Evaluation of cyclosporine levels in pediatric patients submitted to hematopoietic stem cell transplantation in the Hospital de Clínicas de Porto Alegre

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Abstract

Introduction: Hematopoietic stem cell transplantation (HSCT) is a complex procedure, in which stem cells from the bone marrow, peripheral blood or umbilical cord are inserted in the recipient. In order for the recipient receive these cells without developing Graft versus Host Disease (GvHD), immunosuppressive therapy should be followed. A well known drug used in this therapy is cyclosporine (Cya), whose mechanism of action is the T cells proliferation reduction, by the inhibition of interleukin-2. This drug presents high variability in the absorption rate, plus it follows metabolism by the cytochrome enzymes. Pharmacokinetic studies in the pediatric population using this drug are scarce. There are main differences in the pharmacokinetic parameters between solid organs and hematopoietic stem cells recipients. **Materials and Methods**: In this study we reviewed the medical records from the pediatric patients at the Hematological Service of the Hospital de Clínicas de Porto Alegre. We divided these patients into two groups: a) patients whose Cya levels were not used to dose adjustment based on pharmacokinetic analysis (n= 11) and b) patients whose Cya levels were used to dose adjustment based on pharmacokinetic analysis (n =11). **Results**: The medical records were accessed in order to obtain the patients' demographics data and other relevant information such as renal function, grafting day and Cya mode of use and concomitant drugs intake. The indication for the treatment with intravenous (IV) Cya was the prophylaxis against GvHD. Most patients from both monitored and non-monitored groups presented CyA seric levels between the expected therapeutic window (100 ng/ml – 400 ng/ml), however in the literature it was found that in the period just after HSCT these levels should be higher (>200 ng/ml), in order to prevent the GvHD event. **Conclusions**: We observed that in the monitored group the levels out of the therapeutic window were above the expected, while in the non monitored group these levels were below the accepted ones. Our hypothesis is that due to the Pharmaceutical Service there was a better awareness by the health professionals in not letting Cya levels below 100ng/ml, as this could lead to GvHD development.

Key-words

Drug monitoring, cyclosporine, hematopoietic stem cell transplantation, pediatrics.

Introduction

Hematopoietic Stem Cell Transplantation (HSCT) is the process in which an infusion of stem cells, coming from donators, related or not, occurs. The stem cells might come from the bone marrow, peripheral blood or umbilical cord[1]. Before the transplantation happens the patient should undergo the conditioning regimen, also called chemotherapy, which could be a myeloblative or non-myeloblative conditioning, being the last the most preferable. An ideal non-myeloablative regimen should allow the grafting process to occur with the least post-transplant toxicity, in order to avoid high grades of Graft versus Host Disease (GvHD) [2], which is certainly the most important complication from an HSCT. Acute GvHD is one of the most frequent mortality causes in patients who underwent HSCT. This disease is the immunologic process in which mature donor T cells interact with host and donor antigen presenting cells, thus causing the release of pro-inflammatory cytokines. These cytokines might later lead to host tissue damage by T cell activation [3].

GvHD may occur some days before the grafting or even months later. This disease can be acute, in which the symptoms may be dermatological, hepatological or gastrointestinal lesions. In chronic GvHD the symptoms might be located in only one organ or in many sites[1]. Thus, due to possible bad prognosis, these patients should receive efficient prophylactic therapies in order to avoid GvHD occurrence. However, it is also important that the Graft against Leukemia process occurs (GvL). A balance between GvHD and GvL might be the ideal outcome after HSCT. It was found in the literature that when used together, methotrexate and cyclosporine might promote less GVhD cases[4]. This combination is one of the most used in HSCT pediatric centers[5], and that was also the choice in our studied hospital, based on its current protocol[6].

Cya is a cyclic peptide which contains 11 amino acids residues with high immunosuppressive activity. This mechanism of action is related to the T cells proliferation reduction, due to interleukin 2 (IL-2) gene inhibition. Calcineurin is a phospatase activated when a T cell interacts with an antigen. This enzyme, thus, activates IL-2 production.

Cyclosporine-immunophilin complex binds to and inactivates calcineurin, which leads to T helper cells inactivation and, as a consequence, inhibition of IL-2 production, resulting in an immunosuppressive activity [7].

