (related to metabolic elimination). A better parameter is the concentration/dose ratio, which is directly proportional to elimination clearance at steady state. Concentration/dose ratio (C/D ratio) of ciclosporin were calculated from trough blood ciclosporin levels at steady state divided by the daily dose. We only used

ciclosporin levels associated with CRP measured on the same day. Each Cp-CRP or C/D ratio-CRP pair was measured at several distinct time points during the hospital stay for each patient. Due to variable length of hospital stay and modification in ciclosporin dosing regimen, the number of data differ among patients.

The authorization to conduct research from the Clinical Data Warehouse of Hospital of Rennes was given by Commission Nationale Informatique et Liberté (CNIL; Authorization number 2020–028 issued on February 27, 2020).

### Statistics

The mean and standard deviation (SD) are used to represent continuous data that were regularly distributed, and the median (M) and interquartile range (IQR) are used to express continuous data that were not normally distributed. Categorical variables are reported as frequencies or percentages.

The Kolmogorov–Smirnov test was used to assess the normality of the distribution of the continuous variables. If the assumption of normality was violated, non-parametric statistics were used. To avoid imbalance in the statistical analysis due to the difference in the number of Cp-CRP or of C/D ratio-CRP pairs between patients, individual data were summarized as median by CRP class or by week except in the mixed effect linear regression.

To study the comparison between week one to four on laboratory parameters and the potential association of inflammation intensity as a categorical variable (no-to-mild, moderate, and severe inflammation groups) with either the C/D ratio of ciclosporin or the ciclosporin blood levels, groups were compared with a Kruskal and Wallis test with a Dunn's multiple-comparison test for the comparison among the groups.

A retrospective longitudinal mixed effect linear regression analysis was carried out to evaluate the contribution of inflammation to the variability of ciclosporin concentrations and ciclosporin C/D ratio, accounting for patient variability by adding a random effect term. Univariate mixed effect linear regression was performed with post-transplant delay, CRP (as a categorical and continuous variable), DDI, gender, BMI, height, weight, route of administration, albumin, hematocrit, and Aspartate Transaminase (AST)–, respectively, as covariates. Multivariate mixed effect linear regression was performed, and covariate were selected if  $P < .2$  and could be added with higher  $P$ -value if the literature suggested an implication. Ultimately, CRP as categorical variable, DDI, albumin, hematocrit, AST, and post-transplant delay as the continuous time variable were chosen as covariates.

Statistical analysis was performed using GraphPad Prism version 10.2.1 for Mac OS (GraphPad Software,

Boston, MA), and using R software (version 3.6.3) for the mixed linear regression analysis. A  $P$ -value  $< .05$  was considered significant.

## Results

### Demographics

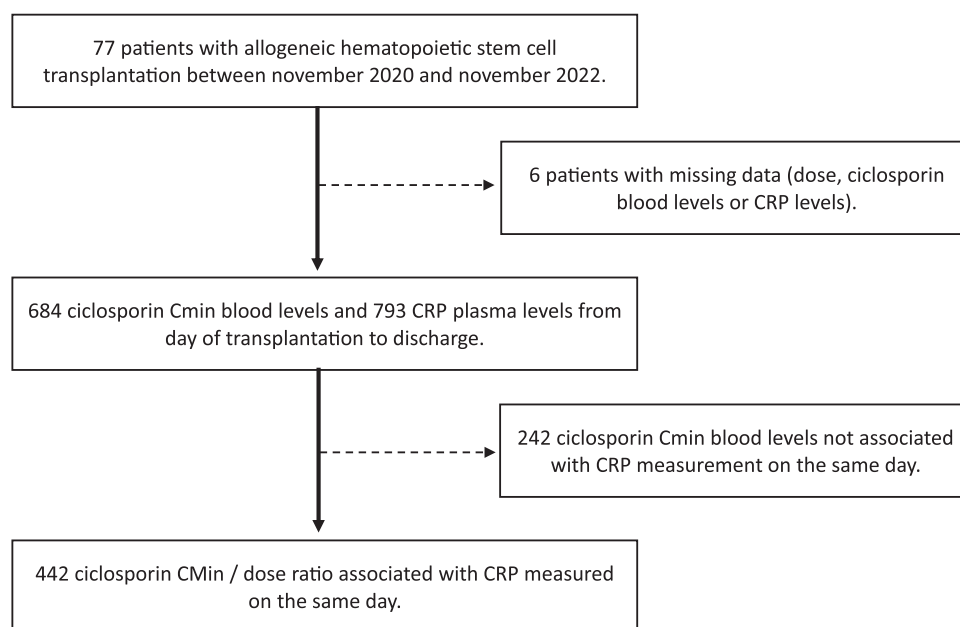
A total of 77 patients were included in the study, but 6 were excluded due to a lack of data on the ciclosporin dosage, ciclosporin blood levels, and plasma CRP levels, which prevented the calculation of the C/D ratio and CRP on the same day (see flow chart of the study population in Figure 1). The median age of the patients (37% female) was 55.5 years old with a majority (45%) being treated for acute myelogenous leukemia. The median duration of hospital stay from the day of transplantation to discharge was 25 days (min–max: 9 to 100 days, mean: 28.6 days). The characteristics of the population are shown in Table 1.

