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**ABSTRACT**

*Background:* Pediatric patients with steroid-resistant nephrotic syndrome (SRNS) and focal segmental glomerulosclerosis (FSGS) may relapse and current second line agents include mycophenolate mofetil. However, there is no current information about the use of the sodium salt of mycophenolic acid (SMPA) in this population.

*Objectives:* We conducted a prospective study on the efficacy and pharmacokinetics of SMPA in children with FSGS.

*Patients and Methods:* Patients without NPHS2 pathogenic variants received SMPA at dosages between 460 to 720 mg/m2/d for 12 months after previous treatments failure. Clinical and biochemical assessments were performed. Blood samples were obtained after the first dose and at steady state (3 months after the onset of treatment) and total and free mycophenolic acid (MPA) was quantitated using HPLC-UV.

*Results:* Two patients showed partial remission after the 12-month period of SMPA treatment with a notable decrease in proteinuria and an increase in serum albumin levels. Maximum MPA concentrations after the first dose and at steady state were 11.6 µg/mL and 10.5 µg/mL, respectively, without drug accumulation. Maximum MPA free levels after the first dose and at steady state were 192.9 and 120.6 ng/mL, respectively. MPA levels became undetectable after 4 hours of the administration in all cases.

*Conclusions:* SMPA is a promising agent for pediatric patients with SRNS and FSGS but SMPA schedule of treatment should be revised with shorter intervals of administration and higher doses than those used in the present study in order to attain higher systemic exposures and accumulation of the immunosuppressant drug. Further efficacy and pharmacokinetic studies should be performed to confirm these findings.

**Implication for health policy/practice/research/medical education:**

Pediatric patients with steroid-resistant nephrotic syndrome (SRNS) and focal segmental glomerulosclerosis (FSGS) may develop end-stage renal disease. Pharmacological treatment of these patients is still a challenge to preserve kidney function. Despite mycophenolate mofetil has been proposed as a new treatment, side-effects may lead to treatment withdrawal and data on clinical response is scarce. Here we provide evidence of the safety, efficacy and pharmacokinetics of the enteric coated formulation of mycophenolate sodium in pediatric nephrotic syndrome and FSGS.


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1. Background

Nephrotic syndrome is characterized by heavy proteinuria with hypoalbuminemia, edema and dyslipidemia (1). The incidence of nephrotic syndrome (NS) in pediatric patients in Argentina is 2 new cases per 100,000 children showing lower values when compared to other populations (2,3). Focal segmental glomerulosclerosis (FSGS) is the most frequent histological finding in patients with nephrotic syndrome and about 40% of these develop end-stage renal disease. Nonetheless, pharmacological treatment of children with FSGS is still a challenge to achieve proteinuria control and preserve kidney function (4). In this scenario, renin-angiotensin-aldosterone system plays a key role as the expression of the angiotensin type 1 and type 2 receptors are higher in patients with proteinuria justifying the additional use of inhibitors and/or blockers of this system (4,5). Even though some patients with FSGS respond to steroid treatment, about 25% of them are resistant to corticosteroids and thus, are subjected to second line immunosuppressive treatment including cyclosporine and cyclophosphamide (4-8). Nonetheless, long-term treatment with immunosuppressant agents is associated with adverse events with significant morbidity.

A novel pharmacological alternative treatment for these children is mycophenolic acid (9,10). Mycophenolate mofetil (MMF) is widely used to prevent acute rejection in kidney pediatric renal transplantation. The immunosuppressive activity of mycophenolic acid (MPA), the active form of MMF, is mediated by inosine monophosphate dehydrogenase (IMPDH) inhibition and therefore, interfering with the de novo synthesis of purine nucleotide. More recently, MMF has emerged as a second-line immunosuppressant agent for adult and pediatric patients with nephrotic syndrome. However, clinical response to MPA is scarce and controversial with a significant proportion of pediatric patients with FSGS remaining with persistent proteinuria (11-14).

