The therapeutic potential of indoleamine 2.3-dioxygenase in kidney transplantation

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Chapter 5

Early posttransplant tryptophan metabolism predicts long-term outcome of human kidney transplantation


submitted
Abstract

Background: Chronic transplant dysfunction (CTD) is the leading cause of long-term loss of the renal allograft. So far, no single test is available to reliably predict the risk for CTD. Monitoring of tryptophan metabolism through the enzyme Indoleamine 2.3-dioxygenase (IDO) has been previously proposed to predict acute rejection of human kidney transplants. Here we investigate the potential of IDO to predict the long-term outcome of human kidney transplantation.

Methods: During the 2 year follow-up blood, urine and kidney biopsies were collected from 48 renal transplant patients. Concentrations of kynurenine and tryptophan in serum and urine were measured 2 weeks, 6 months and 2 years after transplantation. Kynurenine to tryptophan ratio (kyn/trp) was calculated as an estimate of IDO activity. To evaluate the histological changes and IDO expression, PAS staining and immunohistochemistry for IDO was performed on biopsies taken at 6 months and at 2 year follow-up. Correlation analysis between tryptophan, kynurenine, kyn/trp ratio, clinical data and immunohistochemistry was performed.

Results: Urine IDO activity 2 years after transplantation was increased as compared to 2 week values, whereas serum IDO activity decreased. In 2 year biopsies IDO expression was found in infiltrating inflammatory cells and in the glomeruli. The amount of IDO staining did not correlate with the histologic damage in the renal biopsies, nor with the levels of tryptophan, kynurenine and kyn/trp ratio in serum and urine. Serum level of kynurenine 6 months after transplantation predicted the serum creatinine 2 years after transplantation. Additionally, the urine level of tryptophan 2 weeks after transplantation predicted the serum creatinine 6 months and the estimated creatinine clearance 2 years after transplantation.

Conclusion: Early serum and urine levels of tryptophan and kynurenine predicted the 2 year outcome in patients after transplantation. Therefore, these parameters might offer a novel route for early diagnosis of CTD.
**Introduction**

The short-term outcome of renal transplantation has improved during the past 20 years. The introduction of cyclosporine in the early 80’s and the use of newer and a combination of immunosuppressive drugs such as mycophenolate mofetil and mTOR (mammalian target of rapamycin) inhibitors have substantially increased the rate of short-term graft survival. However, long-term graft survival has not noticeably improved. Among all causes of long-term graft failure, chronic transplant dysfunction (CTD) is the most common indication for re-transplantation. The cause of CTD is multifactorial, with allore sponsiveness, diabetes mellitus, hypertension and nephrotoxicity of immunosuppressants having a role herein. Clinically, hypertension, increased levels of serum creatinine and proteinuria are found in the patients with signs of CTD. The definite diagnosis requires histological analysis of a kidney biopsy in which interstitial fibrosis, tubular atrophy and glomerulosclerosis are indicators for CTD. Although needle biopsy of the graft is the most sensitive diagnostic method, there is a 5-10% risk of biopsy-associated complications, such as hematoma, infections (including sepsis and peri-renal abscess) and even loss of the renal graft.

As currently no single test is available to accurately predict the risk for CTD, the search for a suitable biomarker for CTD is still ongoing. In contrast to biopsies, such a marker would allow close monitoring of the development of CTD, especially in high risk patients. Consequently, the use of renal graft biopsy for graft monitoring may be minimized. Additionally, immunosuppressive therapy could be tailored according to the individual risk profile.

Monitoring of tryptophan (trp) metabolism through the enzyme Indoleamine 2.3-dioxygenase (IDO) has been previously proposed to predict acute rejection in renal transplant patients. IDO catalyzes the initial and rate-limiting step of tryptophan oxidative catabolism with formation of several intermediaries, collectively referred to as kynurenines (kyn). The rate of tryptophan degradation, expressed as the kyn/trp ratio, has been used as a good estimate of enzymatic activity of IDO.

