Sirolimus-based regimen is associated with decreased expression of glomerular vascular endothelial growth factor

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Abstract

Background. Sirolimus (SRL) is a potent immunosuppressant used in organ transplantation. It is known to decrease vascular endothelial growth factor (VEGF) synthesis, making it an interesting treatment option for transplant patients who develop Kaposi sarcoma or other malignant diseases. Because VEGF plays a key role in glomerular function and vascular remodelling, we determined the effect of SRL on renal VEGF expression.

Methods. Using immunohistochemistry and quantitative image analysis, we examined renal VEGF expression in routine kidney biopsies performed at 1 year post-transplant in the CONCEPT study, a prospective randomized study comparing a cyclosporine (CsA)-based regimen to a SRL-based regimen in association with mycophenolate mofetil (MMF).

Results. A total of 74 patients were included in this substudy; 35 were randomized to the CsA group and 39 to the SRL group. Using continuous variables, the mean percentage of glomerular VEGF expression at Week 52 was significantly lower in the SRL group (14.7 ± 13%) compared to CsA group (21.2 ± 14%; P = 0.02). The percentage of glomerular VEGF expression at Week 52 was not influenced by recipient or donor age, gender, renal function, CsA dose, CsA blood level, SRL dose or SRL blood level. It was significantly lower in patients with a proteinuria over versus below 0.5 g/day (11.58 ± 7.9 versus 19.45 ± 15.53; P = 0.036).

Conclusions. There is emerging evidence that the VEGF system can play either a beneficial or a detrimental role depending on the specific pathologic situations. Therefore, modulating the renal VEGF axis by using an SRL-based regimen may influence the evolution of kidney injury associated with renal transplantation.

Keywords: kidney transplantation; sirolimus; vascular endothelial growth factor

Introduction

The introduction of mammalian target of rapamycin (mTOR) inhibitors to the treatment of renal transplant patients has increased the repertoire of immunosuppressive protocols. The mTOR inhibitors have proven their efficacy and safety in numerous studies and are used either de novo or as a substitute in the follow-up treatment after renal transplantation [1–6]. The various advantages and disadvantages of using mTOR inhibitors at different time-points following renal transplantation led to the concept of sequential immunosuppression [7]. We recently reported a new strategy to combine sirolimus (SRL) with mycophenolate mofetil (MMF) early after transplantation (CONCEPT study) [8]. The introduction of SRL after 3 months was associated with an improvement in renal function at 1 year compared to the cyclosporin (CsA)-based protocol.

SRL is an antifungal macrolide that displays potent antiproliferative activities, producing immunosuppressive effects. Binding of SRL to the intracellular immunophilin FKBP12 blocks the activity of mTOR, resulting in potent inhibition of downstream signalling and of progression from the G1 to the S phase of the cell cycle. mTOR controls the phosphorylation of proteins that regulate the cell cycle and is also involved in the regulation of the production of growth factors including vascular endothelial growth factor (VEGF), which plays a key role in endothelial survival [9] and signalling as well in pathological angiogenesis [10]. The mTOR inhibitor, SRL, inhibits the production of VEGF in different tumour cell lines in vitro and in vivo [11]. In contrast, the calcineurin inhibitors can promote VEGF messenger RNA stability and synthesis in renal cancer cells [12].
In the kidney, VEGF is principally produced by the visceral glomerular epithelial cells (podocytes) [13, 14]. Reduction of VEGF production by podocytes in transgenic mice leads to proteinuria and endotheliosis, which are glomerular lesions that occur in preeclampsia and in thrombotic microangiopathy (TMA) [15, 16]. Notably, the risk of TMA has been reported to be significantly increased in patients receiving a SRL-based regimen [17]. We hypothesized that alteration of VEGF production by podocytes is one mechanism by which SRL may increase the risk of renal TMA due to our observation that renal VEGF expression during SRL-induced TMA was significantly lower than VEGF expression in normal transplanted kidneys [18]. SRL, by changing VEGF renal production, promotes existing endothelial cell lesions, providing a 'second hit' and increasing the risk of the development of TMA. However, the effect of mTOR inhibitors on VEGF synthesis by podocytes in normal transplanted kidney is unknown. It has been shown that mTOR inhibitors decrease the production of VEGF in cultured human podocytes in vitro and attenuates the increased expression of renal VEGF in vivo in rat models of renal injury [19–21]. However, there is no data examining normal allograft kidneys. On the other hand, in allografts with persistent injury, there is an excessive turnover of graft vascular endothelial cells and VEGF may be a mediator for the initiation of this leukocyte-induced angiogenesis [22] via the mTOR-Akt signalling pathway [23], suggesting that mTOR inhibitors may be beneficial. Therefore, to determine the effect of immunosuppressive agents on renal VEGF expression, we examined glomerular VEGF expression in routine kidney biopsies performed at 1 year post-transplant in the prospective randomized CONCEPT study that compared a Cyclosporine based regimen to an SRL-based treatment in association with MMF.

