CLINICAL SCIENCE

Pharmacokinetics of cyclosporin - a microemulsion in children with idiopathic nephrotic syndrome

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OBJECTIVE: We present a prospective study of a microemulsion of cyclosporin to treat idiopathic nephrotic syndrome in ten children with normal renal function who presented cyclosporin trough levels between 50 and 150 ng/ml and achieved complete remission with cyclosporin. To compare the pharmacokinetic parameters of cyclosporin in idiopathic nephrotic syndrome during remission and relapse of the nephrotic state.

METHOD: The pharmacokinetic profile of cyclosporin was evaluated with the 12-hour area under the time-concentration curve (auc0-12) using seven time-point samples. This procedure was performed on each patient during remission and relapse with the same cyclosporin dose in mg/kg/day. The 12-hour area under the time-concentration curve was calculated using the trapezoidal rule. All of the pharmacokinetic parameters and the resumed 4-hour area under the time-concentration curve were correlated with the 12-hour area under the time-concentration curve. ClinicalTrials.gov: NCT01616446.

RESULTS: There were no significant differences in any parameters of the pharmacokinetic of cyclosporin during remission and relapse, even when the data were normalized by dose. The best correlation with the 12-hour area under the time-concentration curve was the 4-hour area under the time-concentration curve on remission and relapse of the disease, followed by the 2-hour level after cyclosporin (c2) dosing in both disease states.

CONCLUSIONS: These data indicate that the same parameters used for cyclosporin therapeutic monitoring estimated during the nephrotic state can also be used during remission. Larger controlled studies are needed to confirm these findings.

KEYWORDS: Cyclosporin-A; Nephrotic Syndrome; Children; Pharmacokinetics; Area Under Curve.

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INTRODUCTION

Idiopathic nephrotic syndrome (INS) is characterized by heavy proteinuria (urinary protein above 50 mg/kg/day), hypoalbuminemia (serum albumin below 2.5 g/dl), edema, and hyperlipidemia; the syndrome occurs mainly in children. It is generally classified as steroid-sensitive nephrotic syndrome (SSNS) and steroid-resistant nephrotic syndrome (SRNS), according to the patient's response to therapy. Most patients respond to steroid therapy and show a favorable outcome. However, 10–20% of patients fail to respond and may progress to end-stage renal failure (1). Children who fail to respond to steroids or become steroid-dependent (SDNS) may be treated with immunosuppressive agents. This group of patients continues to pose a therapeutic challenge. There is considerable diversity in the use of immunosuppressive drugs, with differences in combinations, administration modes, and regimens. However, in children, there is a lack of evidence regarding the best schedule that should be adopted (2,3). The optimal strategies with the least toxicity remain to be determined (4).

Cyclosporin A (CSA), a calcineurin inhibitor immunosuppressive agent, has been widely used to treat with SRNS and SDNS patients. Studies have shown that CSA is effective in inducing remission in patients with SRNS and SDNS with toxicity steroid signs (2,5-7). However, CSA is associated with a high rate of relapse after its withdrawal, nephrotoxicity, and CSA dependence (8).

CSA can reduce proteinuria by immunological and non-immunological mechanisms. The best-understood mechanism involved in CSA-mediated immunosuppression consists of inhibiting cytokine synthesis, particularly interleukin-2 (IL-2) and its IL-2R receptor (9). CSA and the cyclophilin complex bind to and inhibit calcineurin activity toward phosphorylated proteins. Consequently, the cytosolic nuclear factors of activated T-lymphocyte (NFATs) cannot be dephosphorylated to enter the cell nucleus, where they bind to both the Fos and Jun family proteins and the distal IL-2 promoter NFAT site to facilitate gene transcription (10). The non-immunological CSA

No potential conflict of interest was reported.

mechanisms that are involved in reducing proteinuria are the reduction in the glomerular filtration rate, which leads to a decreased filtered load of protein, and the reduction of the permeability of the glomerular capillary wall for albumin (11). In addition, CSA has been shown to directly decrease the synaptopodin degradation rate and to stabilize the podocyte (12).

It is well known that the CSA has a narrow therapeutic window (13,14). In clinical practice, the pharmacokinetic (PK) profile can provide an indicator of the appropriate CSA dose to obtain an efficient effect and to try to avoid adverse events (15).

