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Population pharmacokinetics of Cyclosporine in Egyptian hematopoietic stem cell transplant recipients: Dose individualization approach.

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Abstract:
Aim*: The objective of the study is to develop a population pharmacokinetic model for the Egyptian allogenic hematopoietic stem cell transplant (allo- HSCT) patients that can be practically applied to adjust the dose of cyclosporine (CsA) on individual basis and provide guidelines for dose adjustment in patients with special pharmacokinetic profiles, whose dose adjustment is extremely difficult and commonly found in the acute post-transplantation period.*
Patients and methods: *A total of 295 patients undergone HSCT, of whom 207 were an index group to develop the model and 88 were the test population for external validation. Patients were treated with CsA given twice daily by intermittent intravenous infusions over 2 hours till oral administration is possible then shifted to oral route. Patients were routinely monitored for CsA steady state trough concentration obtained just before initiating the infusion three times weekly. Pharmacokinetic parameter estimation was performed using nonlinear mixed effect modelling implemented in the Monolix® software* (version 3.3.3, Lixoft, France). *A one compartment model with first order disposition model was used as a structural model. The influence of demographic variables, medication coadministration and pathological conditions on the individual estimates of clearance and apparent volume of distribution was tested. The final model was internally validated using Uppsala prediction corrected visual predictive check (PcVPC) & cross validation and results of analysis from the test population was compared to those of the index group for seek of external validation of the model.*
Results: *A total of 1452 CsA steady state trough concentration were analyzed for purpose of model development, Clearance (CL) was 23 L hr^{-1} (relative standard error [RSE] ±29%) with an inter-individual variability (ω^2) of 39.2%. The apparent volume of distribution (V) was 917 L (RSE ± 8%) with an inter-individual variability (ω^2) of 55%. The covariate analysis identified age, renal status and albumin status as individual factors influencing the CL of CsA. The population model was validated by internal and external approaches, and was demonstrated to be effective and stable.*
Conclusion: *A population Pharmacokinetic model was proposed to estimate the individual cyclosporine clearance for Egyptian allo-HSCT patients. The derived final regression model reasonably predicts the clearance values and hence it can be utilized to individualize CsA doses for prompt and adequate achievement of target blood concentrations of CsA.*

Keywords – *Cyclosporine, Dose individualization, HSCT, Population pharmacokinetics.*

I. INTRODUCTION

Allogenic hematopoietic stem cell transplantation (allo-HSCT) is a widely used modality for treatment of many malignant & non-malignant disorders [1][2]. One of the potential complications of this approach is the alloreactive effect of the grafted stem cells known as graft-versus host disease (GVHD) [3]. Immunosuppressive strategies, usually based on Cyclosporine A(CsA) alone or in combination is commonly used in allo-HSCT patients to prevent the immunologic complications of GVHD [4]–[6]. However, the clinical use of CsA is highly challenging due to the large interindividual variability of the pharmacokinetic behavior together with narrow therapeutic window [7], making dose adjustment is critically important. Several studies evaluated the relationship between serum CsA Concentrations and toxicity or GVHD. Majority of these studies found that high serum CsA levels strongly correlates with toxicity including neurotoxicity, cardiovascular and nephrotoxicity, while lower levels associated with increased risk of GVHD and loss of stem cell graft [8]. This challenges makes the individualization of CsA doses based on patient's specific individual pharmacokinetic (PK) parameters significantly important for prediction of CsA exposure [9]. Therapeutic drug monitoring of CsA is well established in clinical practice in settings of renal, liver, cardiac, lung and bone marrow transplantation, however the process of achieving , maintaining or modification of specific target concentration still a hard task. CsA is highly protein bound, primarily to RBCs, lipoproteins and albumin, metabolized by

CYP450 3A4 in the liver to active and inactive metabolites and primarily eliminated through biliary excretion [10]. Pharmacokinetics of CsA is highly complex and affected by many factors like: demographics, concurrent medications and type of primary disease for which transplantation was indicated. Moreover, complexity of CsA pharmacokinetics is obviously increasing in critically ill patients in the acute post-transplantation phase due to presence of additional several clinical factors simultaneously affecting the in-vivo behavior of CsA [11]. This made a very little research investigating the pharmacokinetic behavior of CsA in acute post-transplantation period. Few models were developed to predict CsA exposure, most of them built using very small number of patients who were on stabilized CsA therapy usually in outpatient settings [7][11][13], and therefore their application is not suitable to be extrapolated to the acute post-transplantation phase.

The goal of the study is to predict the individual exposures of CsA through development of population pharmacokinetic model using only steady state trough concentrations that provides a basis for personalized dose adjustment using patient's specific factors, and providing ways of appropriate dosing of CsA in special subpopulations usually encountered in the acute phase post-HSCT like acute renal failure (ARF) and hypoalbuminemia.

II. PATIENTS AND METHODS

1. Patients:

Patients were recruited from those with related or unrelated allogeneic stem cell transplantation in the bone marrow transplantation unit of Nasser Institute for Research and Treatment at Shoubra; Cairo, Egypt . Informed consent was taken from the patients prior the enrollment in the study. Patients were selected from those who were admitted to the bone marrow transplantation unit of the hospital from June 2013 to January 2016. The study was approved by the research and ethics committee of the faculty of pharmacy, Helwan University and the Institutional Review Board (IRB) of the local ethics committee of the Nasser Institute which followed the tents of declaration of Helsinki.

Patients were enrolled in the study when CsA therapy alone or in combination was indicated for GVHD prophylaxis. Patients included in the study were aged 2–65 years, clinically stable after a first HSCT. The primary disease types included were: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), biphenotypic leukemia (BPL), myelodysplastic syndrome (MDS), thalassemia, severe aplastic anemia (SAA), Fanconi anemia (FA), combined immunodeficiency syndrome (CIDS). Patients were excluded from the study if they: refused to sign the informed consent, had inaccurate times of sampling or dose administration, developed earlier GVHD or microangiopathy, had a history of hepatic or renal failure, had severe psychiatric conditions, pregnant or breastfeeding women .

2. Methods

The study was a prospective cohort study, patients were splitted into 2 groups: first group is the index group for purpose of model development and the second group is a validation group for purpose of external validation of the final model. Informed consent was taken from the patients prior the enrollment in the study.