### Laboratory Parameters

The median number of Cp and CRP measurements per patient and per stay was 8 and 9, respectively. However, Cp and CRP were not systematically measured on the same day in all patients throughout their stay. In the study, we only used in each patient the Cp (or C/D ratio) and CRP measured on a same day but at different time of the stay. The median number of pairs Cp or C/D ratio and CRP per patient was 6 (min–max: 1 to 19). As a whole, the total number of independent pairs (Cp or C/D ratio and CRP) was 442 in the 71 patients. All patients began with IV dosing and were then switched to oral dosing during hospitalization (mean switch time to oral was 18.7 days, during week 3). The final case mix is 310 IV doses and 137 oral doses.

The glomerular filtration rate was within the normal range throughout the hospital stay, with a significant ( $P < .0001$ ) gradual decline from 109.6 mL/min at week 1 to 96.6 mL/min at week 5 (Figure 2a). Up to the median post-transplantation day (day 25), the median GFR remained above 87 mL/min. Thirteen patients exhibiting a GFR below 60 mL/min with three of them exhibiting a GFR below 30 mL/min. The albumin levels were in the lower part of normal levels (median = 37 g/L, min–max = 24.7–46.5 g/L). Hematocrit was, as expected, clearly below normal levels (median = 28.7%, min–max = 20%–39.5%). AST levels were in the normal range (median = 20 UI/L, min–max = 7–20 UI/L).

The median CRP levels at the initiation of ciclosporin were close to the upper bound of the no-to-mild range ( $<40$  mg/L).<sup>12</sup> There was considerable inter-individual variability in the CRP levels (Figure 3), which increased after transplantation and then decreased by week 3 post-transplantation showing a significant decrease between week 1 and week 4 ( $P < .0001$ ) (Figure 2b). The majority of patients exhibited



**Figure 1.** Flow chart of the study population.

either one or more episode of no-to-mild inflammation ( $n = 15$  patients) or one or more episode of no-to-mild and moderate inflammation ( $n = 44$  patients). Episodes of severe inflammation were encountered in nine patients (Table 1).

#### Drug–Drug Interactions with Ciclosporin

The drugs with potential DDI with ciclosporin most frequently used were fluconazole, posaconazole, and letermovir, the latter being always associated with an antifungal agent (Table 1).

Although rarely employed, colchicine which is contraindicated with ciclosporin (Micromedex Merative Company, retrieved November 24, 2023 from <http://www.micromedexsolutions.com>), was used in two patients, in conjunction with fluconazole, for brief periods (2 and 4 days). This combination contributed minimally to DDI.

#### Ciclosporin Blood Levels

Ciclosporin levels exhibited a significant ( $P < .0001$ ) consistent increase over time, reaching a plateau at week 4 (Figure 2c). The median and min–max ciclosporin levels in the no-to-mild ( $n = 66$ ), moderate ( $n = 55$ ), and severe ( $n = 9$ ) groups were  $200 \mu\text{g/L}$  [81–358],  $176 \mu\text{g/L}$  [53–625], and  $253 \mu\text{g/L}$  [100–461], respectively, and were not significantly different (Figure 4a, illustration of the comprehensive analysis on the impact of inflammation on ciclosporin concentration, which is fully detailed in Table 2). Univariate analysis showed that ciclosporin concentrations were positively associated with post-transplant delay, and drug–drug interactions (DDIs; Table 2). Multivariate analysis showed that ciclosporin

concentrations were not associated to any variables (Table 2).

#### Ciclosporin Concentration/Dose Ratio and Metabolism

The ciclosporin C/D ratio exhibited a significant ( $P < .0001$ ) increase over time, reaching a plateau at week 3 (Figure 2d). The median C/D ratio after IV and oral administration were 0.7 (min–max: 0.18–6.9, IQR: 0.50–1.10,  $n = 310$ ) and 0.6 (min–max: 0.2–2.9, IQR: 0.50–1.00,  $n = 137$ ), respectively. These values were not statistically different ( $P = .4565$ , Figure S1).

The median C/D ratio in the presence or the absence of a DDI were 0.7 (min–max: 0.18–6.9, IQR: 0.50–1.10,  $n = 376$ ) and 0.6 (min–max: 0.2–2.9, IQR: 0.40–0.90,  $n = 72$ ), respectively ( $P = .0035$ , Figure S2).