Different factors may explain the variability in the pharmacological response to MPA. Mutations in more than 20 genes have been identified in monogenic forms of steroid-resistant NS, most of which encode proteins related to the normal function of glomerular filtration in podocytes (15,16). NPHS2 gene encodes podocin that is a transmembrane protein and has been related to 18% of steroid resistant NS cases in childhood (16-18). Other factors related to sodium salt of mycophenolic acid (SMPA) response include hypoalbuminemia and high inter-individual variability in the pharmacokinetics of the drug (19,20). In this sense, underexposure to the drug may lead to the lack of efficacy but overexposure to the incidence of adverse events that lead to treatment discontinuation. In order to enhance the efficiency and safety of MMF therapy, therapeutic drug monitoring is performed in renal transplant patients but the therapeutic range for MPA systemic exposure is still under debate (20-22).

In addition, side-effects associated with MMF may lead to treatment withdrawal. Thus, the enteric coated formulation of mycophenolate sodium (SMPA) that delays the absorption until the drug reaches the small intestine may be a choice for reducing gastrointestinal side effects but evidence about its clinical usage and advantages in nephrotic syndrome and FSGS should be provided. Scarc data has been reported on the pharmacokinetics of MPA in children and even less information is available in pediatric patients with FGSC (23).

2. Objectives

The present study aimed to evaluate in a cohort of pediatric patients with FGSC without podocin SNHS2 gene mutations the efficacy of the immunosuppressant treatment and the pharmacokinetics of MPA.

3. Patients and Methods

3.1. Study population

Patients with steroid-resistant idiopathic nephrotic syndrome and FSGS were treated at the Nephrology Service at Hospital de Pediatría SAMIC JP Garrahan. Eligibility criteria included (a) idiopathic nephrotic patients with FSGS histologically confirmed, (b) less than 16 years old, (c) resistant to treatment with steroids and to cyclophosphamide concomitant to blockade of the renin-angiotensin-aldosterone system, (d) glomerular filtration rate > 60 mL/min/1.73m², (e) a urine protein-to-creatinine ratio >2 mg/g and (f) serum albumin <2.5 g/dL. Patients were excluded if they presented (a) secondary causes of nephrotic syndrome, (b) congenital nephrotic syndrome, (c) chronic or systemic infections at the time of relapse, hepatitis B or C, VIH positive, (d) cystic fibrosis, celiac disease and malabsorption syndrome caused by parasites infection, (e) non-compliant patients or family, (f) identification of pathogenic gene mutations in NPHS2.

3.2. Ethical issues

The present study followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board (Protocol #588). Informed consent was signed by parents or guardians of participants included in the study.
3.3 Drug administration and study procedures

Patients were treated with prednisone (2 mg/kg/d) for 4-6 weeks and followed by a single dose on alternate days for 4-6 weeks and discontinued. After confirmation of resistance to prednisone treatment, cyclophosphamide (2 mg/kg/d) was administered for 8-10 weeks concomitant to enalapril (0.1-0.3 mg/kg/d) and thereafter losartan (1-2 mg/kg/d). In case of persistent proteinuria, patients received another cycle of steroids as described. Steroid and cyclophosphamide resistant patients were included in the study and were treated with sodium mycophenolic acid at 460 mg/m² to 720 mg/m². Nutritional support was provided according to the hospital guidelines. Patients were evaluated on a monthly basis with clinical assessment and laboratory tests including hemoglobin level, blood count, platelet count, serum creatinine, serum albumin, serum cholesterol, and 24-hour proteinuria and protein/creatinine ratio (Up/c).

3.4 Outcome

Complete response was defined as 24-hour urinary protein <5 mg/kg/d or an Up/c ratio <0.2 mg/g, normal plasma cholesterol, and plasma albumin >3.5 g/dL. A partial response was defined as 24-hours urinary protein less than 50% of baseline values, and plasma albumin >3 g/dL. Resistance was defined as persistent nephrotic syndrome after 6 months of treatment. Relapse was considered if urinary protein levels >50 mg/kg/d for 5 consecutive days after a partial or complete remission.