Cya is a very lipophilic drug with high variability in terms of absorption. It presents a large volume of distribution (4 - 5 L/kg), low clearance (5–10 ml/kg/min)[8] and it passes through liver biotransformation, due to cytochrome P450 3A4 enzymes[9]. Pharmacokinetics studies about Cya in the pediatrics context are scarce in the literature. Some prospective studies based on this population, who underwent HSCT, suggest the dosing regimens of 2 mg/kg given intravenously at a constant rate 2 h infusion twice a day[5,9]. There is a very large range of the best serum trough levels to be achieved described in literature, depending on covariates such as age, underlying disease, body weight, among others. Even though trough levels measurements are the most used method in order to estimate pharmacokinetic parameters for patients using Cya, there are many studies which have not found this laboratory result to be a good dosage predictor. This is observed not only for HSCT patients [5,9,10,11], but also for patients submitted to solid organs transplantations[12]. Even though, trough levels measurement is not the best approach in Cya monitoring, this is the most used method due to its practical performance[11]. It is also important to relate that there are differences in the pharmacokinetic parameters between solid organs transplantation and HSCT [13]. Shultz *et al* [10] reports that children and adults who received stem cells presented delayed Cya absorption in relation to patients who received solid organs. Moreover, patients who underwent HSCT had changes in the gastrointestinal tract, due to inflammation processes like mucositis, leading to high Cya exposure. The pediatrics population also presents some differences in the pharmacokinetic parameters in

relation to adults, which are important to be noticed when studying pharmacokinetic models in this population. Newborns undergo fast changes due to their maturation process, which causes huge variability in pharmacokinetics and pharmacodynamic approaches [14]. Neonates and infants tend to have a reduced plasma protein binding, what might increase the drug distribution in the body. Consequently, this difference in the volume of distribution might lead to a wrong interpretation of the drug plasma levels measurements. It is recommended that drugs highly metabolized by the liver, such as Cya, should be administered with care before the age of 2 months and changes in dosing should be ascertained mainly by drug monitoring [15]. For these reasons drug monitoring techniques development is essential in the pediatric population who underwent HSCT using Cya as prophylaxis against GvHD.

In the present study, demographical characterization of the HSCT pediatric patients treated at Hematological Service of the Hospital de Clínicas de Porto Alegre, who received cyclosporine during the period from January 2015 to October 2017 was described and the impact of dosing adjustments of intravenous Cya was evaluated.

Methods

Study design and population

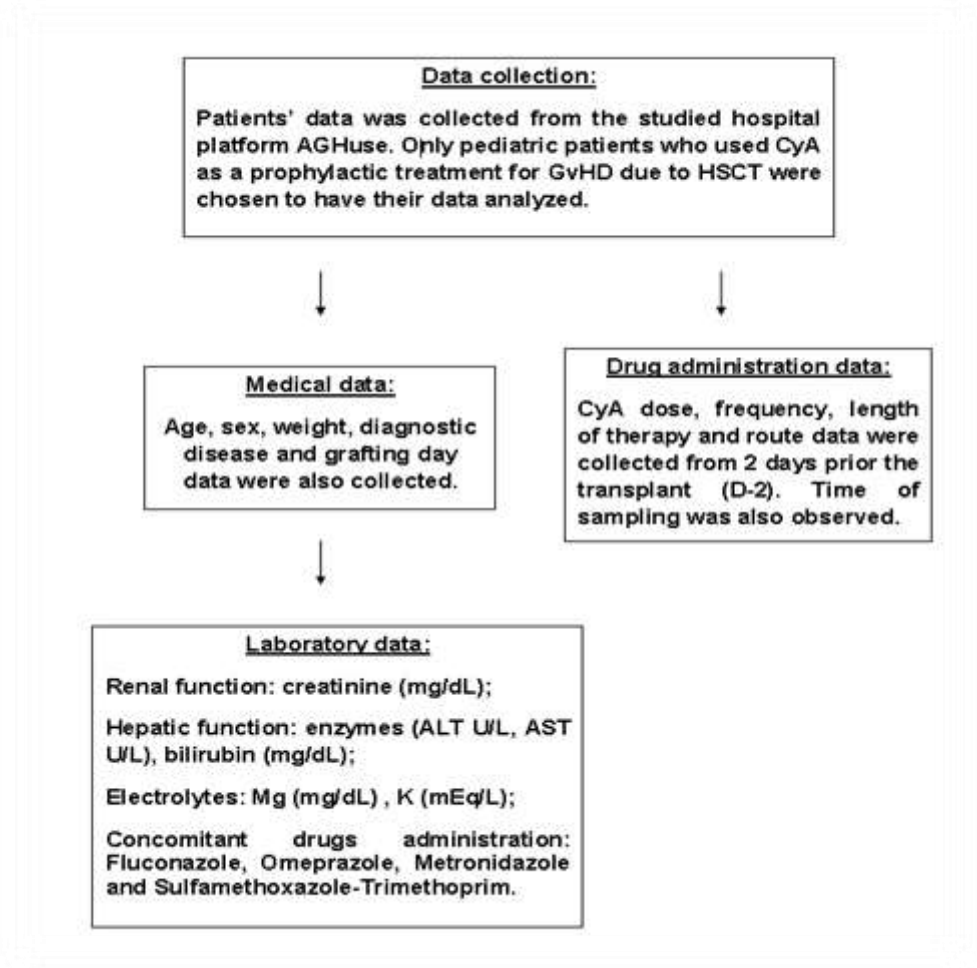
The study was performed at Hospital de Clínicas de Porto Alegre (HCPA), a general hospital located in Porto Alegre city, Brazil. The data was collected retrospectively in the platform called AGHuse from patients aged 0 – 14 years of age, who were given intravenously Cya between January 2015 and November 2017 in order to prevent the GvHD development following HSCT. Patients were selected by a search in the platform AGHuse by drug, route of administration and hospital ward. Pediatric patients who were taking Cya, but did not undergo HSCT at HCPA were excluded. The patients (n = 22) were split in two groups: a) patients whose Cya levels were used to dose adjustment with empirical analysis (11) and b) patients whose Cya levels were used to dose adjustment with pharmacokinetic analysis (11). A total of 180 whole blood levels of Cya were evaluated. Both patients' populations were statistically equal in terms of age and weight. This fact was verified by the non-parametric Mann