The median and min–max C/D ratio in the no-to-mild (0.66, [0.29–2.4],  $n = 66$ ), moderate (0.58, [0.25–3.0],  $n = 55$ ), and severe groups (1.3, [0.62–4.67],  $n = 9$ ), were significantly different when compared the severe group ( $P = .0267$  between mild and severe, and  $P = .00186$  between moderate and severe, Figure 4b is the illustration of the comprehensive analysis on the impact of inflammation on the C/D ratio and ciclosporin metabolism, which is fully detailed in Table 3).

The univariate analysis demonstrated a positive association between the ciclosporin C/D ratio and the post-transplant delay, as well as a positive association between the ratio and CRP as a categorical variable and DDIs (Table 3).

The multivariate analysis indicated that ciclosporin C/D ratio was positively associated with post-transplant delay, but not with CRP considered as a continuous variable (Table 3). The ciclosporin C/D



**Table 1.** Patient Characteristics (Median and IQR)

Gender	n (%)		
Male	45 (63%)		
Female	26 (37%)		
<i>Demographic</i>	<i>Median</i>	<i>IQR</i>	<i>Min-max</i>
Age (years)	55.5	44–63	18–70
Weight (kg)	79.5	68.7–91.0	41–133
Height (m)	1.72	1.65–1.78	1.47–1.90
BMI (kg/m <sup>2</sup> )	26.2	23.7–30.8	18.3–41.0
Length of stay post graft (day)	25	22–32	9–100
<i>Underlying disease</i>	n (%)		
Acute myelogenous leukemia	32 (42%)		
Myelodysplastic syndrome	12 (16%)		
Non-Hodgkin lymphoma	10 (13%)		
Myelomonocytic leukemia	9 (12%)		
Acute lymphoblastic leukemia	7 (9%)		
Hodgkin lymphoma	2 (3%)		
Other	5 (6%)		
<i>Laboratory parameters (at the initiation of ciclosporin treatment)</i>	<i>Median</i>	<i>IQR</i>	<i>Min-max</i>
Albumin (g/L)	37	34.1–39.3	24.7–46.5
AST (UI/L)	20	13–25	7–120
Hematocrit (%)	28.7	25.1–32.6	20–39.5
CRP (mg/L)	32.9	11.4–63.5	1.2–187.5
GFR (mL/min)	108	95–119	51–155
<i>Number of patients with episode(s) according to the CRP class during hospitalization</i>	n (%)		
No-to-mild inflammation	15 (21%)		
No-to-mild and moderate inflammation	44 (62%)		
No-to-mild, moderate and severe inflammation	6 (8%)		
Mild and severe inflammation	1 (1%)		
Moderate inflammation	3 (4%)		
Moderate and severe inflammation	2 (3%)		
<i>Drug treatments: Number of patients with a CYP3A4 inhibitor within the hospitalization stay</i>	n (%)		
<i>Antifungal agents (AFs)</i>			
Fluconazole	63 (89%)		
Posaconazole	10 (14%)		
Voriconazole	5 (7%)		
Isavuconazole	2 (3%)		
<i>Others</i>	n (%)		
Letermovir	20 (28%)		
Letermovir and an AF at the same time	20 (28%)		
Colchicine and AF or letermovir	2 (3%)		

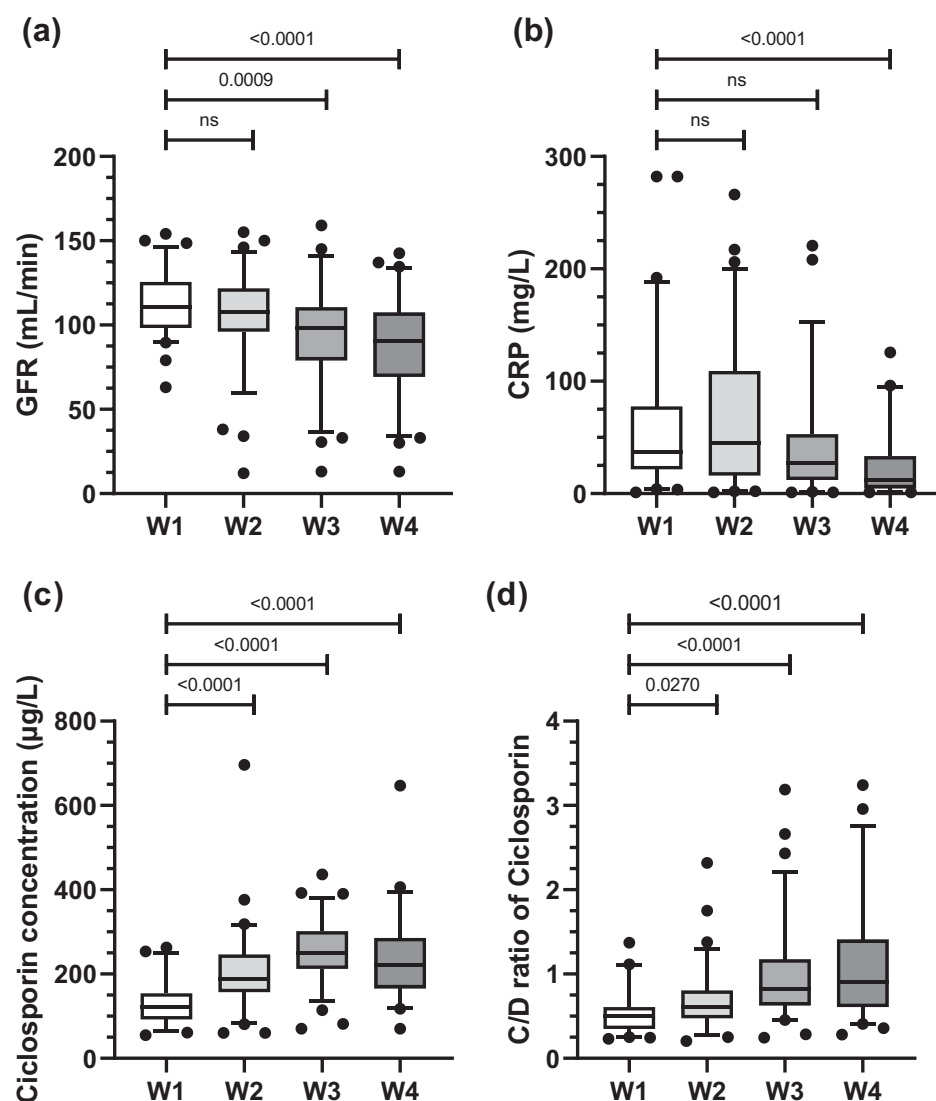
ratio was found to be positively associated to with post-transplant delay and with CRP considered as a categorical variable (Table 3).