Materials and methods

Patients

Data were obtained from patients enrolled in the CONCEPT study. The CONCEPT study was a prospective, open-label, randomized, multi-center study that evaluated the conversion of a Cyclosporine based regimen to an SRL-based regimen 3 months after transplantation. The inclusion/exclusion criteria, detailed immunosuppressive regimens and baseline characteristics of the study cohort have been described previously [8]. Additional inclusion criteria for this histologic analysis study are (i) One year of follow-up was completed during which graft function was maintained and the assigned study medication was continuously administered; (ii) surveillance biopsy was successfully carried out at Week 52; and, (iii) biopsy specimen contained at least five glomeruli. This histological study was conducted in 12 of the 16 centres that had participated in the CONCEPT study. These 12 centres routinely performed surveillance biopsies following kidney transplantation. This ancillary study was reviewed and approved by the Institutional Review Board of Tours on 22 February 2005. Written informed consent was obtained from all study participants. Patient care and study conduct complied with good clinical practice and the Declaration of Helsinki guidelines.

Immunosuppressive regimen

All patients received a humanized anti-IL-2 receptor monoclonal antibody intravenously (daclizumab, Zenapax®; Roche) combined with 2 g mycophenolate mofetil (CellCept®, Roche) daily, cyclosporine (Neoral®, Novartis), and steroids. Patients were randomized at Week 12 between continuation of CsA with targeted C2 of 500–800 ng/mL (CsA group) or receiving SRL (SRL group). The SRL dose was adjusted to maintain a CO blood level between 8 and 15 ng/mL from Week 12 to Week 39, then between 5 and 10 ng/mL after Week 39. Discontinuation of the use of oral steroids was planned to occur at Month 8.

Study design

The glomerular VEGF expression at Week 52 post-renal transplantation was analysed according to clinical characteristics including randomization group, serum creatinine, glomerular filtration rate (eGFR) estimated according to a simplified modification of diet in renal disease equation, proteinuria, donor, and recipient age. Immunohistochemistry

Protocol renal biopsies performed at Week 52 were fixed, embedded in paraffin, sectioned and stained for light microscopy in each center (12 centers). Paraffin blocks were then harvested in one center (Reims) and kept until VEGF analysis was performed. Immunohistochemical studies were performed using the indirect immuno-peroxidase staining technique on a paraffin-embedded section with a haematoxylin counterstain and a Ventana Benchmark XT automatic analyser (Ventana Medical Systems, Tucson, AZ). Paraffin sections (4-μm) were cut the day of VEGF immunostaining. Sections were placed on poly-L-lysine-coated slides (SuperFrost III®), deparaffinized using the EZ-Prep kit (Ventana Medical Systems) at 75°C for 4 min, subjected to antigen retrieval for 8 min at 95°C in Tris buffer cell-conditioning solution® (pH 7.5) (Ventana Medical Systems) and treated at 37°C for 4 min with the UltraView Inhibitor® (Ventana Medical Systems) to block endogenous peroxidase. Sections were then incubated for 1 hour at 37°C with a monoclonal mouse antibody to VEGF (clone C1, Santa Cruz Biotechnology, Santa Cruz, CA) applied to the slide at a 1:750 dilution in antibody diluents (Ventana Medical Systems). Immunohistochemical staining was performed using an HRP kit (Universal HRP Multimer kit, Ventana Medical Systems). The complex was then visualized with a hydrogen peroxide substrate and 3,3'-diaminobenzidine tetrahydrochloride as the chromogen. The slide were rinsed with a Tris-based buffer solution, counter-stained with haematoxylin, post-counterstained with an aqueous solution of buffered lithium carbonate (Bluing Reagent, Ventana Medical Systems) and mounted. Negative controls were prepared by replacing the primary antibody with normal mouse serum.