2.1. Treatment protocol and drug administration:

Based on hospital protocol, all patients received cyclosporine A CsA (Sandimmune®, IV, 10mg/ml, Novartis, Rotterdam, the Netherlands) at a dose of 3 mg/kg/day as IV intermittent infusion over 2 hours every 12 hours from the day before transplantation (day −1) until the oral intake was possible then shifted to oral form (Sandimmune®, oral solution, 2mg/ml, Novartis, Rotterdam, the Netherlands) at a dose of 5 mg/kg/day divided as two daily doses and maintained till day 180 then gradually tapered off, the dose was adjusted in order to maintain a trough goal of 150–300 ng/dl. Dose adjustments were made empirically based on TDM; If CSA level exceeded the target range, the dose is reduced by 20% and if the level dropped less than the target level, the dose was increased by 20%. Methotrexate (MTX) was given at a dose of 15 mg/m2 IV on day +1, then 10 mg/m2 on days +3, +6, and +11. For FA patients, MTX was replaced by anti-thymocyte globulin (ATG).

2.2. Blood sampling and measurements of cyclosporine concentrations:

Whole blood sample of 1 ml was drawn into vacutainer tubes containing EDTA just before initiation of the IV infusion to obtain the trough levels. Only concentrations obtained 4 days following start of CsA therapy were used in population pharmacokinetic analyses to ensure attainment of the steady state. Steady state was assumed to be reached in 5 half-lives after regular dosing is started (median half-life = 4.43 hours) [13], only concentrations obtained after 4 days from the start of CsA administration were used in the analysis. Samples were stored at 2-8 °C until analysis. CsA in whole blood was assayed using Emit ® 2000 cyclosporine specific immunoassay supplied by Siemens Healthcare Diagnostics Inc., SYVA®, USA.

The lower limit of quantification of the assay is 40 ng/ml. This level represents the lowest concentration that can be distinguished from 0 ng/ml with a confidence level of 95%. Between 40 and 500 ng/mL, the precision coefficient of variation ranged from 3.8% to 11.8%. Concentrations higher than 500ng/ml were diluted, then the results of assay were multiplied by the dilution factor. Concentration points that were

below lower limit of quantitation (BLLQ) were treated as a left censored data by adding a column "CENS" to the original data set and coding BLQ data with one, zero otherwise. Monolix® proposes to sample the BLQ data from the conditional distribution of the concentrations, then applies the method of maximum likelihood to impute for BLQ data points with higher accuracy.

2.3. Structural model development:

Nonlinear mixed effects modeling using stochastic approximation with expectation maximization (SAEM) algorithm implemented in Monolix® (version 3.3.3, Lixoft, France) was used for population pharmacokinetic analysis. A one compartment model with first order elimination was hypothesized to describe CSA pharmacokinetic behavior. To avoid estimation of negative values of CL & V; these parameters were assumed to be log normally distributed and exponential model was used to describe interindividual variability. Equations 1, 2 were used to describe the structural model [14].

$$(1) CLij = TVCL.e^{\eta_i CL}$$

$$(2) Vij = TVV.e^{\eta_i V}$$

Where: TVCL, TVV are the typical population mean values for CL, V respectively. Subject no. denoted with i and observation no. denoted with j. η_i,CL, η_i,V are random variables that distinguish the i^{th} individual's parameter from the population mean as predicted by the regression model and are assumed to be independent and normally distributed with zero mean and variance ω^2. The magnitude of interpatient variability in the structural parameters was expressed as ω^2.

2.4. Choice between competing models:

A modified form of the traditional forward inclusion-backward elimination was used to compare between candidate models and submodels. Instead of inclusion or exclusion of a model based on change in OFV only which usually results in inclusion of covariates that may potentially deteriorate the precision of final parameter estimates or model diagnostics; we developed through this study a novel comprehensive way of evaluating the model based on OFV, change of the conditioning no. of the correlation matrices of fixed & random effects, reduction of interindividual variability, extent of η shrinkage & improvement of model visual diagnostics.

On the basis of this model discrimination criteria we have developed a novel scoring system providing adequate metric measure for different competing models during each step in full pharmacometric model development to allow the comparison between the models in quantitative terms, and hence facilitate the selection of the best model. The scoring system was developed by giving more weight (higher scores) to the most influential model assessment metrics. Table (1) summarize the scoring system from model discrimination criteria. Each model will be evaluated numerically by calculating its discrimination score, then models with higher scores will be chosen over those with lower scores.

Table (1): Model discrimination scoring system for choice of pharmacometric model describing CSA exposure

Model discrimination criteria	
Pharmacometric parameter	**Score**
Δ -2LL (OFV)	2
P-value*	2
RSE reduction of the parameters of fixed and random effects.	2
Conditioning No. of Fixed effects**	1
Conditioning No. of random effects**	1
η Shrinkage reduction	1
Improvement of visual model diagnostics	1

* P-value of the competing models will be calculated based on Wald test or log likelihood test, models with p-value less than 0.05 will be given a score of 1.

**Score of 1 is always given for the models whenever the absolute value is less than 1000.

LL: log Likelihood, **OFV:** objective function value, **RSE:** relative standard error.

2.5. Covariate model development:

The effects of the covariates including: age, gender, weight, post-transplant day, bilirubin status, renal status, albumin status, stem cell dose, serum creatinine and coadministration of phenytoin and/or methotrexate

were modeled to test the effects of this covariates on precision of estimated parameters of fixed and random effects, reduction of the interindividual variability, η shrinkage and over all goodness of fit.

Some continuous covariates may not follow a strictly linear relationship and exhibiting a complicated nonlinear function with multiple parameters, making its introduction into covariate model is not feasible. Transformation into dichotomous categorical covariates was employed through the division of continuous variable into two groups and model each group having its own multiplier [11].

Covariates like renal functions were dichotomized into acute renal failure (ARF) group if serum creatinine (≥2 mg/dl) or Non-ARF otherwise, albumin status into hypoalbuminemic if albumin < 3 mg/dl or normoalbuminemic otherwise, and finally hyperbilirubinemic if total bilirubin ≥ 2 mg/dl and Non-hyperbilirubinemic otherwise during the course of study.

The inclusion or exclusion of different covariates will be based on its model discrimination score with covariates with higher scores will be pushed up in the selection process in favor of those with lower scores. The addition of covariates will be in a stepwise way, till deterioration of model diagnostics which is considered an end point for further covariate addition.

2.6. Model validation:

Internal validation of the final model was double checked using Uppsala prediction corrected visual predictive checks (PcVPCs) and cross validation. PcVPC is a graphic generated from 1000 simulated data set developed from our original dataset design using Monte-Carlo simulations, differs from traditional VPCs in that the dependent variable has been subject to statistical prediction correction before the statistics are calculated. Comparing the 5^{th} percentile, median and 95^{th} percentile of the simulated data to those of the observations will give a clear picture about the quality of the predictive performance. Monolix® graphically displays the outlier area as red colored area of the PcVPC, The wider outlier area usually indicates model misspecification or poor predictive performance.