Whitney U test, whose U value for weight was equal to 36 and the U value calculated for age was equal to 47 ($p = 0.05$). The study was approved by the Research Ethics Committee of Federal University of Rio Grande do Sul and HCPA (#2.672.571). In Figure 1 it can be observed a scheme with the steps followed for the patients' data collection.

Review of medical record

The medical records found in AGHuse Platform were accessed in order to obtain the patients' demographics data (age, gender, weight, diagnostic disease) (Table 1) and other relevant information such as renal function, grafting day and Cya mode of use and concomitant drugs intake (Table 2). The concomitant drugs were chosen based on their capacity of changing Cya serum levels and frequency of use by the studied population. Thus, data from omeprazole, fluconazole, metronidazole and trimethoprim/sulfamethoxazole therapies were also accessed.

Figure 1: Scheme used for the patients' data collection process.



The demographics data from the patients is shown in Table 1.

Table 1: Characteristics of the patients who used Cyclosporine.

Patient Code	Sex	Disease	Monitored by pharmacokinetic equations		Age (years)	Weight (kg)
			Yes	No		
1	M	AML	x		9	45.45
2	F	ALL /AML	x		14	59.93
3	F	ALL	x		1	7.06
4	F	ALL	x		8	25.53
5	F	ALL	x		14	61.94
6	F	MDS	x		9	32.59
7	M	ALL	x		3	15.74
8	F	SCID	x		1	10.95
9	F	HLH	x		11	54.9
10	M	SCA/BT	x		9	25.21
11	M	LL	x		8	39.34
MEAN	_____	_____	_____	_____	7.9	34.42
12	F	ALL/MDS		x	5	22.53
13	M	ALL		x	8	23.00
14	M	ALL		x	5	26.44
15	F	AG/NSI		x	6	16.33
16	M	ALL/AML		x	1	11.52
17	M	ALL / MDS		x	14	32.34
18	M	HIES/AML		x	1	10.57
19	F	AML		x	4	18.9
20	M	AML/ALL		x	14	41.42
21	M	HIgMI		x	10	24.94
22	F	MDS/AML		x	1	10.72
MEAN	_____	_____	_____	_____	6.27	21.70
MEAN	_____	_____	_____	_____	7.09	28.06

*ALL = acute lymphoblastic leukemia AML= acute myeloid leukemia NSI = non-specific immunodeficiency MDS = myelodysplasic syndrome HLH = hemophagocytic lymphohistiocytosis HIES= hyperimmunoglobulin E syndrome SCID = severe combined immunodeficiency SCA = sickle cell anemia BT= beta thalassemia LL= lymphoid leukemia HIgMI= hyperimmunoglobulin M immunodeficiency AG = agranulocytosis

As it can be observed in Table 1, in the monitored group (patients 1 – 11) there were 4 boys and 7 girls. The average age of the studied patients was 7.9 years and they weighed in average 34.42 kg. In the non-monitored group (patients 12 – 22) there were 7 boys and 4 girls. Their average age and weight

were, respectively, 6.27 years and 21.70 kg. 81.82% patients in each group were diagnosed with a malignant disease.