Quantitative image analysis

The image analysis system, which basically consists of a microscope (Axioskop 2 Mot plus, Karl Zeiss, Germany), a colour digital camera (Axiocam MRC5, Karl Zeiss, Jena, Germany), a computer, and the KS300 Software by Karl Zeiss (Germany), was used to determine the intensity of the immunochemical staining. Stained tissue sections were viewed and acquired using the ??40 times objective. The entire tissue section was screened for glomeruli. For each slide, the haematoxylin counterstain differentiated the positive area (brown) from the negative area (blue), and the microscopic field was divided into very small, equal-sized elements (pixels) in a two-dimensional array, each associated with a value. To calculate the area fraction of a particular immunostained component, a threshold was applied to each image at a constant level that distinguished between the stained component (rendered red) and the unstained background (rendered blue). The proportion of red to blue pixels in the image was then calculated as a percentage. After merging each glomerulus, the computer calculates the percentage of the immunostained area. The reproducibility of VEGF immunostaining quantification by image analysis was tested. Ten paraffin-embedded biopsies were cut, stained and quantified at three different periods. The measure of reproducibility is given by the mean deviation standard (σ = (σ1 + σ2 + ... + σn)/n) and by the coefficient of variation (σ/μ). Immunohistochemical staining and staining quantification were performed in a blinded fashion.

Statistical analysis

Continuous values were expressed as mean ± SD. Dichotomous data were presented as percentages. The chi-square test was applied for dichotomous data, and unpaired t-tests were used to compare continuous variables.

Results

Study population

A total of 74 patients were included in the ancillary study, 35 were randomized to the CsA group and 39 to the SRL
group. Demographics and baseline characteristics at the time of transplant and randomization were not different between the two groups (Table 1).

Clinical outcome

Delayed graft function occurred in 24% of cases and was similar in both groups. Biopsy-proven acute rejection occurred after randomization in five patients in the CsA group (14%) compared to nine patients in the sirolimus group (23%; P = NS). Mean serum creatinine at Week 52 was significantly lower in the SRL group compared to the CsA group (P = 0.05). Mean eGFR at Week 52 was significantly higher in the SRL group compared to the CsA group (P = 0.04).

Immunosuppressive regimen

At Week 52, the mean value of cyclosporine and sirolimus doses was 221 ± 45 mg/day and 3.18 ± mg/day, respectively. Mean targeted blood levels were achieved for both SRL and CsA (Table 2). The mean mycophenolate mofetil dose was slightly lower in the SRL group than in the CsA group (not significant). Corticosteroids were withdrawn in 72% of the CsA group and in 80% of the CsA group (not significant).

VEGF immunostaining quantification

In agreement with previous studies, immunohistochemical staining of transplanted kidney localized VEGF protein to visceral epithelial cells in the glomeruli (Figure 1). Endothelial cells of renal arteries were also noted but the level of expression was much lower than that found in glomeruli. To test the reproducibility of VEGF immunostaining quantification, we analysed three sections from 10 biopsy specimens prepared on three separate days. The reproducibility was high, with a mean standard deviation estimated from repeated measurements of 1.16% and a coefficient of variation of 4.9%.

Using continuous variables, the mean percentage of glomerular VEGF expression at Week 52 was significantly lower in the SRL group (14.7 ± 13%) compared to the CsA group (21.2 ± 14%; P = 0.02) (Figure 2). The level of VEGF protein expression in the glomeruli was classified into four quartiles. Compared with the highest quartile, VEGF levels in the lowest quartile were significantly associated with SRL treatment (Table 3). The percentage of glomerular VEGF expression at Week 52 was not influenced by recipient or donor age, gender, renal function, CsA dose, CsA blood level, SRL dose or SRL blood level. No difference in VEGF expression was detected between extreme quartiles of cyclosporine C2 levels (data not shown). When we compared patients with proteinuria over versus below 0.5 g/day, we found that VEGF expression in podocytes was significantly lower in patients with proteinuria >0.5 g/day. This result was observed in SRL and CsA groups but reached significance only in the CsA group (Table 4).