To ensure adequacy of internal validation a second additional method was applied, cross validation, where 10 % of the data set was randomly eliminated and the population parameters are re-estimated, standard errors and relative standard errors (RSE) and the values of parameter estimates and their corresponding precision were compared to those of the final model. Stable models generate parameter estimates that are close in terms of value and precision to those of the final developed model.

An external validation was applied to evaluate the model using a test group having similar characteristics to the index group, receiving the same formulation of CsA. The mean population parameters with their corresponding standard errors were used to calculate the p-value from a two-tailed unpaired t-test to assess the statistical significance of the difference between the parameter estimates from index and test group. External validation of a model is accepted when there is a statistically non-significant difference between the parameter estimates of index group & validation group (p-Value less than 0.05).

III. RESULTS

3.1 Baseline Characteristics:

Two hundred seven patients were studied as an index group for purpose of model building. Based on the indication for HSCT, they we classified as: ALL 13 (6.3%), AML 59 (28.5%), BPL 7 (3.4%), CML 10 (4.8%), MDS 14 (6.8%), SAA 48 (23.2%), thalassemia 17 (8.2%) and miscellaneous group including all other indications 39 (18.8%). Table (2) summarizes the main demographic and clinical data of index group.

Table (2): Demographics and clinical features of the index group.

Characteristic	**Population study (n=207) (Mean ± S.D)**	**Range**
Gender (Male/Female), %	140/67, 67.63% / 32.37%	-
ABW (kg)	50.123 ± 30.96	7-148
Age (years)	20.32 ± 14.869	3-59
SCD (10^6 cell/kg)	9.6 ± 6.51	1-45
PTDs (days)	24.76 ± 8.01	4-64
CsA data		
CsA dose (mg/day)	70 ± 42.68	3 – 240
CsA trough concentrations (ng/mL)	190 ± 119	4-1500
No. of observations	1452	-
Average observations per individual	4.9	-
Disease type (indication for HSCT) no.of cases,%		
ALL	13 (6.3%)	
AML	59 (28.5%)	

BPL	7 (3.4%)
CML	10 (4.8%)
MDS	14 (6.8%)
SAA	48 (23.2%)
Thalassemia	17 (8.2%)
Miscellaneous group	39 (18.8%)
Medication coadministration (no.of cases),%	
No. of patients administered phenytoin (%)	104 (50.2%)
No. of patients administered MTX (%)	152 (73.4%)

ABW: Actual body weight, **ALL:** Acute lymphoblastic leukemia, **AML:** Acute myeloid leukemia, **BP:** Biphenotypic leukemia, **HSCT:** Hematopoietic stem cell transplantation, **MTX:** Methotrexate, **MDS:** Myelodysplastic syndrome, **PTDs:** Post-transplant days, **SAA:** Severe aplastic anemia, **SCD:** Stem cell dose, **SD:** Standard deviation.

3.2. Population pharmacokinetic modelling:

1452 steady state trough concentrations collected from the studied population were used to build the pharmacokinetic model, concentration-time plots for the whole data range are displayed in fig.1. A one compartment model with first order elimination provides the best fit for our data points. The model was parameterized in terms of clearance (CL) & volume of distribution (V). Constant additive error model was used to describe the residual unexplained variability.

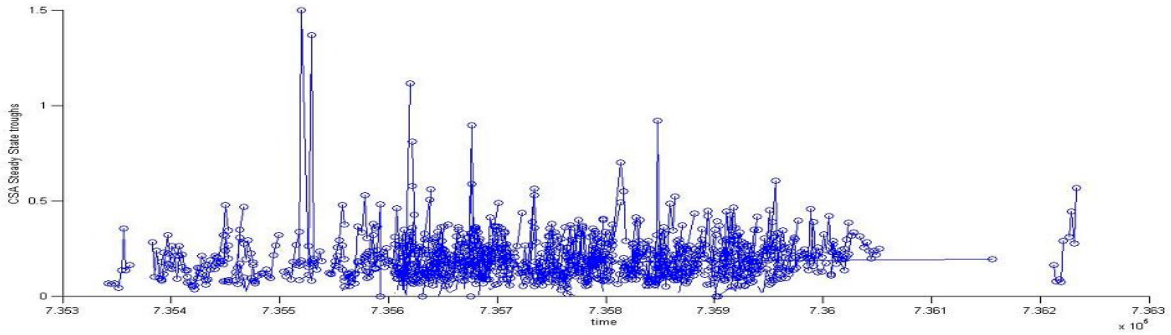

Figure (1): Spaghetti plot of CsA steady state concentrations (mg/L) vs time (hr) of the pooled data points segmented by individuals. Time axis (hours) scaled according to the internal scaling system of Monolix®.

Covariates were screened individually and was evaluated using the model discrimination scores, covariates whose p-value developed from log likelihood ratio test (LRT) & Wald test more than 0.05 were given a score of zero and they were permanently excluded from the final regression equation. Only statistically significant covariates were pushed up for covariate model building and was ordered in terms of their separate model discrimination scores. Table (3) demonstrates the significant covariates ordered according to model discrimination score.

Table (3): Descending order of the studied covariates according their corresponding model discrimination score:

Covariate studied	Model discrimination score
Renal status	8
Albumin status	8
Age	8
Phenytoin administration	7
Weight	7
Disease Type	7
PTD	5
Bilirubin status	0
SCD	0
MTX	0
Gender	0

MTX: Methotrexate, **PTD:** Post-transplant day, **SCD:** Stem cell dose.

Only renal status, albumin status & age were included in the final covariate model (as they poccess the highest scores) and it was found that the addition of further covariates may result in significant worsening of model diagnostics, increase in the conditioning number more than 1000, distortion of parameters distribution curve and decrease of parameters precision. The final regression equation for prediction of CsA CL (L/hr) was:

$$(3) Cl = 23 * Age(years)^{0.0237} * e^{0.738 * renal\ status - 0.718 * albumin\ status}$$

Renal status will be given a value of 0 if S.cr for patient is less than 2 mg/dl, value of 1 if S.cr for patient is more than 2 mg/dl, also albumin status will be given a value of 0 if albumin is more than 3 g/dl and value of 1 if albumin level drops to less than 3 g/dl during the post-transplantation period.