IDO has been documented to be critically involved in establishing immune tolerance against paternal antigens in pregnant mice and in inducing T-cell unresponsiveness. Furthermore, several studies indicate IDO activity or levels of its substrate and/or metabolites to associate with or predict acute rejection. Brandacher et al. documented elevated serum and urinary kyn/trp ratios during acute rejection of human kidney transplants. Further, Lahdou et al. found that increased pre-transplantation serum kynurenine levels predict acute kidney allograft rejection in humans. Additionally, IDO activity correlates with the
severity of chronic kidney disease\textsuperscript{13}. There is yet no data regarding IDO activity or tryptophan metabolism in relation to the development of CTD or in long-term, uncomplicated renal transplantation. As a first step towards assessing the potential use of IDO activity as a biomarker in patients with CTD, we analyzed the levels of tryptophan and kynurenines in both serum and urine samples during a follow-up period of 2 years in kidney transplant patients. Moreover, level of rejection (Banff score) and IDO expression in renal biopsies was assessed using (immuno)histochemistry and correlation and multivariate regression analyses were performed between tryptophan metabolism and long-term renal outcome.
Material and Methods

Study design and patient population

Forty eight patients (thirty two male and sixteen female, aged between 18 and 70 years), receiving a kidney transplant were included in a 24 month, prospective, randomized trial. The immunosuppressive treatment prior to the transplantation and during the follow-up was previously described\(^2\). Briefly, the peri-operative immunosuppressive regimen consisted of 20 mg badiliximab i.v. prior to transplantation and on day 4 and two doses of 50 mg prednisolone i.v. during the first 48 hrs. During the first 6 months after transplantation, all patients received oral prednisolone (P) and triple-drug therapy consisting of cyclosporine A (CsA), mycophenolic sodium (MPS) and everolimus (EVL) (Table 1). After 6 months, the patients were randomized to double therapy with P/CsA, P/MPS, or P/EVL. Patients with histologic features of rejection continued on the triple-drug medication. Immunosuppressive drug exposure was closely monitored and its level was adjusted when necessary. Scheduled renal biopsies were performed at 6 months and 2 years after transplantation.

Table 1. Immunosuppressive therapy during the follow up. During the first six months after transplantation all patients received triple therapy consisting of CsA, MPS and EVL. The drug regimen thereafter consisted of double therapy with P/CsA, P/MPS, or P/EVL or continuation of the triple-drug medication (triple therapy). P-prednisolone; CsA-cyclosporine A; MPS-mycophenolate sodium; EVL-everolimus

<table>
<thead>
<tr>
<th>first 6 months after transplantation</th>
<th>after 6 months</th>
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<tbody>
<tr>
<td>triple therapy (CsA+MPS+EVL)</td>
<td>P/CsA</td>
</tr>
<tr>
<td>patients (n)</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>6</td>
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<td>12</td>
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</table>

Clinical parameters

Serum and urine from these patients were collected at 2 weeks (2 wk), 6 months (6 mo) and 2 years (2 yrs) after transplantation. Clinical parameters (blood pressure, body weight) as well as serum creatinine and albuminuria were measured at each time point. Estimated creatinine clearance was calculated according to the Cockroft-Gault formula \((140 – \text{age}) \times \text{body weight} \times \text{gender coefficient/serum creatinine})^{13}. 

Tryptophan metabolism predicts long-term renal
Tryptophan and kynurenine measurement

The concentration of tryptophan and kynurenines in serum and urine was measured by a high-throughput on-line solid-phase extraction-liquid chromatographic-tandem mass spectrometer (XLC-MS/MS), as described earlier\textsuperscript{21}. Briefly, fifty microliters of serum or urine were pre-purified by automated on-line solid-phase extraction, using strong cation exchange (PRS, propylsulphonic) cartridges. Chromatographic separation of the analytes and deuterated analogues occurred by C18 reversed phase chromatography. Mass spectrometric detection was performed in the multiple reaction-monitoring mode using a quadrupole tandem mass spectrometer with positive electrospray ionization. Detection limit was 30 nmol/l for tryptophan and 1 nmol/l for kynurenines. Finally, kynurenine/tryptophan (kyn/trp) ratio was calculated as an indirect estimate of IDO activity.