In Table 2 we can observe that in the monitored group by pharmacokinetic equations (patients 1 – 11) the average serum creatinine range was 0.28 – 0.67 mg/dL and in the non-monitored group by pharmacokinetic equations (patients 12 – 22) was 0.19 – 0.53 mg/dL. The reference value for seric creatinine is found in a range between 0.5 – 1.0 mg/dL for children aged 3 to 18 years old, while the range is between 0.3 – 0.7 mg/dL for children under 3 years old[16]. Drugs such as omeprazole, fluconazole, metronidazole and trimethoprim/sulfamethoxazole might be able to interfere with CyA levels. Frequently the patients from both groups had to take some of these drugs during CyA intravenous therapy. Fluconazole and trimethoprim/sulfamethoxazole are recommended by the hospital's protocol. Fluconazole is a prophylatic treatment against various fungi infections, while trimethoprim/sulfamethoxazole is used against *Pneumocystis carinii* infection [6]. Both omeprazole and metronidazole were not recommended by the current hospital protocol. Table 2 shows that 63.64% of the total number of patients used omeprazole during CyA IV therapy, while 86.36 % took fluconazole during this period, 86.36% metronidazole and only 18.18 % had to take trimethoprim/sulfamethoxazole during the same period. Only two of the patients had to use the four drugs during CyA IV therapy and both of them are part of the monitored group.

Table 2: Patients' data related to renal function, grafting process and use of Cyclosporine and concomitant drugs.

Patient Code	Disease	Serum Creatinine (range) (mg/dL)	Grafting day	Route		Days using IV route	Drugs used during CyA intravenous therapy			
		IV		IV	Oral		OMZ	FCZ	MTZ	TMP -SMT
1	AML	0.3 – 0.44	D + 13	x	x	15	x	x	_____	_____
2	ALL /AML	0.25 – 0.49	D + 24	x	x	30	x	x	x	_____
3	ALL	0.17	D + 34	x	x	41	_____	x	x	_____
4	ALL	0.17 – 0.33	D + 21	x	x	29	x	x	x	_____
5	ALL	0.69 -1.23	_____	x	_____	17	x	_____	x	_____
6	MDS	0.2 – 0.37	D + 15	x	x	28	_____	x	x	_____
7	ALL	0.17 – 0.37	D + 25	x	x	40	x	x	_____	x
8	SCID	0.17 – 0.29	D + 22	x	x	29	_____	x	_____	_____
9	HLH	0.54 – 1.57	_____	x	_____	17	_____	x	x	_____
10	SCA/BT	0.17 – 0.31	_____	x	_____	24	x	x	x	x
11	LL	0.23 – 1.78	D + 22	x	x	27	x	x	x	x
MEAN		**0.28 – 0.67**				**27**				
12	ALL/MDS	0.17 - 0.91	D + 31	x	x	27	_____	x	x	_____
13	ALL	0.16 – 0.22	D + 22	x	x	25	x	x	x	_____
14	ALL	0,17 - 0.21	D + 17	x	x	24	x	x	x	_____
15	AG/NSI	0.17 - 2.00	D + 29	x	x	32	x	x	x	_____
16	ALL/AML	0.17 – 0.33	D + 18	x	x	25	_____	x	x	_____
17	ALL / MDS	0.21 – 0.56	D + 20	x	x	21	x	x	x	_____
18	HIES/AML	0.17 – 0.24	D+ 17	x	x	27	x	x	x	_____
19	AML	0.17 – 0.19	D + 14	x	x	15	_____	x	x	x
20	AML/ALL	0.23 – 0.34	D + 15	x	x	16	x	x	x	_____
21	HIgMI	0.27 – 0.48	D + 15	x	x	26	x	x	x	_____
22	MDS/AML	0.17 – 0.3	_____	x	x	38	_____	x	x	_____
MEAN		**0.19 – 0.53**				**25.09**				
MEAN	_____	0.23 – 0.60	_____			26.05	_____			

*OMZ = omeprazole, FCZ = fluconazole, MTZ = metronidazole, TMP-SMT = trimethoprim/sulfamethoxazole

Method for quantification of Cya in serum

The blood samples were collected from pediatric patients by the nursery service, in tube containing EDTA, and the immunoassay method was used for quantification of Cya levels in the whole blood of patients by using the Architect ci4100 system (Abbot®). The measurement range for drug's levels in this system is between 25 – 1500 ng/mL.

Evaluation of the agreement of the cyclosporine seric levels after intravenous intake

According to the HCPA's Allogenic Hematopoietic Stem Cell Transplantation Protocol, Cya administration in HSCT pediatric patients starts two days before transplantation (D-2) by continuous infusion of 24 h, with the first dose defined by body weight (2 mg/kg). This drug is usually combined with metotrexate and cyclosporine target for *steady-state* level is defined in the range of 100-400 ng/mL. The value of volume of distribution was assumed as 4 - 5 L/kg and the bioavailability was 0.30[8]. Based on the dose administered and levels of Cya determined in the patients (C_{ss}), the pharmacokinetics parameters were calculated by the equations showed in the Figure 2 and the pharmacokinetic parameters values estimated for each patient are shown in Table 3.