There were no significative differences in ciclosporin C/D ratio when grouping by CRP class and DDI status (Figure S3).

## Discussion

Our research in allogeneic HSCT recipients demonstrated that ciclosporin blood concentrations remained unaffected by inflammation, whereas its metabolism, as estimated by the C/D ratio, decreased with severe inflammation. While some patients exhibit high intra-individual variability in CRP levels, the majority of the overall variability is attributable to inter-individual differences (Figure 3).

An early study involving six patients undergoing HSCT hypothesized that inflammation-induced inhibition of ciclosporin metabolism occurred.<sup>11</sup> This study reported that blood levels after IV continuous infusion increased and paralleled IL-6 levels. In contrast, a more recent study identified CRP as a novel covariate of ciclosporin CL. One study indicated that CL of ciclosporin (after IV or oral administration) in allogeneic HSCT recipients, increased with CRP levels with a stratification of the CRP based on a cut-off of 10 mg/L. However, it should be noticed that the recipient's inflammation status did not show significant inflammation, as indicated by a median CRP levels of 4.8 mg/L, ranging from 1.4 to 238 mg/L.<sup>13</sup> This study did not align with the established understanding that inflammation downregulates CYP450 metabolism and efflux transporter activity.<sup>10</sup> As such, inflammation

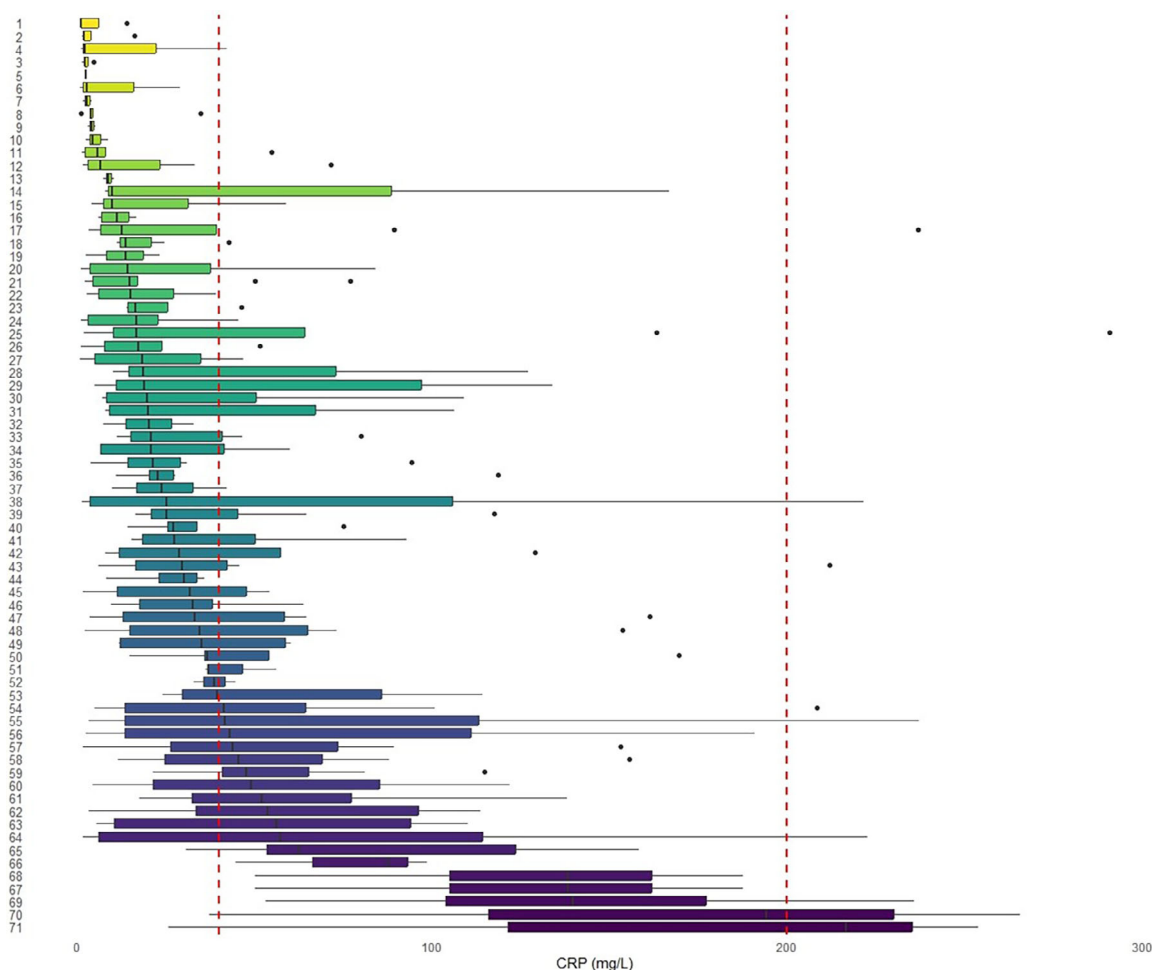


**Figure 2.** Glomerular filtration rate (a), CRP (b), cyclosporin trough concentrations (c), and cyclosporin C/D ratio (d) as box (median and 25th and 75th percentiles) and whisker (5th and 95th percentiles) in patients with allogeneic hematopoietic stem cell transplantation during weeks post-transplantation. The filled circles represent outliers.

should decrease hepatic and intestinal metabolism of cyclosporin (by inhibiting CYP3A4), and increase absorption by inhibiting ABCB1 intestinal efflux leading to an increased bioavailability and a decrease in systemic clearance of cyclosporin.