3.3. Model Evaluation:

Despite of the substantial variability in the CsA trough concentrations profile from one patient to another, fig.2 demonstrates that individual fits from the final model have a very good fit for different patients' profiles. Goodness of fit was further evaluated using graphics of Observed concentrations vs PRED & IPRED (fig.3) and the scatter plots of weighed residuals versus time and predicted concentrations shown in fig.4. The plots of observed vs population & individual predicted concentrations demonstrates no pattern of bias except in levels more than 500 ng/ml where there is an obvious bias in the plots, however this bias didn't seriously affect the overall quality of our model; as the distributions of individual weighed residuals (IWRES) & normalized distribution prediction error (NDPE) for the final model were nicely normal and almost centered around zero indicating overall goodness of fit (fig.5). This was also obvious from Quantile –Quantile (Q-Q) plots of IWRES & NDPE shown in Fig.6, which demonstrate acceptable linearity.

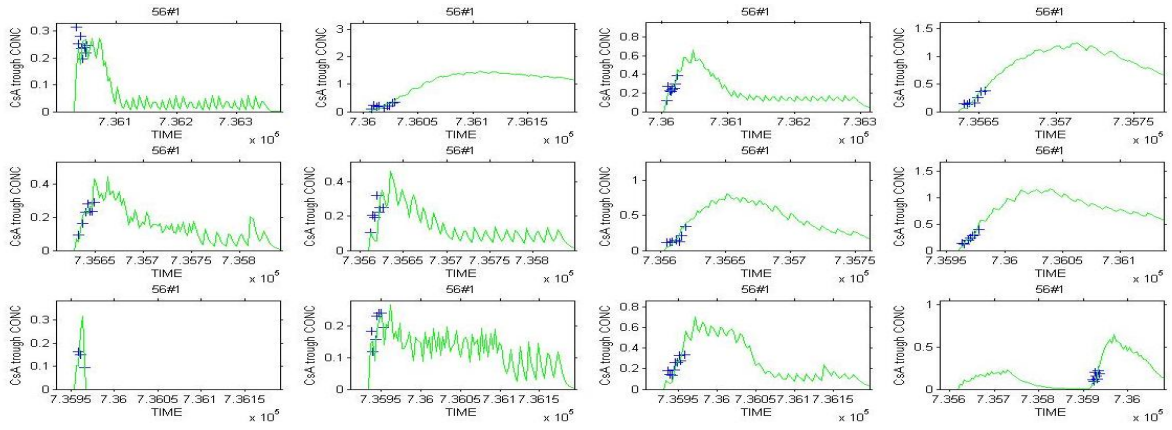

Figure (2): Examples of individual fits of the final model. Concentration (mg/L) plotted versus time (hours) .Green curves are the real observed CsA trough concentrations & blue crosses are the model predicted concentration.

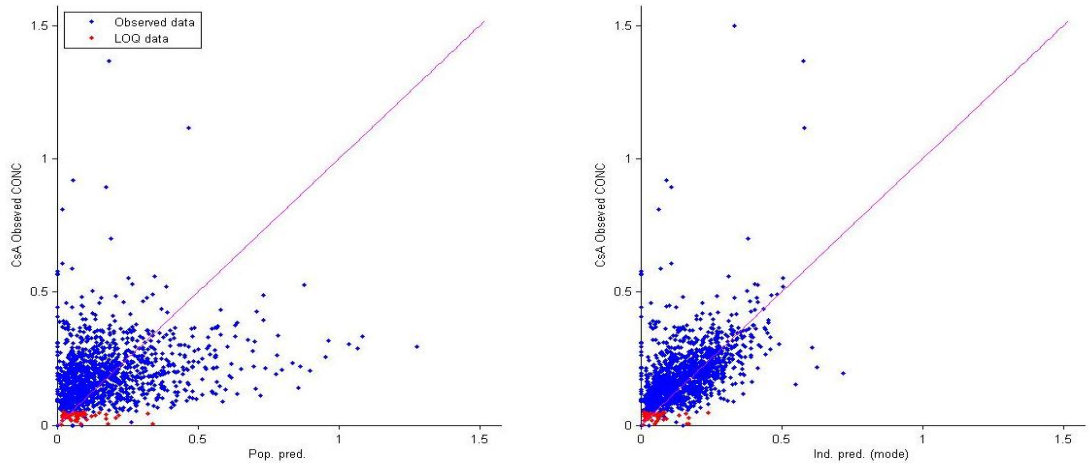

Figure (3): Plots of observed CsA concentrations (mg/L) versus population predicted (left) and individual predicted concentrations (right). Concentrations lower than the limit of quantitation are red-colored.

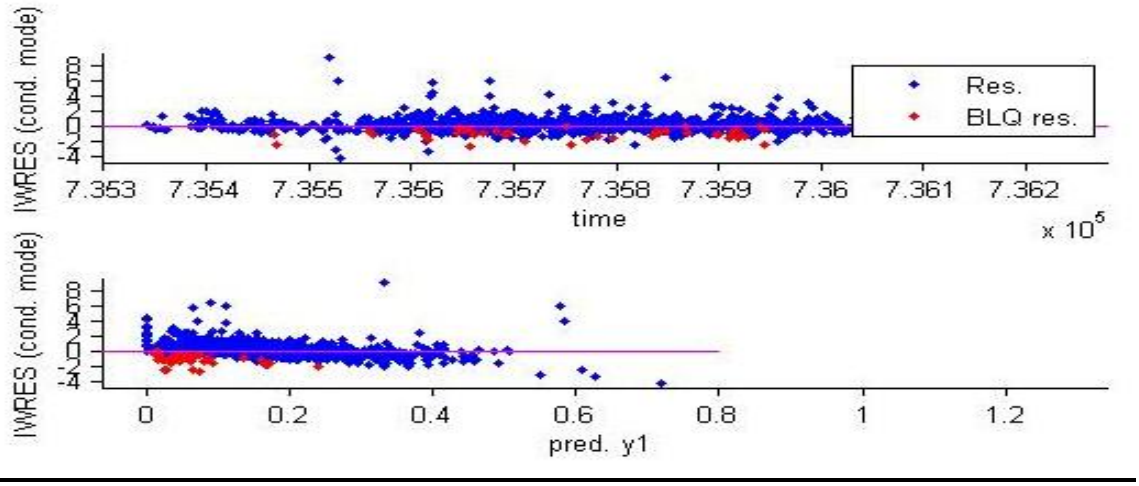

Figure (4): Plots of IWRES vs time (hr) & predicted concentrations (mg/L) from the final model