Histology and Immunohistochemistry

PAS staining was performed on biopsies taken at 6 months and at 2 year follow-up, according to the standard protocol. Immunohistochemistry for IDO was performed on biopsies taken at 6 months and at 2 years after transplantation. Normal kidneys were used as controls. Biopsies were dewaxed and subjected to antigen retrieval by 15 min incubation in 0.1M Tris/HCl buffer, pH 9.0, at 80°C. Mouse hybridoma anti-IDO primary antibody was used to assess the IDO expression. For immunohistochemistry, a three-step immunoperoxidase technique was used, according to standard techniques. Peroxidase activity was developed using 3, 3’-diaminobenzidine tetrachloride and H2O2. The cortical staining was measured using an Aperio-Image Scope based protocol and these values were further used in the correlation analysis.

Statistical analysis

Data are presented as mean ± SEM in case of normal distribution and as median (interquartile range) in case of skewed distribution. Significance was tested with the ANOVA for repeated measures followed by a least significant difference post hoc test, or with the Friedman test, as appropriate (SPSS). The potential relationships between tryptophan and kynurenine concentrations and kyn/trp ratio and clinical parameters were analyzed using Pearson’s parametric correlation test or Spearman’s non-parametric test as appropriate (SPSS). Multivariate linear regression analyses with forward stepwise procedure were performed to identify significant predictors of long-term renal outcome (plasma creatinine and albuminuria as dependent variables) among the concentrations of tryptophan and kynurenine and kyn/trp ratio 2 weeks and 6 months after transplantation.
(independent variables). To avoid multicollinearity, the independent variables which were in correlation were excluded from data set and included to another data set where no inter-variable correlation occurred. Differences were considered significant at p<0.05.
Results

Patients, assessment of the biopsies and clinical follow-up

Table 2 gives an overview of the demographic characteristics of the patients.

Table 2. Demographic characteristics of the patients

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<table>
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<tbody>
<tr>
<td>Number of patients (male/female)</td>
<td>48 (32/16)</td>
</tr>
<tr>
<td>Age in years (mean ± SEM)</td>
<td>49.2 ± 2.0</td>
</tr>
</tbody>
</table>

Cause of end-stage renal disease (number of patients)

- Glomerulonephritis     12
- Polycystic kidney disease    10
- Urological (postrenal) causes      7
- Hypertensive nephropathy     3
- Vascular                        2
- Diabetes mellitus            1
- Other                          13

Donor (number of patients)

- Living nonrelative donor    10
- Living relative donor       9
- Cadaveric donor             29

Mean duration of cold ischemia (hrs)  11.1 ± 1.2

Body weight, blood pressure, serum creatinine and albuminuria were monitored during the 2 year follow up. These data are depicted in Table 3. Body weight increased significantly at 2 years as compared to 2 week values. There were no significant changes in systolic blood pressure between all three time points however diastolic blood pressure was significantly elevated at 6 months and 2 years after transplantation, compared to the values at 2 weeks after transplantation. Serum creatinine and estimated creatinine clearance decreased at 6
months after transplantation and remained at this level until the end of the follow-up. A small but significant increase in albuminuria was seen at 6 months and 2 years after transplantation, compared to the second posttransplantational week. There were no significant differences between the four arms of immunosuppressive treatments (data not shown).

Table 3. Clinical parameters, serum creatinine and albuminuria during the follow-up. Data are expressed as mean ± SEM in case of normal distribution and as median (interquartile range) in case of skewed distribution; * p<0.05 versus 2 weeks.