Therefore, it is recommended that therapeutic drug monitoring (TDM) be used to determine adequate dosing (8). Nevertheless, most studies have been performed on adults and organ transplant recipients. General guidelines for dosage administration and therapeutic monitoring in nephrotic syndrome [NS] are still needed, particularly for children (14).

Many factors can influence the cyclosporin PK profile (CSA-PK), and there is almost no information regarding whether there are changes in CSA-PK during the remission and relapse periods of the NS. Edema, metabolic changes in the gastrointestinal tract, and changes in hepatic metabolism and drug volume distribution could contribute to a different PK profile during these two diverse states of the NS. If this is so, then different TDM might be necessary during each of these periods. Furthermore, it is important to find a drug that is effective and safe (13,16).

This study aimed to verify the possible differences in the CSA-PK parameters in nephrotic children during both the remission and relapse periods of the NS and to try to find the best point in the area under the time-concentration curve (AUC) that correlates with the 12-hour area under the time-concentration curve (AUC_{0-12}) in both states.

MATERIALS AND METHODS

This was a prospective study of children with INS who were followed in the Pediatric Nephrology Unit of the Instituto da Criança -HCFMUSP. The study included children who were diagnosed with SRNS and SDNS, who were prescribed CSA to treat INS, and who once achieved complete remission with CSA according to the guidelines of the International Society of Kidney Diseases in Children (ISKDC); the inclusion criteria included normal renal function as evaluated by creatinine clearance estimated by stature (17) $\geq$90 ml/min/1.73 m^2, with CSA trough levels (C0) between 50 and 150 ng/ml (1). We decided to adopt this value range for C0 because in most reports of CSA treatment in children with INS, the CSA trough levels are maintained between 50 and 120 ng/mL (2,7). All of the patients were subjected to renal biopsies before the CSA introduction. The exclusion criteria were renal and hepatic function abnormalities, the presence of infectious disease, clinical or histological signs of CSA nephrotoxicity, and suspicions of non-compliance.

In this study, the definitions and criteria of ISKDC were adopted in relation to NS, remission and relapse (1).

The patients were evaluated weekly in the first month and monthly thereafter. The following exams were performed: urinalysis, 24-hour proteinuria, hematological counts, serum creatinine, cholesterol and triglycerides, serum protein and albumin levels, and liver enzymes. The patients received along with prednisone to induce remission, according to the ISKDC guidelines (1).

The study was approved by the Local Ethics Committee, and the parents signed informed consent documents before the children were enrolled in the study.

Pharmacokinetics

We performed CSA-PK evaluations in patients who fulfilled the inclusion criteria. The procedure was evaluated through the 12-hour area under the time-concentration curve (AUC_{0-12}). The patients were required to take their evening CSA dose at 7 pm on the previous day and to fast after an early dinner. They were kept in observation on the following day. The CSA trough level was collected at 7 am immediately before the patients received their morning doses of CSA, and the time-concentration curve collection began. The PK studies consisted of collecting whole blood samples through peripheral venous access before (trough level or C0) and 1, 2, 4, 6, 8, and 12 hours after the drug administration (C1, C2, C4, C6, C8, and C12, respectively). On the same day, the hematological count and the serum creatinine, cholesterol, albumin, and 24-hour proteinuria and creatinine clearance levels were collected.

The same procedure was performed for each patient during the remission and relapse periods with the same dose of CSA (mg/kg/day). The PK studies were performed at least 72 hours after the introduction the drug or after the dose modification if the C0 reached 50-150 ng/ml.

Relapse was defined as the presence of proteinuria >50 mg/kg/day. Remission was defined as proteinuria <5 mg/kg/day and serum albumin >2.5 g/dl (1). The PK study was performed at least four days after the characterization of the remission or relapse periods.

The blood CSA concentration was measured with a monoclonal antibody fluorescence polarization immunoassay using the Abbott TDxFLx cyclosporine monoclonal whole blood assay (18).

The AUCs were calculated using the trapezoidal rule. The C0 through C12 variables were used to calculate the AUC_{0-12}, and the C0, C1, C2, and C4 variables were used in the construction of the 4-hour area under the time-concentration curve (AUC_{0-4}). Cmax was defined as the highest concentration and Tmax as the time to achieve Cmax.