Discussion

The present study was designed to determine the effect of SRL on renal VEGF expression in renal transplant recipients. We examined glomerular VEGF expression in routine kidney biopsies performed at 1 year post-transplant in the prospective randomized CONCEPT study comparing a cyclosporine based regimen to a SRL-based treatment plan [8]. Our results demonstrate that the mean percentage of glomerular VEGF expression at Week 52 was significantly lower in the SRL group (14.7 ± 13%) compared to the CsA group (21.2% ± 14%; P = 0.02). Moreover, our results show that most patients with the lowest glomerular VEGF expression (first quartile) were in the SRL-treated group. These results indicate that SRL decrease VEGF synthesis or that cyclosporine promote VEGF overexpression in normal transplanted kidney.

VEGF-A is a dimeric glycoprotein. Alternative exon splicing of a single VEGF gene results in at least six different isoforms. VEGF<sub>121</sub>, VEGF<sub>165</sub>, and VEGF<sub>189</sub> are the most abundantly expressed isoforms. VEGF<sub>121</sub> is secreted and freely diffusible, VEGF<sub>165</sub> is sequestered in the extracellular matrix and VEGF<sub>189</sub> is cell associated [24]. The three isoforms are recognized by the mouse monoclonal antibody anti-VEGF-A Clone-1 used in the current study. As shown by immunostaining, the VEGF protein localized predominantly to the cytoplasm of podocytes. Focal expression of VEGF can also be detected in endothelial cells of the renal arteries, but the level of expression is much lower and less consistent than that found in the glomeruli [25]. Therefore, we limited VEGF immunostaining quantification to glomeruli.

We found a high interindividual variation of glomerular VEGF expression that ranged from 4.4 to 55.39% in the

Table 1. Study population and characteristics of renal function at Week 52

<table>
<thead>
<tr>
<th></th>
<th>CsA group (n = 35)</th>
<th>SRL group (n = 39)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (% male)</td>
<td>63</td>
<td>64</td>
<td>ns</td>
</tr>
<tr>
<td>BMI</td>
<td>25.7 ± 5</td>
<td>24.3 ± 3</td>
<td>ns</td>
</tr>
<tr>
<td>Recipient age</td>
<td>47 ± 9</td>
<td>48 ± 11</td>
<td>ns</td>
</tr>
<tr>
<td>Donor age</td>
<td>42 ± 14</td>
<td>40 ± 12</td>
<td>ns</td>
</tr>
<tr>
<td>Number of delayed graft functions</td>
<td>6</td>
<td>4</td>
<td>ns</td>
</tr>
<tr>
<td>Creatininemia µM/L at Week 52</td>
<td>123 ± 38</td>
<td>107 ± 31</td>
<td>0.05</td>
</tr>
<tr>
<td>eGFR simplified MDRD formula (mL/min)</td>
<td>57 ± 18</td>
<td>65 ± 18</td>
<td>0.04</td>
</tr>
<tr>
<td>Proteinuria (g/day) at Week 52</td>
<td>0.21 ± 0.29</td>
<td>0.39 ± 0.6</td>
<td>ns</td>
</tr>
<tr>
<td>Number of episodes of BPAR</td>
<td>5</td>
<td>9</td>
<td>ns</td>
</tr>
</tbody>
</table>

*MDRD, modification of diet in renal disease.