3.4.1 NPHS2 molecular analysis

Genomic DNA was isolated from peripheral blood cells using the salting-out method (24). All 8 exons of NPHS2 gene were amplified by PCR using flanking intron primers as previously described by others (25). Mutation analysis was performed by direct sequencing using the ABI PRISM Big Dye Terminator sequencing kit and an ABI Prism 3130 analyzer. The obtained sequences were compared to other reference published sequences (NCBI accession number NM_014625.2).

3.5 Pharmacokinetic studies

Blood samples for pharmacokinetic studies were taken immediately after the first dose of SMPA, and at three and six months after the start of treatment. Three milliliters of blood were collected in EDTA-containing tubes before and at 1, 2, 3, 4, 6, and 8 hours after the first or the morning dose of SMPA on steady state. Samples were immediately centrifuged to separate plasma. About 200 µL of each plasma sample was added into the reservoir of a Centrifree Micropartition System (Millipore, Merek KGaA, Germany) and centrifuged at 2000 g for 40 minutes to obtain the ultra-filtrate or free SMPA. Both plasma and ultra-filtrate samples were stored at -20°C until quantitation of total and free SMPA by a modified and validated HPLC method coupled with UV detection (26). The analysis was performed with an HPLC system equipped with an Agilent 1100 liquid chromatography pump and an Agilent UV detector set at a wavelength of 214 nm. Separation chromatography was performed using a Nova-pack C18 reverse-phase column (150 mm × 3.9 mm, 4 µm particle size; Waters - Milford, United States) coupled to a C18 Phenomenex security guard pre-column. The limit of quantitation for free and total SMPA was 25 ng/mL and 50 µg/mL, respectively. The linear range was defined between 25 and 250 ng/mL for free MPA and between 50 µg/mL to 5000 µg/mL for total SMPA.

3.6 Statistical analysis

Clinical data were reported as medians with an associated range for continuous parameters; categorical variables were expressed as percentages. Total and ultra-filtrate MPA pharmacokinetic parameters were calculated using standard non-compartmental and consisted of the concentration of the drug at the trough or before dosing (C0), the maximum plasma concentration (Cmax), the time to Cmax (Tmax), and the area under the concentration versus time profile (AUC).

4 Results

4.1 Patient characteristics and clinical assessment

A total of 20 patients diagnosed with steroid-resistant idiopathic nephrotic syndrome and FSGS were included. Thirteen of 20 patients were in chronic renal failure and were excluded from the study. Therefore, the 7 patients that retained normal renal function and fulfilled the inclusion criteria were included in the study. Demographic and clinical data just before the start and after 12 months of SMPA treatment are shown in Tables 1 and 2, respectively. Before SMPA treatment, all patients have received prednisone, cyclophosphamide, enalapril and losartan. Thereafter, patients received sodium mycophenolic acid at a dosage between 460 mg/m² and 720 mg/m². The median (range) time that elapsed between the diagnosis of nephrotic syndrome and the start of SMPA treatment was 33 months (22-142) as shown in Table 1. Partial remission was achieved in 2 patients after 1 and 2 years of the 12 month-treatment period with SMPA. Specifically, proteinuria decreased from 153.7 mg/kg/d
to 26.5 mg/kg/d and from 47.2 mg/kg/d to 20.0 mg/kg/d while serum cholesterol levels returned to normal levels of 205 mg/dL and 213 mg/dL, respectively. The serum albumin notably increased in these two patients from 1.3 g/dL to 2.1 g/dL and from 2.3 g/dL to 3.4 g/dL, respectively. The time elapsed between onset of nephrotic syndrome and the start of SMPA treatment in the two patients that showed late remission was 142 and 22 months.

One patient showed a significant decrease in proteinuria from 53.4 mg/kg/d to 36.4 mg/kg/d at 1 year after the start of SMPA treatment. However this decrease was not accompanied by an increased in serum albumin and thus was not considered as a response. Five patients that did not show a response to SMPA treatment remained with normal renal function, none required dialysis and all patients were alive up to 2 years of follow-up.