Figure 2: Strategies for Cya dose adjustment by intravenous dosing:

Protocol recommendations	**Dose adjustment by Hospital Pharmacy Service**
Based on the Cp_{ss} levels of Cya determined in the blood, if the values achieved were between: • 0-100 ng/mL increase the dose in 50%; • 400-500 ng/mL decrease the dose in 25%; • 500-600 ng/mL stop the administration up to 12 h and restart with 50% of the last dose.	$MD_{new} = \frac{Cpss_{desired}}{Cpss_{obtained}} MD_{adm}$ $CL = ke.Vd$ (eq. 1) $t_{1/2} = \frac{0.693}{ke}$ (eq. 2) Vd = 2.4 x weight (kg) (eq.3)

Results

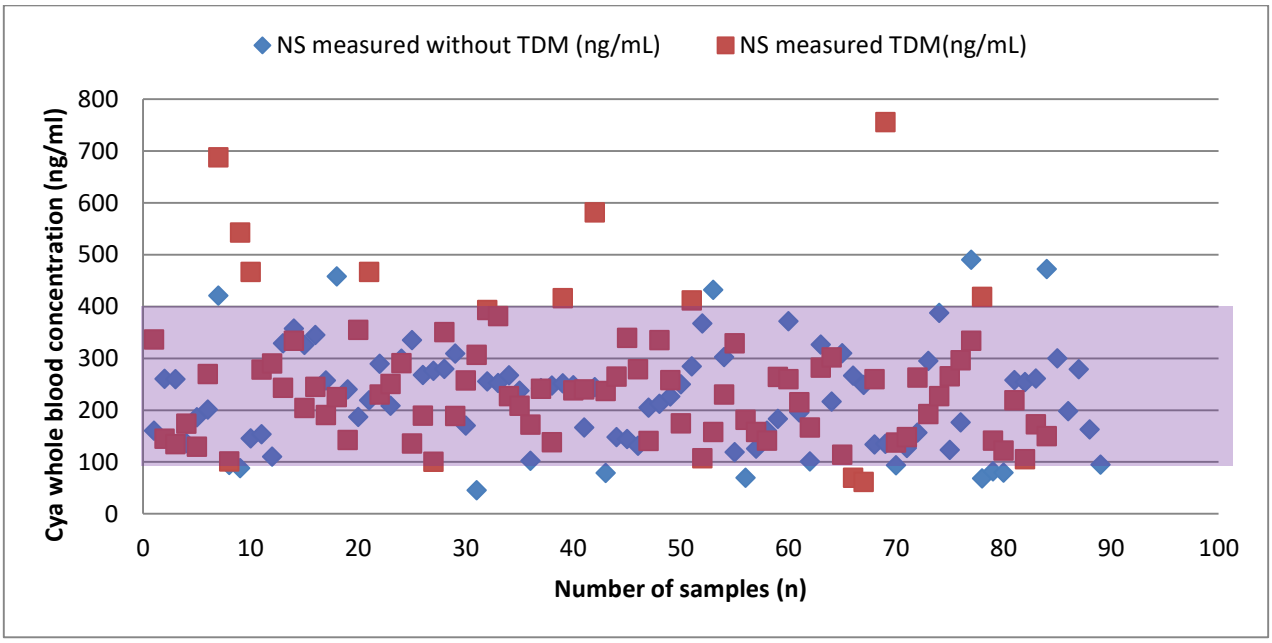
A total of 22 pediatric patients had their medical records relating to intravenous Cya prescription analyzed. All these patients started to receive Cya intravenously 2 days before HSCT, according to the protocol used in the hospital where the medical records were searched. In this population, eleven patients received this prophylactic treatment after January 2017 which means they had their Cya levels and symptoms also reviewed by the Clinical Pharmacy Service. Their results were compared to other 11 patients who did not have their Cya levels monitored by the Clinical Pharmacy Service and had the dose adjustment based on the Hospital protocol (Figure 2). The patients were submitted to Cya IV with 24 hours infusion in average during 26 days, which corresponds to 173 samples. For the patients who had the Cya levels monitored the average of days using Cya IV was 27 days and 84 samples. On the other hand the patients who did not have the CyA levels monitored by the Clinical Pharmacy Department used Cya IV during, in average, 25 days and 89 samples were collected. The indication for the treatment with Cya IV was the prophylaxis against GvHD that occurs frequently in patients who undergo HSCT. According to the hospital protocol Cya IV is the first choice as a pharmacological prophylaxis for this disease.