Table 2. Drug doses and blood levels (C2 CsA and C0 SRL) at Week 52

<table>
<thead>
<tr>
<th></th>
<th>CsA group (n = 35)</th>
<th>SRL group (n = 39)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients with steroids</td>
<td>7</td>
<td>11</td>
<td>ns</td>
</tr>
<tr>
<td>CsA daily dose (mg/day)</td>
<td>221 ± 45</td>
<td></td>
<td></td>
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<tr>
<td>CsA blood levels (ng/mL)</td>
<td>761 ± 252</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRL daily dose (mg/day)</td>
<td>3.18 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRL blood levels (ng/mL)</td>
<td>10 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMF daily dose (g/day)</td>
<td>1.9 ± 0.3</td>
<td>1.75 ± 0.47</td>
<td>ns</td>
</tr>
</tbody>
</table>
CsA group and from 0 to 62.5% in the SRL group (Figure 2). This variation was not due to the technical procedure because the immunostaining was highly reproducible on identical test material with a low coefficient of variation. Sampling error cannot be excluded since there is no data showing that a single biopsy is representative of the entire allograft with regard to VEGF expression. However, to limit the impact of sampling error, we only include renal biopsies with at least five glomeruli in our analyses. Intra-renal factors such as acute rejection [18], chronic rejection [26], chronic CsA nephrotoxicity [27] and ischaemia-reperfusion [28] have been shown to modulate the production of glomerular VEGF. None of these factors had been identified in our patients at the time of biopsy. Furthermore, we did not find any correlation between VEGF expression and recipient age, donor age, gender, renal function, CsA dose, CsA blood level, SRL dose or SRL blood level. The absence of an association between VEGF expression and CsA or SRL blood levels may be due to the fact that immunosuppressive agents levels were relatively homogeneous due to the protocol observance. When patients were subdivided according to those having above or below 0.5 g/day of proteinuria, we found that VEGF expression in podocytes was significantly lower in patients with proteinuria >0.5 g/day. Interestingly, this result is in agreement with the proteinuria effect observed with the use of angiogenesis inhibitors therapies in humans [29]. The causes of the interindividual variation of VEGF expression remains unclear. The VEGF gene is highly polymorphic and certain polymorphisms are associated with alterations in the expression of VEGF [24]. Some VEGF polymorphism may participate in the interindividual variation of renal VEGF expression observed in our study. The renal VEGF expression level is probably the result of genetic, intrarenal and systemic factors. Immunosuppressive treatment is one of these factors. Finally, the expression of VEGF does not necessarily reflect its bioactivity. Indeed, the bioactivity of glomerular VEGF is regulated by numerous local factors such as an inhibitory VEGF splice variant, VEGF-C, TGF-β and endothelin-1 [30]. VEGF expression is one factor among many in the local glomerular environment that regulate VEGF bioactivity.

Fig. 1. Glomerular VEGF expression in protocol renal biopsies performed at Week 52 from three patients included in the SRL-treated group (A, B and C) and three patients included in the CsA group (D, E and F). The VEGF immunostaining quantification using image analysis was 3% in Patient A, 23% in B, 56% in C, in 8.8% in D, 22% in E and 58% in F. Results are expressed as the percentage of the glomerular area fraction. Magnification: ×200.

Fig. 2. Glomerular VEGF expression in protocol renal biopsies performed at Week 52 in the CsA group (n = 35) and the SRL group (n = 39). Results are expressed as percentage of the glomerular area fraction.
VEGF is a growth factor that exerts a paracrine survival effect on glomerular endothelial cells. Reduction of VEGF production in podocytes in transgenic mice leads to proteinuria and endotheliosis, the glomerular lesions occurring in preeclampsia and in TMA [15, 16]. Conversely, exogenous VEGF treatment accelerates renal recovery in an experimental model of TMA [31]. In humans, treatment of malignant tumours with VEGF antagonism can lead to proteinuria and TMA [15]. It is therefore possible that the downregulation of renal VEGF observed in patients with SRL-based regimen is a predisposing factor for TMA. This interpretation is supported by an experimental study in rats, where the mTOR inhibitor, everolimus, has been shown to reduce glomerular VEGF and exacerbate the acute phase of glomerular endothelial injury in a model of TMA [20]. Interestingly, in this study, once the cause of acute endothelial injury was stopped, everolimus therapy did not disturb the long-term recovery of TMA lesions. This result indicates that mTOR inhibitors do not induce TMA alone but may act as a predisposing factor of TMA by rendering the glomerular endothelium more susceptible to injury or less easy to repair. In addition to its angiogenic function, VEGF exerts an autocrine survival effect on podocytes [32]. Therefore, inhibition of VEGF production by SRL may also predispose to podocyte injury and to focal segmental glomerulosclerosis, lesions that are observed in renal biopsies of SRL-treated patients [33]. While the downregulation of renal VEGF may be a predisposing factor for glomerular injury, the upregulation of renal VEGF may also participate in glomerular lesions. Indeed, podocyte-specific overexpression of VEGF in transgenic mice resulted in a collapsing glomerulopathy [16]. Furthermore, an increase in the production of VEGF has been proposed to play a significant role in the development of glomerular hypertrophy and albuminuria in experimental diabetic nephropathy [34] and in the renal ablation model [19]. Notably, in the rat model of reduced renal mass, SRL had a favourable effect. It attenuated the increased expression of renal VEGF, reduced the incidence of glomerulosclerosis and halted the progression of proteinuria [19]. In the CONCEPT study, we reported that conversion from CsA to SRL 3 months after transplantation significantly improved renal function at 1 year [8]. We recently showed that the renal benefits associated with conversion of CsA to SRL, at 3 months post-transplantation, were maintained 4 years post-transplantation [35]. However, mean proteinuria was significantly increased in the SRL group. One may speculate that determination of renal VEGF expression at 3 months post-transplantation before conversion of CsA to SRL may be one way to predict the risk of proteinuria. This hypothesis is under investigation.