As shown in Table 1, the median dose of SMPA treatment calculated for all studied patients was 655 mg/m^2/d (486-742) or 720 mg/d (360-1440). The two patients that showed partial remission received 742 mg/m^2/d (equivalent to 1440 mg/d) and 735 mg/m^2/d (equivalent to 720 mg/d). The 5 patients that did not respond to SMPA treatment received a median dose of 493 mg/m^2/d (equivalent to 360 mg/d).

No patient experienced hematological, gastrointestinal or other adverse event related to SMPA treatment during the entire follow-up period.

### 4.2. Molecular genetic analysis

No pathogenic variants were found in the patients included in the present study. Genetic variants observed in the study population were single nucleotide polymorphisms (SNPs) previously published in public databases (NCBI, GNomAD, ClinVar). Seven of the eight genetic variants were found in heterozygous state while p.A318A variant, described as the most frequent one in the general population, was found in homozygous state in one patient. In addition, six variants are classified as benign in ClinVar database and according to in silico analysis (Mutation Taster, Human Splicing Finder). Finally, two variants, c.-51G>T in 5’UTR region and p.R229Q are classified as variants of uncertain significance (Table 3). Specifically, p.R229Q is only pathogenic in combination with other variants in exon 7 or 8 and thus, a single heterozygous mutation could not be by itself acknowledged as a causative mutation (27).

### 4.3. Pharmacokinetic analysis

In all seven patients, pharmacokinetic studies were performed after the first dose of SMPA. At 3 months after starting SMPA treatment, the pharmacokinetic studies could not be done in three patients because of difficult vascular access (n = 1) and due to the lack of consent (n = 2). Thus, a total of four complete concentration versus time profiles of SMPA three months after treatment initiation were available. The mean pharmacokinetic parameters of MPA and free MPA were shown in Table 4. After the first dose, the median (range) MPA and free MPA Cmax was 11.6 µg/mL (5.6-21.6) and 192.9 ng/mL (79-121), respectively. The concentration versus time profiles of SMPA three months after treatment initiation were available. The pharmacokinetic studies were performed after the first dose of SMPA. At 3 months after starting SMPA treatment, the pharmacokinetic studies could not be done in three patients because of difficult vascular access (n = 1) and due to the lack of consent (n = 2). Thus, a total of four complete concentration versus time profiles of SMPA three months after treatment initiation were available. The mean pharmacokinetic parameters of MPA and free MPA were shown in Table 4. After the first dose, the median (range) MPA and free MPA Cmax was 11.6 µg/mL (5.6-21.6) and 192.9 ng/mL (79-121), respectively. The concentration versus time profiles of SMPA three months after treatment initiation were available. The mean pharmacokinetic parameters of MPA and free MPA were shown in Table 4. After the first dose, the median (range) MPA and free MPA Cmax was 11.6 µg/mL (5.6-21.6) and 192.9 ng/mL (79-121), respectively. The concentration versus time profiles of SMPA three months after treatment initiation were available.
administration, there was no accumulation of the drug as the median (range) Cmax at steady state was 10.5 µg/mL (4-10.7), which is almost the same value obtained after the first dose (Table 4, Figures 2A and 2B for total and unbound MPA, respectively). The same trend was observed with the AUC after the first dose and three months from MPA treatment start as depicted in Table 4. MPA free fraction was close to 2% at all times after the first oral dose as depicted in Figure 3A. In addition, a clear relationship between the AUC of the total and the free MPA was observed as shown in Figure 3B.

Based on the pharmacokinetics profile and maximum plasma concentration of MPA after the first and the 3-month dose for the first patient, an amendment for the study protocol was presented to the local IRB in order to change the dose. The dose was increased from