<table>
<thead>
<tr>
<th></th>
<th>2 weeks</th>
<th>6 months</th>
<th>2 years</th>
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<tbody>
<tr>
<td>Body weight (kg)</td>
<td>74.4 ± 2.0</td>
<td>79.4 ± 2.1</td>
<td>81.0 ± 2.1*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>143.6 ± 2.8</td>
<td>145.4 ± 2.3</td>
<td>150.4 ± 1.9</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>81.1 ± 1.5</td>
<td>90.7 ± 2.1*</td>
<td>91.1 ± 1.6*</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>102.0 ± 1.7</td>
<td>109.0 ± 1.9*</td>
<td>110.9 ± 1.8*</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>164 (332)</td>
<td>118.5 (48.2)*</td>
<td>114 (68)*</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>49.4 (37.8)</td>
<td>72.5 (28.9)*</td>
<td>65.2 (33.8)*</td>
</tr>
<tr>
<td>Albuminuria (mg/24 hrs)</td>
<td>34 (5)</td>
<td>42 (2.5)*</td>
<td>40 (4)*</td>
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</tbody>
</table>

Based on histological analysis of the renal biopsy at 6 months follow-up, from the 48 patients studied, 3 patients suffered from acute rejection (Banff score 1A), 5 patients were on the borderline of acute rejection, 15 patients showed slight reactive changes and 15 patients did not show any significant histological changes. For six patients no biopsy was performed. Four biopsies showed no adequate or no kidney tissue.

Table 4 depicts the results of the histological analysis at 2 year follow-up. Two patients showed clear signs of acute rejection (Banff 1A), 25 patients showed a borderline acute rejection pattern and 23 patients showed various degrees of interstitial fibrosis and tubular atrophy (IFTA). In 15 patients both signs of borderline acute rejection as well as IFTA were found. No significant changes were found in 10 biopsies. For 3 patients no biopsy was available or the biopsy was inadequate.
Table 4. Histologic changes at 2 years. From the 48 patients included, for 3 patients no biopsy was available. IFTA = interstitial fibrosis and tubular atrophy.

<table>
<thead>
<tr>
<th>Histologic changes</th>
<th>Number</th>
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<tbody>
<tr>
<td>Acute rejection Banff 1A</td>
<td>2</td>
</tr>
<tr>
<td>Borderline acute rejection</td>
<td>25</td>
</tr>
<tr>
<td>IFTA</td>
<td>23</td>
</tr>
<tr>
<td>IFTA + Borderline acute rejection</td>
<td>15</td>
</tr>
<tr>
<td>No changes</td>
<td>10</td>
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</table>

Serum tryptophan, kynurenine and kyn/trp ratio

In comparison with 2 weeks after transplantation, the serum level of tryptophan was increased at 6 months and 2 years after transplantation and slightly decreased at 2 years in comparison with 6 months (Figure 1A, p<0.05). Serum level of kynurenine increased at 6 months as compared to 2 weeks and returned to the level of the 2 week by 2 years after transplantation (Figure 1B, p<0.05). Consequently, the serum IDO activity calculated as kyp/trp ratio was not changed at 6 months after transplantation as compared to the 2 weeks level and decreased significantly at 2 years (Figure 1C, p<0.05). No significant differences were found in serum tryptophan, kynurenine and kyn/trp ratio between the four immunosuppressive treatments (data not shown). In summary, IDO activity in serum is decreased at 2 years post-transplantation as compared to 2 week level.