Moreover, VEGF has been found to have proinflammatory properties in vivo and enhanced expression of VEGF has been found in association with T cell, monocyte and macrophage infiltrates in human cardiac allografts [36] and have been correlated with enhanced cardiac allograft arteriosclerosis in experimental models [37]. Targeting VEGF-R reduces myelomonocyte infiltration and cardiac allograft arteriosclerosis [38] and TNP-470, an angiogenesis inhibitor, attenuates the development of allograft vasculopathy [39]. Thus, a balance of the VEGF system seems to be important for glomerular function and glomerular endothelial cell regeneration. There is emerging evidence that the VEGF system can play either a beneficial or a detrimental role depending on the pathologic situation. Therefore, modulation of the renal VEGF axis by SRL-based treatments may influence the evolution of kidney injury associated with renal transplantation.

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**Conflict of interest statement.** The following author of this manuscript has conflicts of interest to disclose as described by Nephrology Dialysis Transplantation: S.G.S. is employee of the study sponsor. The others authors of this manuscript have no conflicts of interest to disclose as described by Nephrology Dialysis Transplantation.

**References**


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**Table 3.** Quartile distribution of glomerular VEGF staining (percentage of area fraction)

<table>
<thead>
<tr>
<th>VEGF staining (%)</th>
<th>SRL treatment (% of patient)</th>
<th>Serum creatinine µM/L at Week 52</th>
<th>Proteinuria (g/day)</th>
<th>Recipient age</th>
<th>Donor age</th>
<th>CsA blood level (ng/mL)</th>
</tr>
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<tbody>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>3 ± 2.9</td>
<td>73*</td>
<td>111 ± 40</td>
<td>0.3 ± 0.47</td>
<td>46 ± 10</td>
<td>41 ± 12</td>
</tr>
<tr>
<td>Q2</td>
<td>12 ± 1.8</td>
<td>56</td>
<td>115 ± 36</td>
<td>0.7 ± 1</td>
<td>49 ± 11</td>
<td>40 ± 13</td>
</tr>
<tr>
<td>Q3</td>
<td>18 ± 2.5</td>
<td>50</td>
<td>125 ± 44</td>
<td>0.26 ± 0.26</td>
<td>49 ± 11</td>
<td>42 ± 12</td>
</tr>
<tr>
<td>Q4</td>
<td>36 ± 10</td>
<td>39</td>
<td>112 ± 19</td>
<td>0.13 ± 0.14</td>
<td>46 ± 8</td>
<td>41 ± 15</td>
</tr>
</tbody>
</table>

*P < 0.05 Q1 versus Q4.

**Table 4.** Glomerular VEGF staining according to proteinuria

<table>
<thead>
<tr>
<th>Proteinuria</th>
<th>VEGF staining (%)</th>
<th>All patients</th>
<th>CsA group</th>
<th>SRL group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinuria &lt; 0.5 g/day</td>
<td>19.45 ± 15.53</td>
<td>24.10 ± 14.81</td>
<td>15 ± 15.22</td>
<td></td>
</tr>
<tr>
<td>Proteinuria &gt; 0.5 g/day</td>
<td>11.58 ± 7.9</td>
<td>12.21 ± 7.45</td>
<td>11.09 ± 9.08</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.036</td>
<td>0.041</td>
<td>0.467</td>
<td></td>
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