<table>
<thead>
<tr>
<th>Genetic variant</th>
<th>Protein change</th>
<th>Gene location</th>
<th>Alleles (n)</th>
<th>Mutation status</th>
<th>Clinical significance</th>
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<tr>
<td>c.51 G&gt;T</td>
<td>-</td>
<td>5’UTR</td>
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<td>c.288C&gt;T</td>
<td>p.S96S</td>
<td>Exon 2</td>
<td>1</td>
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<td>Benign</td>
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<td>c.452-21C&gt;T</td>
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<td>Intron 3</td>
<td>2</td>
<td>Het</td>
<td>Benign</td>
</tr>
<tr>
<td>c.452-46C&gt;T</td>
<td>-</td>
<td>Intron 3</td>
<td>2</td>
<td>Het</td>
<td>Benign</td>
</tr>
<tr>
<td>c.686G&gt;A</td>
<td>p.R229Q</td>
<td>Exon 5</td>
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<tr>
<td>c.873+7A&gt;G</td>
<td>-</td>
<td>Intron 7</td>
<td>1</td>
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<tr>
<td>c.954C&gt;T</td>
<td>p.A318A</td>
<td>Exon 8</td>
<td>5</td>
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<tr>
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<td>1</td>
<td>Het</td>
<td>Benign</td>
</tr>
</tbody>
</table>

Abbreviations: Het, heterozygous; Homo, homozygous.
⁴One patient showed homozygous state (2 alleles).

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>After first dose</th>
<th>At steady state</th>
<th>Steady state-to-first dose</th>
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</thead>
<tbody>
<tr>
<td>Cmax (µg/mL)</td>
<td>11.6 (5.6-21.6)</td>
<td>10.5 (4.0-10.7)</td>
<td>1.04 (0.3-1.9)</td>
</tr>
<tr>
<td>AUC (µg *h/mL)</td>
<td>18.0 (5.6-27.0)</td>
<td>13.1 (10.2-27.0)</td>
<td>1.64 (0.56-2.35)</td>
</tr>
<tr>
<td>C0 (µg/mL)</td>
<td>ND</td>
<td>0.36 (0.14-0.83)</td>
<td>-</td>
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<tr>
<td>Tmax (h)</td>
<td>2 (1-3)</td>
<td>2 (1-3)</td>
<td>-</td>
</tr>
<tr>
<td>Cmax /D (µg/mL /mg/m²)</td>
<td>0.018 (0.013-0.047)</td>
<td>0.014 (0.006-0.029)</td>
<td>1.04 (0.3-1.9)</td>
</tr>
<tr>
<td>AUC/D (µg *h/mL) / (mg/m²)</td>
<td>0.026 (0.015-0.059)</td>
<td>0.036 (0.014-0.037)</td>
<td>1.64 (0.56-2.35)</td>
</tr>
<tr>
<td>C0/D (ng/mL)/(mg/m²)</td>
<td>ND</td>
<td>0.54 (0.19-1.12)</td>
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</table>

Free MPA

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>After first dose</th>
<th>At steady state</th>
<th>Steady state-to-first dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (µg/mL)</td>
<td>192.9 (78.8-367.0)</td>
<td>120.6 (111.8-169.7)</td>
<td>-</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>2 (1-3)</td>
<td>2 (1-3)</td>
<td>-</td>
</tr>
<tr>
<td>AUC (ng*h/mL)</td>
<td>255.0 (131.7-464.3)</td>
<td>295.4 (147.0-439.0)</td>
<td>-</td>
</tr>
<tr>
<td>C0 (ng/mL)</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are expressed as median (range).

Abbreviations: Cmax, maximum plasma concentration; Tmax, time at the maximum concentration; AUC, area under the concentration versus time profile; D, dose; ND, non-detectable or below the limit of quantitation.

Figure 1. Individual plasma concentrations of (A) total and (B) free mycophenolic acid (MPA) over time in seven pediatric patients with nephrotic syndrome and FSGS after the first oral administration of (■) 696 mg/m² (+/- 72), (●) 425 mg/m² (± 60).

Figure 2. Individual plasma concentrations of (A) total and (B) free mycophenolic acid (MPA) versus time profiles after the morning dose at steady state (12 weeks) of (■) 696 mg/m² (+/- 72) and (●) 366 mg/m².
420 mg/m$^2$ to 700 mg/m$^2$. The AUC for total MPA after the first dose, 12 weeks and 24 weeks was 5.6 µg*h/mL, 13.1 µg*h/mL and 18.6 µg*h/mL, respectively while for the Cmax of the initial values were 5.6 µg/mL, 10.7 µg/mL and 15.8 µg/mL respectively. In this case, the concentration versus time profiles after the three studied occasions are shown in Figure 4A where total MPA was depicted versus time and Figure 4B for free MPA profiles.