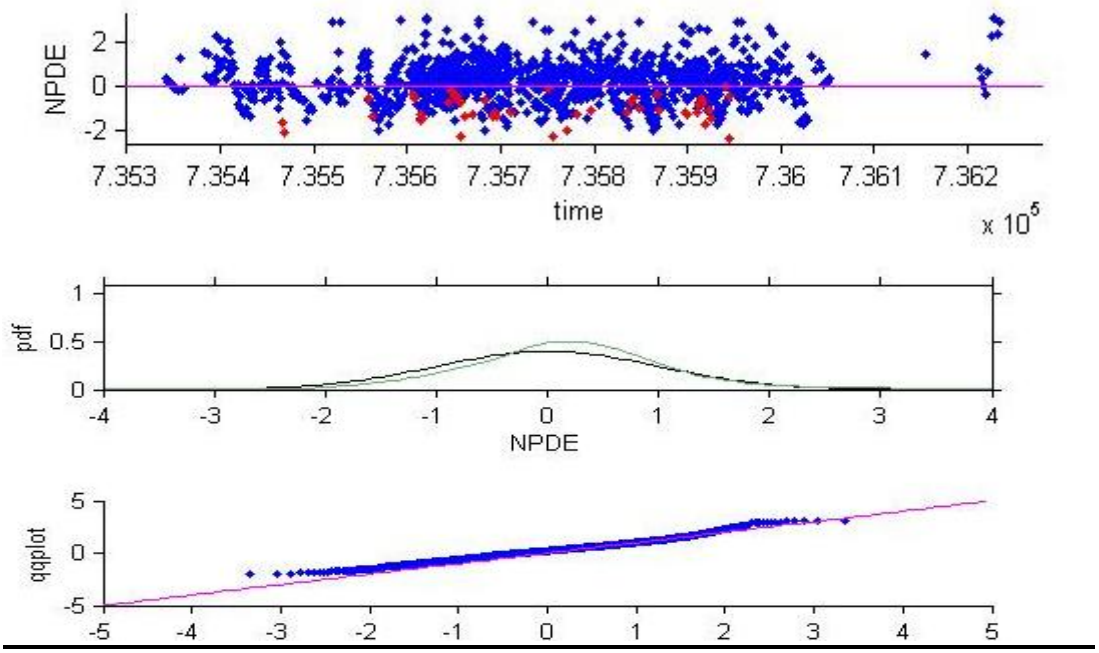

Figure (5): NDPE analysis of the final model including: Plots of NDPE Vs time, NDPE probability density function (PDF) & Q-Q plot respectively.

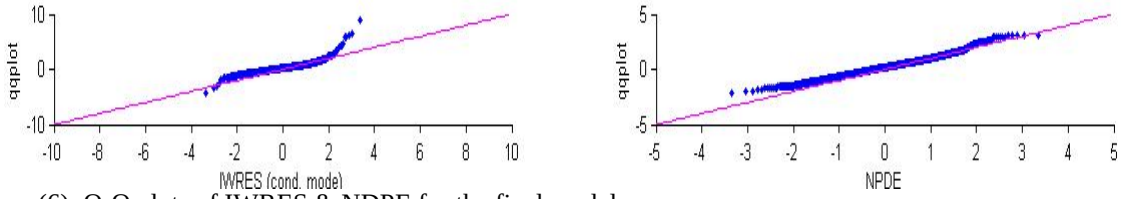

Figure (6): Q-Q plots of IWRES & NDPE for the final model

Parameters distributions (fig.7) display the typical distribution of the estimated population distribution of individual parameters. Estimated CL and V are typically normally distributed around their estimated population value. η Shrinkage was equal to 10 % and 8% for estimates of CL &V respectively. Compared to the base model, η shrinkage of the CL was further reduced by 5 % and this is a good sign for the proper characterization of the final model to the interindividual variations in the studied patients.

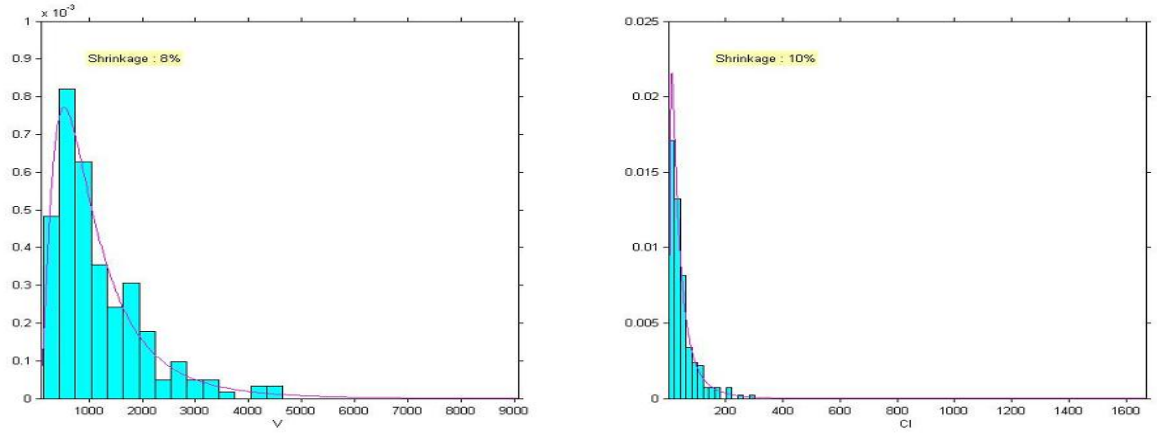

Figure (7): Histograms of parameter distribution of V & CL with their corresponding shrinkages. Estimates of V and CL were plotted versus their corresponding probability density function.

All fixed and random effects parameters converges surely (i.e. with probability of 1) after less than 200 iterations of stochastic approximation with expectation maximization (SAEM) search algorithm indicating good convergence. Different initial estimates of Cl & V were tried to assess appropriateness of convergence yielded the same value and properties of the initial convergence indicating absence of misconvergence due to local minima, finally the convergence assessment of 5 consecutive replicates of convergence assessment shown in fig.8 indicates ideal convergence to a very close values of estimated parameters. Patterns of overlaid convergence assessment demonstrates nearly superimposed graphics for each estimated parameter indicating a good convergence characteristics of the estimation process.