A total of 19 studied patients had their medical records accessed in relation to the treatment with oral cyclosporine. From these patients 8 of them received the treatment with oral Cya after January 2017, while 11 had this treatment before this period. The patients received the treatment with oral Cya when they had clinical conditions that favored this change in route of administration. All the patients selected for this study were taking intravenous Cya before oral Cya.

The averages for the values of some laboratory exams that might be affected due to the intake of CyA were also made. It was found that they were very similar between both of the groups and the references values. The mean of the potassium levels was 4.20 mEq/L for the monitored and 4.24 mEq/L for the non-monitored group, whereas the reference value is a range between 3.6 and 5.0 mEq/L. Magnesium mean value was 1.79 mg/dL for the monitored group and 1.77 mg/dL for the non-monitored patients, and the reference value is between 1.9 and 2.5 mg/dL. The hepatic function for the patients was also

verified based on the AST and ALT mean levels. AST and ALT means were equal to 36 U/L and 37.43 U/L, respectively, for the monitored group; while AST mean value was 39.55 U/L and ALT mean value was 53.22 U/L in the non-monitored group. The reference values for AST in men are between 17 – 59 U/L and for women between 14 – 36 U/L. The reference values for ALT for men are between 21- 72 U/L and for women 09 – 52 U/L. Total bilirubin was also verified because the levels of this compound might indicate hepatic GvHD. The mean of the bilirubin levels for the monitored group was equal to 0.56 mg/dL and 0.43 mg/dL for the non-monitored group, whereas the reference value is in a range between 0.3 – 1.0 mg/dL [17].

Figure 4: Cya whole blood concentrations (ng/ml) measurements in the samples with and without TDM (therapeutic drug monitoring based on pharmacokinetic analysis) during the whole treatment. Shaded area indicates the expected Cya concentrations (ng/ml).



The patients who received the treatment with intravenous Cya after January 2017 showed a mean value of 99. 42 ng/ml, while the patients who had their data collected before January 2017 presented a mean value equal to 127.27 ng/ml. These values should be between 100 – 400 ng/ml. Even though, according to the therapeutic window settled, 99.42 ng/ml is an adequate number, higher levels of CyA are expected in the period just after HSCT. In

Graph 1 it can be observed that 9 values from the 84 monitored group samples were above the maximum expected of 400ng/ml. Only 2 values of the same group were under the minimum expected value of 100ng/ml. Whereas for the non-monitored group there were 89 samples, 5 of these were above the expected number. On the other hand, 10 of these values were below 100 ng/mL.

In Table 3 it can be observed the estimated pharmacokinetic parameters such as clearance (CL), volume of distribution (Vd) and constant of elimination (ke) for each patient. For the patients who had their Cya levels monitored (1 – 11) the mean of the CL range is 8.17 – 23.66 L/h and the mean for the Vd range is 77.94 – 82.48 L. The average for the ke for these patients is 0.22 h^{-1}.These values were similar to the patients who did not have their CyA levels monitored (12 – 22) who presented the average of CL range of 7.26 – 26.84 L/h and a range of Vd equal to 49.39 – 51.79 L. The average for the ke parameter is equal to 0.26 h^{-1} for the non-monitored group. These pharmacokinetic parameters averages from both groups presented values according to the one showed in Table 4 [8].

Table 3: Pharmacokinetic parameters estimate in the studied patients.

Patient Code	CL range (L/h)	Vd (L) range	Ke mean (h^{-1})
1	14.95 – 24.34	106.12 – 106.47	0,17893
2	8.96 – 43.81	137.36- 141.57	0,16111
3	3.75 – 20.39	15.44 – 17.48	0,43947
4	3.04 – 14.36	57.80 – 60.84	0,14938
5	8.74 – 16.00	132.68 – 149.18	0,08613
6	13.41 – 25.12	74.88 – 78.39	0,23762
7	4.37 – 17.57	36.04 – 39.55	0,22152
8	2.68 – 24.78	23.40 – 26.21	0,33727
9	15.80 – 18.88	128.47	0,13496
10	6.19 – 21.65	57.92 – 60.84	0,23464
11	7.96 – 33.36	87.28 – 98.28	0,2565
Mean	**8.17 – 23.66**	**77.94 – 82.48**	**0.2216**
12	9.92 – 22.40	50.31 – 53.35	0.33667
13	8.16 – 29.63	52.65 – 54.05	0.26673
14	4.98 – 7.99	60.37 – 64.35	0.09703
15	5.79 – 26.44	36.97 – 38.61	0.36699
16	1.68 – 9.01	26.56 – 27.03	0.20562
17	10.22 – 32.97	73.48 – 76.05	0.20599
18	2.7 – 10.68	23.99 – 26.26	0.25414
19	9.74 – 21.93	44.23	0.35802
20	15.61 – 91.78	93.83 – 98.98	0.33531
21	9.65 – 26.55	56.86 – 60.84	0.22924
22	1.70 – 15.82	23.99 – 25.97	0.24254
Mean	**7.29 – 26.84**	**49.39 – 51.79**	**0.26348**
Overall mean	**7.71 – 25.25**	**63.67 – 67.14**	**0.24254**