The absolute PK parameters and the dose-normalized parameters were compared. In addition, the following variables were also analyzed upon remission and relapse: cholesterol, albumin, creatinine and hematocrit, creatinine clearance and 24-hour proteinuria. Finally, all points of the time-concentration curve were correlated with the AUC_{0-4} and the AUC_{0-12}, either in absolute values or normalized by dose (mg/kg/day).

Statistical analysis

The data were expressed as means $\pm$ SD or as medians and ranges, when applicable. Parametric tests were employed because the data had normal distributions (according to the skewness and kurtosis coefficients). The different CSA-PK parameters (AUC0-12 and AUC0-4 between remission and relapse) were determined with a paired t-test (significance level $p<0.05$). The correlations between AUC_{0-12} and other points of the curve and the resumed AUC_{0-4} were determined by Pearson's correlation coefficient (r) and the coefficient of determination (r^2). We also compared all of the CSA-PK parameters that were normalized by dose (mg/kg/dose). The same correlations

were performed for the cholesterol and serum albumin levels.

RESULTS

Ten children (mean age at presentation 3.0±1.6 years) were enrolled in the study; none had a history of familial nephrotic syndrome. Table 1 outlines the characteristics of the group. In patients with SRNS, CSA was introduced 8.2±5.6 months after presentation. In SDNS patients, the introduction occurred after 8.1±3.4 years. This difference was because intravenous cyclophosphamide is the first option used in the clinic to treat children with SDNS. During remission, the patients were placed on prednisone, and five patients received the calcium-channel blocker amlodipine; four children received an H2 blocker. During relapse, eight patients were receiving prednisone; six patients were on amlodipine and four on H2 blocker.

Table 2 outlines all of the blood tests performed (serum albumin, hematocrit, cholesterol and creatinine) and the 24-hour proteinuria of the patients during remission and relapse. The NS is well demonstrated in these two distinct phases. Proteinuria, serum albumin and serum cholesterol are significantly different between these two periods (as required by the protocol). There was no significant difference between the creatinine clearances estimated by stature during remission (191.4±52.1 ml/min/1.73 m^2 BS) and relapse (256.9±163.9 ml/min/1.73 m^2 BS) ($p = 0.24$).

During remission and relapse, we could not observe a significant correlation either between cholesterol and $AUC_{0\text{-}12}$ (r -0.26 and r -0.28, respectively), C2 (r 0.03 and r -0.13, respectively) or between albumin and 24-hour proteinuria.

In this study, we did not observe any differences between CSA-$AUC_{0\text{-}12}$ during remission (3324±1094 ng.h/ml) and relapse (3340±880 ng.h/ml) (p = 0.96). There was also no significant difference between the resumed 4-hour area under the time-concentration curve ($AUC_{0\text{-}4}$) (p = 0.98) during remission (1985±623 ng.h/ml) and relapse (1982±631 ng.h/ml). The same result applied when the data were normalized by dose. We did not observe any differences between the CSA-$AUC_{0\text{-}12}$ ($p = 0.84$) and the CSA-$AUC_{0\text{-}4}$ ($p = 0.88$) during remission (1538±517 ng.h/ml and 925±304 ng.h/ml, respectively) and relapse (1574±602 ng.h/ml and 925±458 ng.h/ml, respectively). Figure 1 illustrates the PK curve during remission and relapse. Note that the mean concentration is similar for all time points, causing the two curves cover each other.

Table 2 - Nephrotic Syndrome parameters evaluated during remission and relapse.

Variable	Relapse X±DP Median (range)	Remission X±DP Median (range)	*p*-value
Proteinuria (mg/kg/day)	126.1±58.4* 54.4-220.0	1.8±2.0* 0.0-6.0	**0.0001**
Serum Albumin (g/dl)	2.1±0.9* 0.8-3.5	3.9±0.5* 3.2-4.8	**0.0001**
Cholesterol (mg/dl)	344.2±107.7* 151.0-493.0	209.7±64.3* 136.0-355.0	**0.001**
SCr (mg/dl)	0.36±0.18^{NS} 0.10-0.61	0.39±0.15^{NS} 0.23-0.64	**0.36**
CrCl (ml/min/1,73m^2)	256.9±163.9^{NS} 134-632	191.4±52.1^{NS} 115-243	**0.24**
Hematocrit	38.7±3.8^{NS} 32.9-44.3	38.6±5.1^{NS} 30.6-48.9	**0.96**

*$p<0.05$ (Paired t-test); NS: not significant.