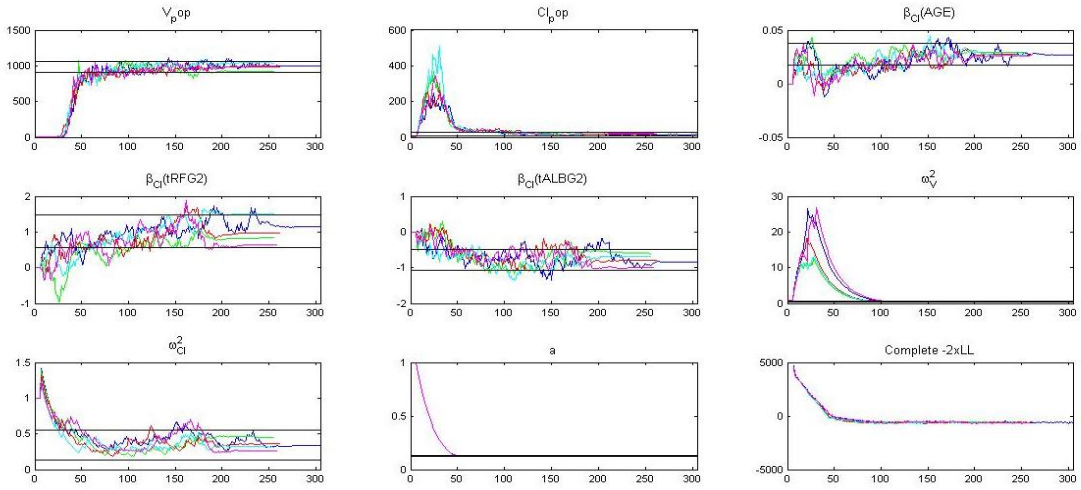

Figure (8): Overlaid convergence assessment of final model parameter estimates

3.4. Final Model parameter estimates:

The results of the structural model & the final model are demonstrated in table (4). Interindividual variability of the clearance (ω^2 CL) was significantly reduced from 139% in the base model to 39.2% in the final model, also η shrinkage reduced by 5 % compared to the base model. Parameter estimates of both fixed effects and random effects were estimated with acceptable precision. The relative standard error (RSE) for the final model estimates didn't exceed 30% of fixed effects parameters & 50% of random effects parameters, which denotes the estimation of model parameters with good precision. Unexplained residual variability in the final model was 12.9%

Table (4): Pharmacokinetic parameters for the measured PK profiles of base model, final model &internal validation

	Base model			Final model		
Parameter	**Estimate**	**S.E ***	**RSE (%)**	**Estimate**	**S.E**	**RSE (%)**
V (L)	1003	76	8%	917	74	8%
Cl (L/hr)	20.2	5.6	28%	23	6.6	29%

β -Cl-Age	-	-	-	0.0237	0.008	32%
β-Cl-Renal status	-	-	-	0.738	0.19	26%
β-Cl-Albumin status	-	-	-	-0.718	0.21	29%
ω^2_V (%)	53.6%	7.6%	14%	55%	8.1%	15%
ω^2_Cl (%)	139%	57%	41%	39.2%	19%	49%
ε (Residual variability)	0.129	0.0028	2%%	0.129	0.0028	2%
η Shrinkage	15%	-	-	10%	-	-

ARF: Acute renal failure**, β:** correlation coefficient, **RSE:** Relative standard error, **ω^2:** interindividual variance.
*Standard errors determined using linearization method implemented in Monolix®.

3.5. Model internal Validation:

PcVPC developed by Monte-Carlo simulation of 1000 data sets shown in fig.9 developed from our dataset, shows a very good prediction of concentrations up to 500 ng/ml with less efficient prediction at higher concentration, this assures the quality of the predictive performance of our final model throughout the therapeutic range, and on other hand support our previously stated limitation about the possible deviance of the predictions from the developed model at CsA concentrations more than 500 ng/ml.

It is also obvious that the median line of empirical distribution which corresponds to 50% prediction interval (P.I 50%) of the observed data points is typically superimposed over the median line of the theoretical distribution which is an ideal condition of predictive performance. To confirm the internal validation, a cross validation was performed and results presented in table (5)

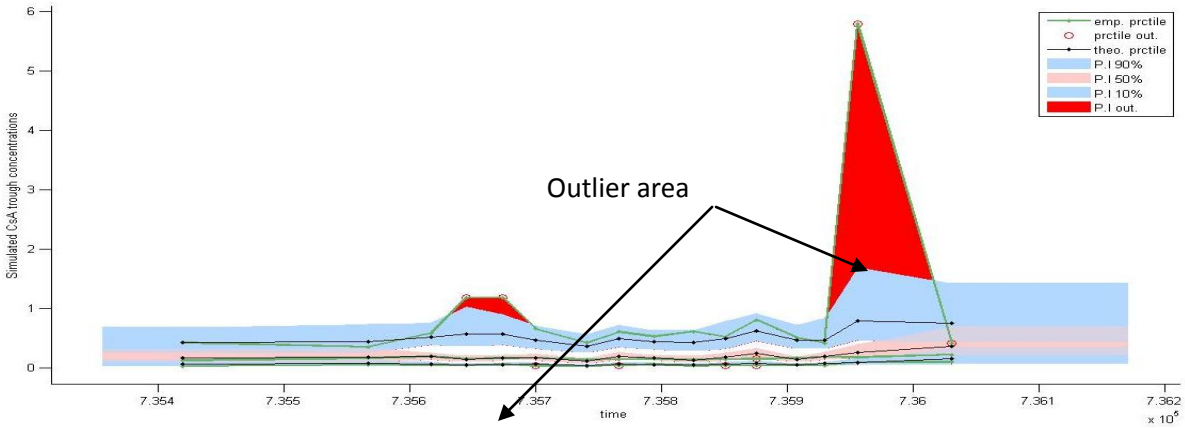

Figure (9): PcVPC of 1000 simulated data sets from the original data set.

Table (5): Results of cross validation including estimates from the index group and the validation group.

Parameter estimate	Parameter estimate from index group (%RSE)	ω^2 of PK parameters of Validation group (%)	CV estimate of the validation group (%RSE)
Cl (pop)	23(38%)	19.1%	20.1(35%)
V (pop)	917(8%)	57%	990(8%)
β -clearance-Age	0.027(32%)	-	0.025 (34%)
β -clearance-ARF	0.738(41%)	-	0.781(40%)
β-clearance-hypoalbuminemia	-0.718(36%)	-	-0.775 (31%)
ε (Residual variability)	0.129(2%)	-	0.129(2%)

ARF: Acute renal failure**, CV:** Cross Validation, **RSE:** Relative standard error, **ω^2:** interindividual variance.