Graph 2 shows us that most patients from both monitored and non-monitored groups have Cya seric levels between the expected therapeutic window (100 ng/ml – 400 ng/ml), however in the literature it was found that in the period just after HSCT these levels should be higher (>200 ng/ml), in order to prevent the GvHD event [18]. 46.15% patients from the non-monitored group presented Cya levels lower than 200 ng/ml until D+10, while 50% patients from the monitored group presented Cya levels under 200 ng/ml.

Figure 5: Cya whole blood concentrations (ng/ml) measurements in the samples with and without TDM until D+10. Shaded area indicates the expected Cya concentrations (ng/ml).

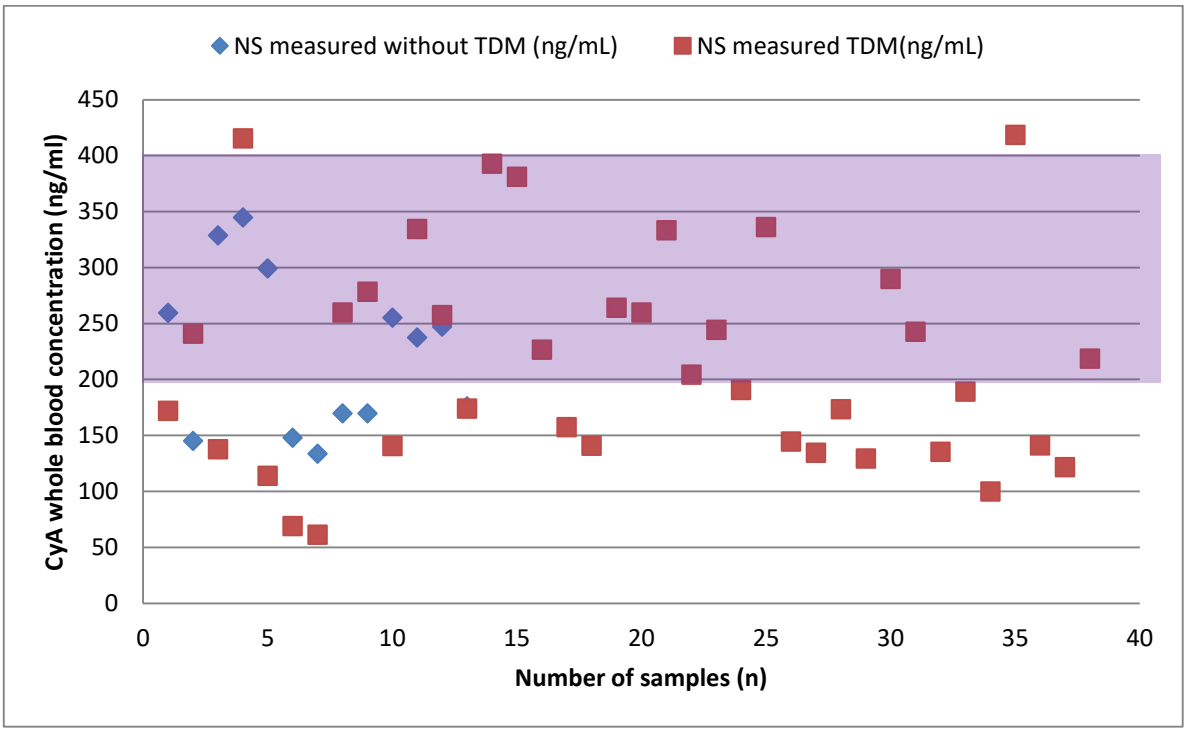


Table 4. Cyclosporine pharmacokinetic parameters estimated for the pediatric population [8].