Lastly, the two patients that developed partial remission showed a total MPA AUC of 13.6 µg*h/mL and 18 µg*h/mL and Cmax values of 11.6 µg/mL and 8.8 µg/mL after the first dose of SMPA. Moreover, free MPA AUC was 368 ng*h/mL and 148.1 ng*h/mL and free Cmax of 329 ng/mL and 78.8 ng/mL.

5. Discussion

The present study shows that SMPA could be a second line option for children with FSGS resistant to steroids. Two patients showed partial remission after the 12-months period of treatment with SMPA and all seven patients treated with this agent are alive with remaining normal renal function. The systemic exposure to MPA was lower than expected and it rapidly disappeared from plasma after intake without showing accumulation at steady state. The low systemic exposure found in this study in addition to the absence of adverse events observed to MPA support an increase in SMPA dose for children with this pathology.

Pediatric patients with NS and FSGS are at high risk of end-stage kidney disease and kidney survival is closely related to the degree of proteinuria remission (1). Therefore, efforts have been focused on pharmacological option to preserve kidney function while controlling proteinuria (4). Initial patient treatment most commonly consists of steroids resulting in remission in some cases. However, lack of response and frequent relapses have motivated the use of other immunosuppressive agents including cyclophosphamide and calcineurin inhibitors but severe toxicities have also limited their use (6-8). Thus, new agents are needed and in this scenario MPA has emerged as a promising candidate (9-11). MMF, the prodrug moiety, has emerged as a promising alternative agent in pediatric NS and FSGS. However, gastrointestinal and hematological side effects to MPA may result in poor patient compliance. Thus, the present study aimed at evaluating the beneficial use of the enteric coated sodium salt of MPA (SMPA) as an alternative immunosuppressive agent for children with FGS non-responsive to steroid and cyclophosphamide.

An important cause of glomerular filtration impairment in NS resides in podocin, a membrane protein in the podocytes encoded by NPHS2 gene. Mutations in NPHS2 are one of the most frequent molecular cause of pediatric NS and might determine steroid resistance (25). Therefore, genetic testing aids the clinicians to guide the pharmacological treatment avoiding exposure to drugs that would only contribute to unnecessary toxicity and consider the renal transplant in those patients with a genetic origin of steroid-resistant nephrotic syndrome (SRNS) (28,29). Nonetheless, scarce data is available about the prevalence of NPHS2 mutations in pediatric Latin American patients (30). In our study, no pathogenic variants in NPHS2 were found and most of the observed polymorphisms were previously reported and classified as benign.

Two patients included in the present study showed a partial remission after 1 and 2 years after treatment with SMPA. Interestingly, these patients reduced the proteinuria in 83% and 57%, serum albumin increased in 61% and 47%, and serum cholesterol returned to normal levels. Both patients received about 740 mg/m$^2$/d of SMPA, greater than the median dose of 500 mg/m$^2$/d that was given to patients that did not respond. Moreover, we did not observe any of the common adverse events to SMPA. This finding could be related to better tolerance to SMPA compared to MMF and/or due to low systemic exposure of MPA. Nonetheless,
the small sample size could have prevented detection of additional SMPA treatment efficacy and also toxicity. Altogether, the efficacy and safety encountered in the present study support an SMPA dose at least of 740 mg/m²/d to attain partial remission.