While cyclosporin levels regularly increased and reached a maximum at week 3 (245 µg/L), the apparent lack of influence of inflammation on cyclosporin levels (Figure 4a) can be attributed to the fact that clinicians have established a meticulous therapeutic drug monitoring program in their daily practice, which swiftly addresses any unexpected changes in blood levels of cyclosporin. This seemingly negligible impact of inflammation conceals a significant effect on cyclosporin metabolism. Indeed, severe inflammation was associated with a higher C/D ratio (Figure 4b).

Multivariate analysis demonstrated a correlation between the C/D ratio and post-transplant delay. This correlation exhibited a consistent increase from week 1 to week 3, suggesting a reduction in cyclosporin clearance (Figure 2d). In HSCT patients, a decrease in cyclosporin CL has been reported in the first 2 weeks post-transplant (50% decrease, in a study involving five patients).<sup>7</sup> A smaller decrease in CL has been reported in a large clinical study (in 130 patients) with a regular decline in CL each week post-transplant up to day 21 post-transplant (the correction factor of CL was 1.46 to 1.20 from the first to third week post-transplant).<sup>8</sup> The origin of the observed decrease in CL over time remained unclear, and potential explanations included reduced hepatic cytochrome P450 metabolism, enterohepatic recycling, or bile formation/excretion.



**Figure 3.** CRP continuous distribution by patient. Boxplot of CRP levels for each patient ( $n = 71$ ), with patients ordered by their median CRP levels in descending order. The red dashed lines represent CRP thresholds at 40 and 200 mg/L describing the categorical inflammatory status:  $<40$  mg/L: Mild; 41–200 mg/L: Moderate;  $>200$  mg/L: Severe.

The current study indicates that increase in inflammation post-transplant may play a role in this decrease in CL post-transplant. Indeed, our findings demonstrated a clear association between the ciclosporin C/D ratio and severe inflammation, which is not unexpected for a low-extraction drug. Hence, inflammation is expected as a nongenetic extrinsic factor involved in the phenocopy conversion observed with ciclosporin in HSCT patients.