Table 3 shows the only patient who exhibited Cmax in the 4th hour (patient 8, during remission). The other patients presented Cmax in either the 1st or 2nd hour. Tmax was 1.8±0.9 h (median = 2) during remission and 1.5±0.5 h (median = 1.5) during relapse.

When the correlations were analyzed between all of the CSA-PK parameters and $AUC_{0\text{–}12}$, both in absolute number and normalized by dose, only the C2 and $AUC_{0\text{–}4}$ had reasonable correlation indices (r/r^2) either for the absolute CSA-PK parameters or for the CSA-PK parameters normalized by dose. Specifically, the correlations identified were as follows: 0.86/0.74 and 0.95/0.90 for the absolute CSA-PK parameters on remission, 0.80/0.64 and 0.93/0.86 on relapse for C2 and $AUC_{0\text{–}4}$, respectively, 0.84/0.70 and 0.94/0.88 on remission for the CSA-PK parameters normalized by dose, and 0.93/0.86 and 0.96/0.92 on relapse for C2 and $AUC_{0\text{–}4}$, respectively (Table 4).

DISCUSSION

It is likely that the inter- and intraindividual variabilities in CSA-PK and the dose requirements are even larger in children than in adults because of the variation in biological maturation (14,19). To achieve comparable exposures, children require higher relative CSA doses compared to adults. Such differences are mainly caused by shorter intestinal surface absorption and a higher metabolic rate for CSA in children (14,16,19-22). Therefore, adult studies cannot be applied to children.

Table 1 - Characteristics of the 10 INS patients.

Patient	Gender	Age (years)	Steroid response	Histology	Time of ISN (months)	CSA dose (mg/kg/day)
1	F	6.8	SR	MCD	5.0	4.5
2	M	10.8	SR	MCD	19.0	6.5
3	M	18.9	SD	FSGS	119.0	4.5
4	F	15.8	SD	MCD	120.0	3.0
5	M	8.0	SR	MCD	5.0	4.0
6	M	10.6	SR	FSGS	12.0	4.0
7	F	6.8	SR	MCD	3.0	3.5
8	F	5.2	SR	MCD	5.0	4.0
9	M	6.8	SD	MCD	35.0	4.8
10	F	13.4	SD	FSGS	116.0	3.8
Mean±SD		**10.3±4.5**			43.9±**52.2**	4.5±**1.1**

F: female; M: male; SR: steroid-resistant; SD: steroid-dependent; MCD: minimal change disease; FSGS: focal and segmental glomerulosclerosis; NS: nephrotic syndrome; CSA: cyclosporine.

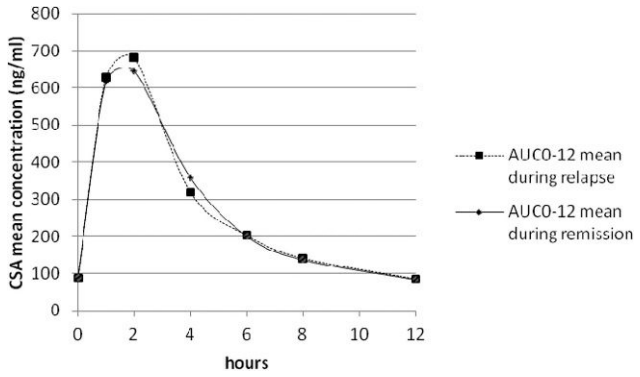

Figure 1 - AUC0-12 during remission and relapse of INS.

In addition, it is unclear whether abnormalities observed during relapse of the NS are able to interfere with its CSA-PK or if the abnormalities could influence the drug prescription (13). Some studies have suggested that edema, hypoproteinemia, and hypercholesterolemia are involved in CSA bioavailability and clearance (13,16). Hypercholesterolemia is particularly important in CSA-PK because the drug is highly lipophilic and binds to blood cells and plasma proteins; the relative distribution depends on the temperature, drug concentration, hematocrit, and plasma lipoproteins (23).

Therefore, in particular, this study attempts to verify the potential differences in the CSA-PK parameters between nephrotic children during remission and relapse of the disease. We employed $AUC_{0\text{-}12}$ as a gold standard because this parameter has been considered the most reliable. However, it is an invasive method that requires the collection of several blood samples, and it cannot be applied in clinical practice (20,22).