Parameter	Value
Target concentration (ng/mL) EV Oral	 100-400 100-200
Oral bioavailability (%)	30
Volume of distribution (L/kg)	4 – 5
Clearance (ml/kg/min)	5 - 10
Free fraction (%)	10
Half-life (h)	6-12

Discussion

Even though Cya is the first choice drug in many hospitals to prevent GvHD, there is still a lack of certainty about how to better predict their serum levels after HSCT in children. It is already well known that trough levels do not represent the best approach to estimate the ideal dose for these patients, when taking oral Cya. In our study it was seen that both groups (monitored and non-monitored) presented *steady state* levels not in accordance with the target one. Moreover, it is important to relate that pediatric patients present some important differences in pharmacokinetic parameters in comparison to adults, which might as well make it more difficult to assure whether the right dose is been given. Lu *et al*[15] provide us with information related to the volume of distribution for children, which is, in general, higher than for adult patients, due to a reduced plasma protein binding. There are also differences in the patients who receive solid organs and hematopoietic stem cells. The stem cell recipients tend to have more damage in gastrointestinal tract than the solid organs recipients. This damage might affect the rate of absorption of drugs. Mucositis, a common inflammation in HSC recipients, might increase Cya exposure. As stated in Table 1 there is a high use frequency of some drugs in patients submitted to HSCT. In our sample these drugs were omeprazole, fluconazole, metronidazole and trimethoprim/sulfamethoxazole. Fluconazole, for example, is able to inhibit

the enzyme CYP 3A4, leading to high Cya concentrations in the patient [8], while trimethoprim/sulfamethoxazole might cause renal damage, thus decreasing Cya effects[19].

In our study we observed in Graph 1 that both monitored and non-monitored patients by pharmacokinetic equations did not have differences in number of serum levels between the accepted therapeutic window. However it was clear that, in the monitored group, most of the levels found not to be in accordance, were above the expected, while in the non monitored group the not correct levels were in many cases below the accepted one. This could be related to a better awareness from the pharmaceutical and medical professionals to not let Cya levels stay below 100 ng/ml, as this might hugely increase the probability of developing GvHD. On the other hand, due to Cya pharmacokinetic characteristics and HSC pediatric recipients conditions, higher levels of this drug might induce a very compromising toxicological process. In Graph 2 we can observe that TDM was not able to let Cya serum levels just after HSCT above 200 ng/ml, as around half of the patients from both groups presented Cya levels under this target. Higher CyA levels in a short period after HSCT are known in the literature to hugely increase the protective effect against GvHD in patients [18]. As presented in Table 3 the pharmacokinetic parameters from both groups seem to be adequate in relation to the reference values for this drug [8].

In order to improve the drug monitoring service conceived by the Clinical Pharmacy Department we suggest a pharmacokinetic population modeling to be implemented in the HSCT service in HCPA. Even though there could be some limitations to implement this approach, such as many levels measurements, this strategy might provide accurate results. Costs with staff and number of hours spent to provide a result that would cause an impact on the dosing process for these patients are very relevant and necessary in a huge hospital reality such as HCPA. A pharmacokinetic model is, thereby, able to provide an understanding in how the drug exposure is and what consequences might be present after its administration in relation to dosing, route and patient's conditions. In a population model we will evaluate how the drug works for each subject and then the parameters measured will be compared between the studied individuals and subjects from other studies, but with similar clinical and

pharmacological conditions [20]. Willemze *et al* [9] showed that determination of AUC (area under the curve) with a two point limited sampling strategy might be an adequate model to preview the cyclosporine exposure in a similar population. For example, with the time-points sampling 0,1,3 or 0,2,3 the r^2 in both cases was equal to 0.99, which demonstrates that with only 3 samples from each patient it is possible to know the accurate Cya exposure. We also suggest that a prospective study should be made, in order to better analyze the following procedures taken by the prescriber after a level out of the therapeutic window.

Conclusions

Our project found some limitations in relation to the number of patients and the accuracy associated to a retrospective study. There are certainly many possible aspects in our patients that might interfere in Cya levels. A review about the initial Cya dose in HSCT patients could be made, as most of the patients were not able to achieve an adequate level for the short period after HSCT. A prospective study should be done in order to provide the necessary information to verify if the suggested dosages based on the pharmacokinetic equations by the Clinical Pharmacy Service were accepted by the prescriber.

Further strategies based on population pharmacokinetic modeling studies could also be implemented for a more ascertained dosing approach. Other drugs that could interfere in cyclosporine levels should also be described, as the studied population need to deal with a large variety of drugs, most of them affecting Cya levels. The dosage is primarily adjusted based on the patient's weight, which might be the best choice. A better control over the sampling process during the drug monitoring step and the engagement of all staff in the process could aid to overcome the failures associated to the level results of Cya in these patients. Furthermore, it is important to relate more drugs hugely used in HSCT patients, in order to analyze how they might interfere with cyclosporine levels.

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