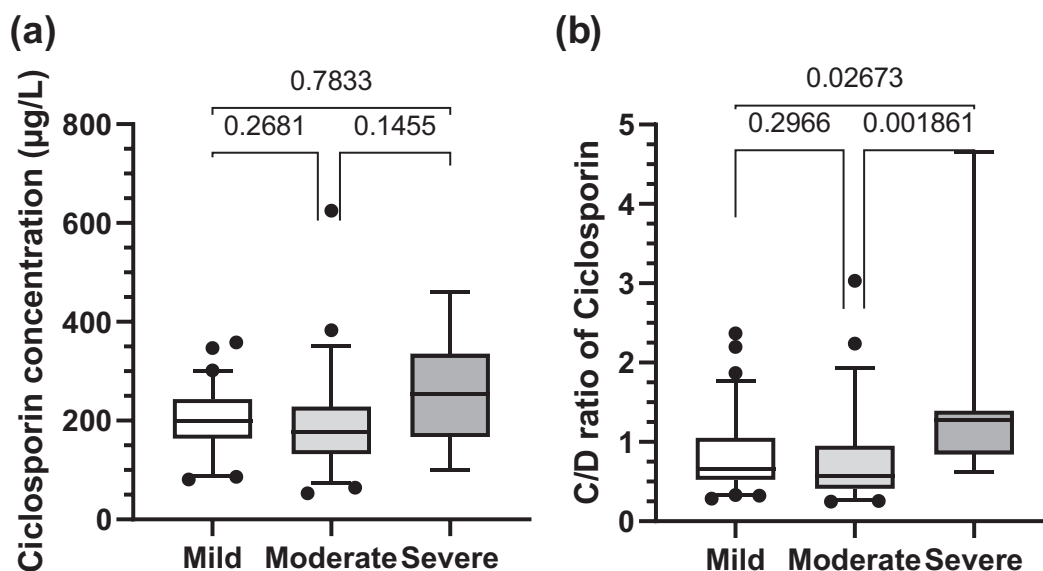
However, the conversion of genotypic extensive metabolizers of CYP3A4 into phenotypic poor metabolizers of ciclosporin may also arise from other external factors such as potential drug–drug interactions (pDDI), which are frequently observed in this patient population, especially via CYP3A4 inhibition by antifungal drugs (Table 1). The drugs with the potential for DDI with ciclosporin that we considered were fluconazole, voriconazole, posaconazole, isavuconazole, letermovir, and colchicine. We did not discriminate between the antifungal drugs even though their inhibitory potential on the CYP3A4 metabolism

may be different, and may be dose dependent and influenced by the route of administration.<sup>14–25</sup>

The renal function was not considered as a covariate since it was considered to have no relevance as a covariate for ciclosporin dosing in solid organ transplant patients.<sup>26</sup> Renal failure, especially at the end stage, has an effect on enzymes and transporters involved in the disposition of drugs. The impact on CYP3A4 is not yet so evident being rated as limited but variable.<sup>27,28</sup> The impact of kidney disease is much clearer on CYP2C8 and CYP2D6, and on OATP hepatic transporters.<sup>29,30</sup>

When pDDI is considered without differentiating the drugs involved, pDDI led to an increase in the ciclosporin C/D ratio that was close to the values reported in the literature. If the median increase was monitored at a moderate level (+17%), the higher increase in the mean ratio (+31%) highlighted the fact that severe reduction in ciclosporin metabolism occurred in some patients (30 patients with a ratio  $>2.0$ , Figure S2). In the univariate and multivariate analyses on the C/D





**Figure 4.** Median ciclosporin trough concentration (a) and median ciclosporin trough concentration-to-dose ratio (b) for patients undergoing allogeneic hematopoietic stem cell transplantation, presented by inflammation status: no-to-mild (CRP < 40 mg/L,  $n = 66$ ), moderate (CRP 40 to 200 mg/L,  $n = 55$ ), and severe (CRP > 200 mg/L,  $n = 9$ ). The data are displayed as box plots representing the median, 25th, and 75th percentiles, with whiskers indicating the 5th and 95th percentiles. See Tables 2 and 3 for detailed analysis on the impact of inflammation on ciclosporin concentration and metabolism.

ratio, the extent and variability of the DDI effect was not as large as in the analysis on ciclosporin concentration. In the analysis on C/D ratio, inflammation status expressed as CRP class is more significant than the DDI effect. The C/D ratio is a better measurement for comparing the inflammation effect against DDIs given that DDIs with ciclosporin are mostly based on CYP inhibition. If there was a DDI effect with ciclosporin, the DDI effect would have appeared. This leads us to conclude that in the HSCT patient population, the CYP3A4 inhibitory potential of interacting drugs may be masked since metabolism is already upstream down-regulated by inflammation so that the inflammation effect is more significant compared to the DDI effect.

Furthermore, the route of administration did not appear to influence the C/D ratio of ciclosporin. The metabolism of ciclosporin is mainly driven (around two thirds) by gut wall CYP3A4 metabolism, as evidenced by pre-systemic metabolism affecting bioavailability, as demonstrated by DDI studies with ketoconazole following a single dose of either IV and PO ciclosporin.<sup>2,31</sup> Nevertheless, the C/D ratio of ciclosporin is determined by the systemic hepatic clearance, which is not influenced by the administration route.