For years, CSA trough levels (C0) have been widely used to monitor CSA dosing (14,21,24). Since the development of Neoral, a micro-emulsified formulation, the bioavailability of CSA has increased, while inter- and intraindividual variabilities have improved remarkably and a new strategy for monitoring CSA was introduced, though mainly in post-transplant adult patients (25).

In several adult reports, C2 has been correlated better than C0 or other time points to the $AUC_{0\text{-}12}$, particularly in transplant patients (26). However, an important random trial involving cadaveric kidney recipients compared C0 and C2 in the first three weeks post-transplant and revealed no advantages in C2 monitoring, although it led to significantly higher CSA doses and blood levels than the C0 monitoring (27). Other reports have considered $AUC_{0\text{–}4}$ a reliable parameter for Neoral dose monitoring in organ transplant patients (25). $AUC_{0\text{–}4}$ was found to correlate better with clinical effects in kidney and liver transplant recipients (28). Many reports of transplanted adults have shown that C2 was the best point of correlation with $AUC_{0\text{–}4}$ and that C0 does not exhibit the same behavior (14,21,29). One report found a correlation coefficient between C2 and $AUC_{0\text{–}4}$ that varied from 0.67 to 0.85 (30).

In transplanted children, C2 has been considered a reliable marker of acute rejection (7). A study of renal transplantation in children reported a good correlation between $AUC_{0\text{–}4}$, C1.25, and C2 with $AUC_{0\text{–}12}$, which was not observed with C0 (14).

Therefore, several studies have suggested C2 as a reliable parameter to be used in transplant patients (adults and children); however, there is no consensus for INS (16), particularly in children (21). The efficacy of C2 as a parameter to fit the CSA dosage and its correlation with $AUC_{0\text{–}4}$ is still uncertain (21). Nozu et al. (21) reported a late absorption peak in 33% of the patients (3–4 hours after drug administration), which supports the wide interindividual variability in the CSA PK profile.

Note that the CSA blood concentration is directly related to its potency and the duration of the calcineurin inhibition effect (19,31,32). Longer calcineurin inhibition and, consequently, longer inhibition of IL-2 production are known to occur during the first 2 hours after CSA administration (14). However, it is a limited strategy for measuring the actual effectiveness of its immunosuppressive action. Currently, pharmacodynamic studies show the real biological effects of the drug (33,34). In addition, polymorphisms in the CYP3A5 and ABCB1 genes have been investigated as modulators of the pharmacokinetic and clinical effects of CSA in Brazilian renal transplant recipients (35).

In this study, the patients received CSA dosages to achieve C0 between 50 and 150 ng/ml in the outpatient clinic evaluation; the patients were then hospitalized, and the blood collections were performed on remission and relapse, while maintaining the same dosage (mg/kg) in both situations. We prefer to employ C0 as an inclusion criterion because a fixed dose could have inter- and intraindividual variability, which is characteristic of CSA. In this protocol,

Table 3 - CSA-PK parameters in 10 INS patients during remission and relapse.

CASO	C0 (ng/ml)		C1 (ng/ml)		C2 (ng/ml)		C4 (ng/ml)		C6 (ng/ml)		C8 (ng/ml)		C12 (ng/ml)	
	R	r	R	r	R	r	R	r	R	r	R	r	R	r
1	116	69	574	358	646	250	412	121	237	93	174	122	98	85
2	114	87	1038	1018	976	708	389	427	314	224	292	179	173	89
3	64	137	613	405	767	730	324	495	181	314	90	209	65	109
4	75	110	621	1204	435	1089	278	247	169	200	101	142	99	93
5	96	57	373	437	490	644	350	239	164	111	121	71	74	42
6	98	76	483	475	453	408	237	253	128	220	83	126	51	72
7	45	59	623	464	728	562	268	248	124	224	51	165	25	110
8	51	63	221	923	234	516	413	378	153	189	91	83	49	52
9	74	111	838	869	808	777	252	406	188	232	135	152	77	93
10	154	112	781	123	908	1131	261	376	348	226	231	163	128	113

R: remission; r: relapse.

Table 4 - Correlation between all pharmacokinetic parameters and AUC_{0-4} with AUC_{0-12} during remission and relapse of the nephrotic syndrome, expressed as absolute values or normalized by dose (mg/kg).