3.6. Model external validation:

A distinct group of patients of similar criteria of the index group were used to evaluate the generalizability of the final model. The validation group comprised of 88 patient of whom 61 (69.32%) were males and 27 (30.68%) were females. Table (6) summarizes the characteristics of validation group.

Comparison was made between parameter estimates of index group and validation group with corresponding p-values estimated by unpaired two tailed t-test, p-values for all pairs of comparisons were > 0.05 indicating that there is no statistically significant differences between estimates of the index group and estimates of the validation group (p-values were 0.88 ,0.46,0.945,0.62,0.56 for estimates of CL, V, β-Cl-Age, β-Cl-ARF, β-Cl-Hypoalbuminemia respectively), so that our model is considered as externally valid, results of the external validation are summarized in table (7).

Table (6): Demographic characteristics of studied patients in validation group:

Characteristic	**Population study (n=88)** Mean ± S.D.	**Range**
Gender (Male/Female), %	61/27, 69.32% / 30.68%	-
ABW (kg)	53.29 ± 30.02	13-114
Age (years)	20.4 ± 14.25	5-56
SCD (10^6 cell/kg)	8.35 ± 4.6	2.7-33
PTDs (days)	25.8 ± 9.31	6-71
Disease type (indication for HSCT) [n,%]		
ALL	8 (9%)	
AML	11 (12.5%)	
BPL	5 (5.7%)	
CML	4 (4.6 %)	
MDS	9 (10.2%)	
SAA	12 (13.6%)	
Thalassemia	10 (11.4%)	
Miscellaneous group	29 (33%)	
Medication coadministration		
No. of patients administered phenytoin (%)	53 (60%)	
No. of patients administered MTX (%)	69 (78.4%)	

ABW: Actual body weight, **ALL:** Acute lymphoblastic leukemia, **AML:** Acute myeloid leukemia, **BP:** Biphenotypic leukemia, **HSCT:** Hematopoietic stem cell transplantation, **MTX:** Methotrexate, **MDS:** Myelodysplastic syndrome, **PTDs:** Post-transplant days, **SAA:** Severe aplastic anemia, **SCD:** Stem cell dose, **SD:** Standard deviation.

Table(7): Comparison between parameter estimates of index group & validation group with corresponding p-values estimated by unpaired two tailed t-test

Parameter ± S.E(%R.S.E)	**Index group (n=207)**	**Validation group (n=88)**	**P-value**
Cl	23±8.6	20.9±11	0.88
V	917 ±74	1010±90	0.46
β-Cl-Age	0.0237±0.0088	0.0247±0.01	0.945
β-Cl-ARF	0.738±0.3	1±0.44	0.62
β-Cl-Hypoalbuminemia	-0.718±0.26	-0.95±0.32	0.56

RSE: Relative standard error, **S.E:** Standard error.

3.7. Applications in dose adjustment in special patient populations:

Since the most of the population clearance estimates in the special patient population (ARF & Hypoalbuminemia) were estimated almost with good precision (RSE < 30%) and due to linear properties of cyclosporine clearance within the therapeutic range; we deduced that:

$$(4) Dose\ correction\ factor = \frac{CL_{Pop}(special)}{CL_{Pop}(normal)}$$

Correction factors of different special subpopulations of HSCT are listed in table (8), including ARF, hypoalbuminemia & combined ARF and hypoalbuminemia patients. The individualized calculated dose should be corrected as follow for those patients:

$$(5) Corrected\ dose = Calculated\ dose \times Correction\ factor$$

Table (8): Dose correction factor for special patient population*

Special patients group	Dose Correction factor
ARF without hypoalbuminemia	2.1
No ARF with hypoalbuminemia	0.49
ARF With hypoalbuminemia	1.02

*Factors were estimated based on the population analysis of each special populations group

However, the application of our model in dose individualization of CsA will not necessitates the use of these corrections; as they are already integrated in our final model regression equation. It is advisable that practitioners applying empirical dosing of CsA should follow these corrections to avoid developing toxicity or subtherapeutic levels of CsA.

IV. DISCUSSION

CsA is characterized by high level of interindividual variability and this hinders the adequate adjustment of the dose and signifies the importance of personalized dosing based on individual pharmacokinetic parameters [15]. The application of population approach in pharmacokinetic analysis of cyclosporine using sparse sampling has a significant contribution to improvement of CsA dosing efficacy [16]–[19]. Therapeutic drug monitoring of CsA based on steady state trough concentrations has been validated in allogenic hematopoietic stem cell transplantation (allo- HSCT) patients [7], and dose individualization of CsA is greatly recommended to be based on the steady state trough levels as a measure of exposure [9].

According to FDA guidelines for population pharmacokinetic analysis, assuming that the sample size is large, the assay and sampling errors are small, and the dosing regimen and sampling times are identical for all patients, distributions of trough level will give a fairly accurate picture of the variability in trough concentrations in the target population. If the three conditions are not met, pharmacokinetic variability will not be sufficiently presented as the data will include other sources of random variation that significantly contribute to the observed spread [20]. For purpose of model qualification, the three conditions were strictly retained during the model building process.

Population pharmacokinetic analysis was performed using nonlinear mixed effects modeling utilizing stochastic approximation with expectation maximization (SAEM) algorithm applied in Monolix® to impute for pharmacokinetic parameters estimates, standard errors & relative standard errors which give an indication of the degree of precision of the estimate. Although NONMEM software is the most widely used software for population PK analyses, stochastic approximation with expectation maximization (SAEM) algorithm implemented in Monolix® used for parameter estimation is proven to be superior to first order conditional estimation (FOCE) methods applied in NONMEM® with reduced bias, increased precision of parameter estimates and little convergence assessment problems in complex models [21][22].

Most of the previous studies investigated the population pharmacokinetics during the stable phase following HSCT where oral therapy is indicated with constant, stable doses. The study of the pharmacokinetic behavior of CsA in the acute post-transplantation phase was extremely challenging for number of reasons; presence of acute organ failures, administration of large number of medications that may affect CsA clearance like antifungals, quinolones, phenytoin or methotrexate, or corticosteroids and high rates of increase, reduction or discontinuation of the medication which induce significant fluctuations in CsA steady state trough concentrations unlike the stable phase of treatment. Our study exclusively investigates the intrinsic changes of pharmacokinetic behavior of CsA in the acute post-transplantation-phase during the first 3 months after HSCT.