In the hospitalization setting, the implementation of a robust therapeutic drug monitoring of ciclosporin blood levels, the influence of inflammation on the metabolism of ciclosporin is not apparent. Nevertheless, monitoring inflammation remains a crucial aspect, as the resolution of severe inflammation can result in decreased ciclosporin concentrations due to the

restoration of CYP3A4 activity, which in turn reduces oral bioavailability and increases systemic clearance. This enhanced comprehension of the influence of inflammation on ciclosporin pharmacokinetics could help clinicians in establishing more precise initial dosages, especially for patients with severe inflammatory conditions. This can be achieved through the use of tools such as population pharmacokinetic models and Bayesian maximum a posteriori estimation. This would enable a reduction in the intra-individual variations in ciclosporin concentrations, and the avoidance of low plasma concentrations of ciclosporin, particularly during the initial weeks following HSCT, which have been associated with an increased risk of developing GVHD.

However, HSCT patients receive ciclosporin in an outpatient setting for 4 to 6 months<sup>1</sup> and frequent TDM is not easy to set up. In this setting, variations in inflammation may also be considered although the main factor of variability is the medication nonadherence to immunosuppressant therapy, which is estimated to be up to 40% in HSCT patients.<sup>32</sup>

#### Strength and Weakness

It must be acknowledged that our study is subject to certain limitations, including its retrospective, single-center nature and the lack of direct measurement of inflammatory cytokines such as IL-6, which induce the production of acute-phase reactant proteins. Instead, we have relied on CRP as a clinical marker of inflammation. Furthermore, we did not consider genetic

**Table 2.** Ciclosporin Concentrations in Allogeneic Hematopoietic Stem Cell Transplantation Recipients During Therapeutic Drug Monitoring Throughout Hospitalization Post-Transplant: Univariate and Multivariate Linear Mixed-Effect Regression Analysis

Characteristic	Univariate			Multivariate		
	Beta	95% CI	P-value	Beta	95% CI	P-value
<b>Post-transplant delay (days)</b>	0.87 40 <sup>1</sup> –101 <sup>2</sup>	0.04, 0.17	.041	0.669	–0.183, 1.521	.12
<b>CRP class</b>						
Severe (>200 mg/L)	—	—	—	—	—	—
Moderate (41 to 200 mg/L)	–50 36 <sup>1</sup> –102 <sup>2</sup>	–115, 15	.13	1	1.000, 16.54	.14
No-to-mild (<40 mg/L)	–38 36 <sup>1</sup> –102 <sup>2</sup>	–103, 26	.2	1	1.000, 22.8	.2
<b>DDI (1/0)</b>						
0	—	—	—	—	—	—
1	40 35 <sup>1</sup> –102 <sup>2</sup>	9.0, 70	.011	30.22	1.000, 62.04	.063
<b>Gender (M/F)</b>						
F	—	—	—	—	—	—
M	–1.1 36 <sup>1</sup> –102 <sup>2</sup>	–29, 27	>.9	—	—	—
<b>Route (IV / ORAL)</b>						
IV	—	—	—	—	—	—
ORAL	–3.2	–26, 19	.8	—	—	—
<b>Albumin (g/L)</b>	0.21 37 <sup>1</sup> –102 <sup>2</sup>	–3.0, 3.4	.9	–0.011	–0.039, 0.016	.4
<b>Hematocrit (%)</b>	2.4 36 <sup>1</sup> –102 <sup>2</sup>	–0.35, 5.2	.085	1.969	–0.903, 4.832	.2
<b>CRP (mg/L)</b>	–0.03 37 <sup>1</sup> –102 <sup>2</sup>	–0.22, 0.16	.8	—	—	—
<b>AST (UI/L)</b>	0.39 37 <sup>1</sup> –102 <sup>2</sup>	–0.60, 1.4	.4	0	–0.008, 0.008	>.9
<b>BMI (kg/m<sup>2</sup>)</b>	–0.53 37 <sup>1</sup> –102 <sup>2</sup>	–2.9, 1.8	.7	—	—	—
<b>Weight (kg)</b>	0.05	–0.71, 0.81	.9	—	—	—
<b>Height (m)</b>	45 37 <sup>1</sup> –102 <sup>2</sup>	–109, 99	.6	—	—	—
<b>Patient.sd.Intercept<sup>1</sup></b>	—	—	—	37.01	—	—
<b>Residual.sd.Observation<sup>2</sup></b>	—	—	—	101.3	—	—

In the univariate analysis, the patient random effect and the residual variability are indicated for each beta value (<sup>1</sup> for patient random effect and <sup>2</sup> for residual variability).

polymorphisms affecting enzymes and transporters involved in ciclosporin pharmacokinetics, which could potentially refine treatment strategies through pharmacogenetic evaluations. Indeed, numerous studies have suggested that single nucleotide polymorphism (SNPs) in cytochrome and transporter genes may influence ciclosporin pharmacokinetics. Consequently, a pharmacogenetic pre-evaluation (CYP3A4, CYP3A5, and ABCB1) could be useful for a targeted treatment of transplanted patients.<sup>33,34</sup> Nevertheless, the study was conducted on a cohort of allogeneic HSCT patients, which enabled the observation of a large number of blood levels, thereby strengthening our findings of an effect of severe inflammation on ciclosporin metabolism.