Pharmacokinetic parameter		Remission				Relapse			
Absolute PK	Normalized by dose (mg/kg/dose)	Absolute PK		Normalized by dose (mg/kg/dose)		Absolute PK		Normalized by dose (mg/kg/dose)	
		r	r^2	*r*	r^2	*r*	r^2	r	r^2
AUC_{0-4}	AUC_{0-4}/dose	0.95	0.90	0.94	0.88	0.93	0.86	0.96	0.92
C0	C0/dose	0.73	0.53	0.80	0.64	0.74	0.54	0.88	0.77
C1	C1/dose	0.82	0.67	0.80	0.64	0.53	0.28	0.65	0.42
C2	C2/dose	0.86	0.74	0.84	0.70	0.80	0.64	0.93	0.86
C4	C4/dose	0.64	0.40	0.82	0.67	0.75	0.56	0.67	0.45
C6	C6/dose	0.96	0.92	0.95	0.90	0.72	0.52	0.78	0.61
C8	C8/dose	0.92	0.84	0.82	0.67	0.58	0.33	0.74	0.54
C12	C12/dose	0.86	0.74	0.76	0.58	0.46	0.21	0.74	0.55

r: Pearson's correlation coefficient; r^2 -determination coefficient.

we were unable to detect a significant difference of the INS between remission and relapse in the following pharmacokinetics parameters: AUC_{0-12}, AUC_{0-4}, or in all points of the curve (C0, C1, C2, C4, C6, C8, or C12). In addition, we were unable to demonstrate a significant influence on the serum levels of cholesterol, albumin or hematocrit and proteinuria on the pharmacokinetics of CSA; the study has shown no significant correlations between these variables and AUC_{0-12}, AUC_{0-4}, and all points of the curve during remission or relapse. Therefore, in our study, we could not detect a significant difference between AUC_{0-12}, AUC_{0-4}, and all points of the curve in the same children during remission and relapse, suggesting that there is no necessity to change the dose when the patient is in relapse or remission.

Medeiros et al. (16) studied seven children with SRNS during remission and relapse, employed a fixed dosage of CSA (6 mg/kg/day), and reported lower CSA exposure during remission, suggesting that the target area under the curve was not the same in both conditions and that a higher dosage could be necessary on relapse. Unfortunately, this study included patients in partial remission of the disease (four cases), which can influence the conclusions.

A similar study conducted with puromycin aminonucleoside-induced nephrotic rats showed a higher drug exposure during relapse, as evaluated by AUC_{0-12}. This study also observed a positive correlation of AUC_{0-12} with cholesterol levels and negative correlations with CSA clearance and with its distribution volume (13). However, it is important to note that this was an experimental study conducted under ideal conditions; therefore, the findings cannot be directly applied to clinical practice (13).

Our study has shown that AUC_{0-4} was the main point in both the remission and relapse states of the disease when compared to AUC_{0-12} ($r = 0.95$ on remission and $r = 0.93$ on relapse) and that C2 was the second parameter identified ($r = 0.86$ on remission and $r = 0.80$ on relapse). Other CSA-PK points had good correlation with AUC_{0-12} during remission; however, this correlation was not observed on relapse. In our study, C2 was the point with a higher correlation with AUC_{0-4} during remission and relapse ($r = 0.98$ on remission and $r = 0.83$ on relapse).

We have observed that C2 appears to be the more adequate parameter to fit CSA dosage in nephrotic children during remission, relapse, and AUC_{0-4}. However, we did not take into account the concomitant drugs used by the patients, such as steroids, calcium-channel blockers, or H2 blockers. We must also emphasize that we could not be certain of the exact time of the drug administration on the previous day. Furthermore, the study used a small sample size, and it was heterogeneous.

Finally, larger prospective controlled studies should be conducted to reproduce these findings and to verify the target value of C2 to continue remission with less toxicity in INS. This important study of CSA-PK in INS in childhood during remission and relapse demonstrated that it is not necessary to change the dosage of CSA in both states; we considered the same reference values in both states.

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AUTHOR CONTRIBUTIONS

Henriques LS contributed to the data collection, review of the literature, statistical analysis, and drafting of the manuscript. Matos FM contributed to the data collection. Vaisbich MH contributed to the data collection, statistical analysis, and final review of the manuscript.

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