In our study, CsA population CL estimate was 23 L/hr with an interindividual variability (IIV) of 39.2%. This was very similar to the findings from Tunisian HSCT population [19] (CL=25.43 L/hr with (IIV) of 38.72 %, European HSCT population (CL=22.3 L/hr, IIV=27.7 %) and (21.9 L/hr, IIV=21 %) [23], Korean populations (CL estimate =21.9L/hr, IIV=40.2%) and American populations (CL estimate=22.3 L/hr, IIV=27.7%) [11]. Estimates from pediatric-HSCT are obviously different where CL=11.3 L/hr, IIV=36 % [24].

Volume of distribution (V) population estimate in Egyptian allo-HSCT was found to be 917 L with IIV = 57%. This results comply with the findings from Chinese HSCT patients reported an estimate of 1080 L with a IIV of 41.5 % [7], and close to the peripheral distribution volume of 1119 L in European renal transplant patients [25], but higher than the values of 133 L in Chinese renal transplant patients [26]. This differences may be related to different genetic characteristics of the populations, population size, disease types, and/or the method of population analysis.

Covariate analysis revealed that gender, stem cell dose, bilirubin status, disease type & methotrexate administration have a non-significant effect on CsA clearance, hence they were eliminated from the final regression equation of the covariate model. Although significantly affecting CL, post-transplant day was excluded from the covariate model due to deterioration of model diagnostics.

Similar to findings of most of the previous studies [11][17], weight (WT) was found to be significantly affecting CsA clearance but was not included in the final model due to lower discrimination score and possible correlation with the age which was included in the covariate model over the weigh due to achieving higher model discrimination score (model discrimination score for WT=7 and for Age =8).

The effect of renal function on cyclosporine CL is controversial. The decline in renal functions post-transplantation was found to have non- significant effect on CsA clearance after renal and stem cell transplantation [27][17], However, our analyses demonstrates that decline in renal function post-HSCT significantly increase CsA clearance. This finding can be clearly interpreted as the acute renal failure (ARF) is a component of the systemic inflammatory response syndrome (SIRS) developed in times of systemic infections [28], which are very common following HSCT, and results in increase the basal metabolic rate of the body, and hence possible CsA clearance.

Effects of hypoalbuminemia were also investigated in the acute phase of post-HSCT. Throughout the studied patients, it was found that development of hypoalbuminemia in the first 30 days post-transplantation was

very common. Albumin levels dropped to less than 3 mg/dl in 149 patient (72%) of the studied population during the acute post-transplantation period, this is thought to be due to many factors: reduction of oral intake due to high rates of mucositis, nausea and vomiting as a result of administering the conditioning regimen, high rates of protein breakdown during the periods of physiological stress or protein loosing enteropathy or nephropathy. Hypoalbuminemia was found as an influential factor of CL of CsA in the acute post-transplantation periods. This can be explained as a portion of serum CsA is bound to albumin and hence in times of hypoalbuminemia the tissue distribution of CsA significantly increases making its elimination rate is slow and disposition from the central compartment is expected to be decreased. Most of the previous studies reported that albumin level has a non- significant effect on CsA clearance [11][13], this is because these studies were performed on stable patients which have very limited risk of hypoalbuminemia compared to acute post-transplantation patient and due to small sample size of the study population that makes the incidence of type II statistical error is relatively high. Although Age demonstrate a significant effect on CsA clearance through our analysis, most of the previous studies found the age is non-significant predictor of CsA disposition [7][11][13][19] this may be due to narrow age span included in this studies, or due to small sample size of the studied population.

It has been accepted that CsA is metabolized by cytochrome P450 3A4 (CYP3A4) [29]. Thus, pharmacokinetics of CsA can be altered by either induction or inhibition of the enzyme [30]. Phenytoin is usually indicated for seizure prophylaxis in patients administering busulfan as a part of the conditioning regimen and has a significant interaction with CsA [31]. Phenytoin was found to significantly increase CsA clearance (p-value=0.016), population clearance was estimated as 45.7 ± 9.5 L/hr in patients administering phenytoin compared to 19.8±6.3 L/hr in patients didn't administer phenytoin, this effect may be explained to be due to the enzyme inducing characteristics of phenytoin to CYP 3A4 which in turn enhances CsA clearance. Based on our results, clinician should be aware that patients on phenytoin therapy will require increasing the dose of CsA by 2.3 times the calculated dose to avoid development of sub-therapeutic level of CsA. Despite of its apparent significant effect on clearance, phenytoin administration was found to deteriorate model diagnostics when added to covariates of higher model discrimination score, so it wasn't included in the final covariate model.

Evaluation of the final model reveals robust and stable characteristics. Plots of observed versus population predicted & individual predicted concentrations of the final model demonstrates good fit up to concentrations more than 500 ng/ml where there is a significant bias, possibly due to deviation of the pharmacokinetic behavior of CsA at this level from the linear disposition assumed in our model to nonlinear one, limited kinetic profiles at this concentration range or decreased assay precision at this level. There was also obvious when investigating visual predictive checks of our data; although the median line of predictions passes through the 50% prediction interval and typically superimposed over the theoretical line which are considered to be ideal findings from a VPC developed from a specific model, there was a significant errors in predicting concentrations higher than 500 ng/ml and this was evident from the VPC as most of predictions of higher concentrations typically fall outside the 90% prediction interval indicating poor predictive performance of trough concentrations exceed this limits. This pattern of deviation was also seen in the model developed in Korean population [13], however the authors didn't account for this bias. Although it is a crucial component of the quality control of population PK studies to present the degree of shrinkage together with VPC [32][33], this guideline was not followed in most of the previous CsA population pharmacokinetic studies and was explicitly demonstrated in our study. Of note, prediction corrected visual predictive checks was used for model internal validation in favor of traditional visual predictive checks (VPCs); as it was proven to be a more informative diagnostic tool than traditional VPCs [34].

One of the major strengths of this study is the prospective design, most of the previous models were developed based on retrospective analysis of data as a method to increase the sample size, however, this approach is known to be subjected to many sources of bias which may adversely affect the robustness of the developed model. Our model utilized the power of the prospective study design which reduced bias, and increased the confidence in the data used in analysis and in the same time include the largest sample size compared to all previous studies that worked on population pharmacokinetic modeling in HSCT patients.

V. CONCLUSION

A population pharmacokinetic model of CsA was developed in Egyptian allo-HSCT patients. Age, renal status and albumin status were found to be the most potential predictors of CsA CL. The model was validated for both pediatric and adult patients so it can provide a basis for the practical dose individualization in different HSCT settings.

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