## Conclusion

The results of our study clearly demonstrated that severe inflammation decreased the metabolism of ciclosporin without significant apparent impact on ciclosporin blood levels. The apparent lack of influence of DDIs on ciclosporin levels and C/D ratio may result from the fact that the CYP3A4 inhibitory potential of interacting drugs is minimized in HSCT patients, due to an upstream downregulation of metabolism by inflammation.

Our research highlights the intricate interrelationship between inflammation, DDIs, and ciclosporin metabolism in allogeneic HSCT recipients, underscoring the pivotal role of targeted therapeutic monitoring in the effective management of these patients.

**Table 3.** Ciclosporin C/D Ratio in Allogeneic Hematopoietic Stem Cell Transplantation Recipients During Therapeutic Drug Monitoring Throughout Hospitalization Post-Transplant: Univariate and Multivariate Linear Mixed-Effect Regression Analysis

Characteristic	Univariate			Multivariate		
	Beta	95% CI	P-value	Beta	95% CI	P-value
<b>Post-transplant delay (days)</b>	0.02 0.40 <sup>1</sup> –0.53 <sup>2</sup>	0.01, 0.02	<.001	0.016	0.011, 0.021	<.001
<b>CRP class</b>						
Severe (>200 mg/L)	—	—	—	—	—	—
Moderate (41 to 200 mg/L)	–0.69 0.43 <sup>1</sup> –0.55 <sup>2</sup>	–1.1, –0.33	<.001	–0.608	–0.958, –0.259	<.001
No-to-mild (<40 mg/L)	–0.52 0.43 <sup>1</sup> –0.55 <sup>2</sup>	–0.88, –0.16	.005	–0.559	–0.907, –0.211	.002
<b>DDI (I/O)</b>						
0	—	—	—	—	—	—
1	0.25 0.42 <sup>1</sup> –0.56 <sup>2</sup>	0.06, 0.44	.011	0.122	–0.065, 0.310	.2
<b>Gender (M/F)</b>						
F	—	—	—	—	—	—
M	–0.14 0.42 <sup>1</sup> –0.56 <sup>2</sup>	–0.38, 0.09	.2	—	—	—
<b>Route (IV / ORAL)</b>						
IV	—	—	—	—	—	—
ORAL	0.04 0.43 <sup>1</sup> –0.56 <sup>2</sup>	–0.09, 0.17	.5	—	—	—
<b>Albumin (g/L)</b>	–0.02 0.42 <sup>1</sup> –0.56 <sup>2</sup>	–0.05, 0.01	.2	–0.011	0.039, 0.016	.4
<b>Hematocrit (%)</b>	0.01 0.43 <sup>1</sup> –0.56 <sup>2</sup>	–0.02, 0.03	.6	0.007	–0.016, 0.031	.5
<b>CRP (mg/L)</b>	0 0.42 <sup>1</sup> –0.56 <sup>2</sup>	0.00, 0.00	.8	—	—	—
<b>AST (UI/L)</b>	0 0.43 <sup>1</sup> –0.56 <sup>2</sup>	–0.01, 0.01	>.9	0	–0.008, 0.008	>.9
<b>BMI (kg/m<sup>2</sup>)</b>	0 0.43 <sup>1</sup> –0.56 <sup>2</sup>	–0.02, 0.02	.7	—	—	—
<b>Weight (kg)</b>	0 0.43 <sup>1</sup> –0.56 <sup>2</sup>	–0.01, 0.01	.9	—	—	—
<b>Height (m)</b>	–0.42 0.42 <sup>1</sup> –0.56 <sup>2</sup>	–1.7, 0.91	.5	—	—	—
<b>Patient.sd.Intercept<sup>1</sup></b>	—	—	—	0.404	—	—
<b>Residual.sd.Observation<sup>2</sup></b>	—	—	—	0.524	—	—

In the univariate analysis, the patient random effect and the residual variability are indicated for each beta value (<sup>1</sup> for patient random effect and <sup>2</sup> for residual variability).

## Author Contributions

David Malnoë: Result presentation; data analysis; statistical analysis; manuscript drafting. Mathilde Bories: Data curation; participation in manuscript drafting. Morgane Jean Pierre: Statistical analysis; participation in manuscript drafting. Tony Marchand: Data analysis; participation in manuscript drafting. Pascal Le Corre: Design of the study; Result presentation; data analysis; supervision; manuscript drafting and editing. All authors reviewed and edited the manuscript, and approved the final version of the manuscript.

## Conflicts of Interest

The authors declare no conflicts of interest.

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Data sharing not applicable—research data cannot be shared as the authors are not authorized to provide access to the data. Indeed, individual participant-level data cannot be adequately anonymized as there is a reasonable likelihood that individual participants could be re-identified.

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## Supplemental Information

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