The therapeutic response to immunosuppressive treatment also depends upon genetic heterogeneity; as patients having genetic mutations do not achieve remission with immunosuppressive drugs (31-33). In addition, lack of response to treatment can be explained by low drug exposure. In this sense, the low levels of albumin and of total and free MPA leads us to speculate that although the drug is rapidly absorbed, it is also eliminated because of low number of sites of binding to plasma proteins due to hypoalbuminemia. Specifically, hypoalbuminemia is a typical feature of patients with SRNS (4). In pediatric patients it has been shown that MPA extensively bound to serum albumin with a mean protein binding of 97% (19,20). Factors that modify albumin levels may impact on MPA binding to albumin, the amount of free MPA, and therefore the efficacy and safety of the drug as only free MPA is pharmacologically active. In our study, free-to-total MPA AUC and Cmax after the first dose was 1.7% and 1.8% and after three months of SMPA treatment it was 2.2% and 1.1%, respectively, in correspondence to other reports in transplant patients. Thus, if a dose increase is proposed to attain therapeutic levels in patients with FSGS treated with SMPA it should be taken into account that this increase may not be accompanied by a proportional and higher free MPA exposure under hypoalbuminemia conditions despite we observed a proportional increase in the systemic exposure of free-to-total MPA. Moreover, we observed a proportional increase in systemic exposure with a 2-fold increase in AUC after almost doubling the dose in one patient that was subjected to dose escalation. Thus, this result demonstrates that systemic exposure of total MPA can increase with dose.

To date, there is no dose recommendation for MMF treatment in pediatric patients with NS and FSGS. Most of the proposed doses have been based on dosages used in pediatric transplant patients and some studies reported the experience in patients with NS but receiving MMF (21,22,34). Children with NS have shown a complete remission if systemic exposure to MPA was 60 µg*h/mL while patients with partial or total relapse showed lower levels at a daily dose of MMF of 600 mg/m²/bid (21). In addition, the same authors proposed a trough concentration of MPA greater than 3 µg/mL to avoid proteinuria recurrence. Others recently proposed a threshold of 45 µg*h/mL for MPA systemic exposure in order to achieve and maintain remission in pediatric patients with idiopathic NS at initial MMF doses of 800-1200 mg/m². Interestingly, it was also emphasized the importance of therapeutic drug monitoring of MMF in achieving the target systemic exposure for MPA in this vulnerable population. In our study, total SMPA exposure was lower than 20 µg*h/mL, both after the first dose and after 3 months of treatment (22). The lack of complete remission in our patients may have been related to low SMPA systemic exposure. After a rapid rise MPA maximum plasma concentration attained a value of about 10 µg/mL but was followed by a quick drop of the drug levels to undetectable after six hours of drug administration after the first dose and less than 1 µg/mL after three months of treatment. Our results show the low or almost no MPA accumulation in the study population. Nonetheless, two patients showed partial remission in our study after 1 and 2 years after SMPA treatment showing MPA AUC values of about 14 µg*h/mL and 18 µg*h/mL after the first dose and one of them of 27 µg*h/mL at steady state (the other patient refused for PK re-analysis). Then, we propose that further studies should be carried out on this population to define the systemic exposure related to partial and complete remission and emphasize the role of pharmacokinetic studies in order to control the immunosuppressive drug exposure and thereby increase the probability of remission in SRNS.

6. Conclusions
In conclusion, based on our results and previous reports about systemic exposure to MPA associated with response in pediatric nephrotic syndrome, it would be advisable to increase the frequency of administration if administering enteric-coated mycophenolate sodium to attain higher than observed systemic exposure but also drug accumulation. We support the use of therapeutic drug monitoring of MPA to optimize drug therapy and attain remission without serious adverse events in children with nephrotic syndrome and FSGS.

Limitations of the study
This study was conducted on a limited number of patients and therefore, further larger studies on pediatric nephrotic patients should be performed.

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**Authors’ contribution**
MGC, JI, VA, PCG, LC, MB and PS conducted the research and contributed to the conception and design of the research. MGC and JI contributed to the acquisition of data. MGC, JI, VA and PS contributed to the analysis of data. MGC, JI, PCG, VA and PS contributed to the drafting of the manuscript and final preparation of the manuscript. All authors read and signed the final paper.

**Conflicts of interest**
The authors declare that they have no conflict of interest.

**Ethical considerations**
Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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**References**
20. Filler G, Alvarez-Elias AC, McIntyre C, Medeiros M. The compelling case for therapeutic drug monitoring...

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