Urine tryptophan, kynurenine and kyn/trp ratio

We also determined urine concentrations of tryptophan, kynurenine and kyn/trp ratio in our patients. The level of tryptophan at month 6 decreased significantly compared to week 2 (Figure 2A, p<0.05) and remained stable at 2 years post-transplantation. The urine level of kynurenine decreased significantly at 6 months compared to week 2 (Figure 2B, p<0.05). Consequently, no change was observed in kyn/trp ratio from week 2 to 6 months. However, the kyn/trp ratio did significantly increase at 2 years in comparison with both 2 weeks and 6 months (Figure 2C, p<0.05). No significant differences were found in urine tryptophan, kynurenine and kyn/trp ratio between the four immunosuppressive treatments (data not shown). In conclusion, IDO activity in urine is increased at 2 years post-transplantation as compared to 2 week and 6 months levels.
Figure 1. Changes in serum levels of tryptophan, kynurenine and kyn/trp ratio during the 2 years follow-up. The level of tryptophan (A) increased at month 6 after transplantation and remained increased thereafter. The level of kynurenine (B) increased at month 6 and again decreased at 2 years. The kyn/trp ratio (C) decreased during the follow-up. * p<0.05 vs 2 wk; # p<0.05 vs 6 mo; trp, tryptophan; kyn, kynurenine; wk, weeks; mo, months; yrs, years.
We further investigated the expression of IDO protein in the 6 month- and 2 year-biopsies, using immunohistochemistry. In normal kidneys, a very limited expression of IDO was found in some distal tubular epithelial cells. In the 6 month-biopsies of the patients with acute rejection, a clear expression of IDO was found in the infiltrating inflammatory cells that morphologically resembled macrophages and dendritic cells (Figure 3A). There was also some expression in glomerular (mostly endothelial) cells (Figure 3B). In the 2 year biopsies of the patients with signs of chronic damage (IFTA), IDO expression was found around the atrophic tubules (Figure 3C). There was also a variable amount of glomerular staining, with some glomeruli showing strong expression of IDO in cells morphologically resembling endothelial cells and mesangial cells (Figure 3D). A very limited expression was found in the tubular epithelial cells.
Figure 3. Localization of IDO in acute rejection (A, B) and chronic transplant damage (C, D). Arrows indicate positive cells, including interstitially located inflammatory cells (black arrows), glomerular endothelial cells (dashed black arrows) and mesangial cells (red arrow).

The amount of IDO staining, as assessed by morphometric analysis (data not shown), did not correlate with the histologic damage in the renal biopsies, nor with the levels of tryptophan, kynurenine and kyn/trp ratio in serum and urine.

*The serum level of kynurenine 6 months after transplantation predicted the serum creatinine at 2 years after transplantation*

Both serum kynurenine level and kyn/trp ratio at 6 months after transplantation correlated with the serum creatinine at 2 years after transplantation (Figure 4A, $R = 0.444$, Figure 4B, $R = 0.410$, $p<0.05$, respectively). Moreover, both serum kynurenine level and kyn/trp ratio at 6 months correlated with the serum creatinine at the same time point ($R = 0.548$, $R = 0.516$, $p<0.05$, respectively). Multiple regression analyses identified serum kynurenine level at 6 months as the only independent predictor for serum creatinine at 2 years (best fitting model: serum creatinine 2 years $= 85.3 + 19.8 \times$ kynurenine 6 months; $R = 0.336$;
p<0.05), when serum tryptophan 2 weeks, kynurenine 2 weeks and kynurenine 6 months were included as independent factors. We did not find correlations between tryptophan or tryptophan metabolites levels and the extent of the transplant damage, as assessed by the renal biopsies (data not shown).

Figure 4. The correlations between serum levels of kynurenine, kyn/trp ratio and serum creatinine. The level of kynurenine 6 months after transplantation correlated with the serum creatinine 2 years after transplantation (A). The serum kyn/trp ratio 6 months after transplantation correlated with the serum creatinine 2 years after transplantation (B). p<0.05; mo, months; yrs, years
The urine level of tryptophan 2 weeks after transplantation predicted the serum creatinine at 6 months and the estimated creatinine clearance 2 years after transplantation

We found that the urine tryptophan level and kyn/trp ratio at week 2 after transplantation correlated with the serum creatinine at month 6 and year 2 after transplantation, in the case of tryptophan the correlation being negative (Figure 5A, R = -0.281, Figure 5B, R = 0.341, Figure 5C, R = -0.319, Figure 5D, R = 0.326, p<0.05, respectively). Additionally, urine tryptophan level at 2 weeks correlated with estimated creatinine clearance 2 years after transplantation (R = 0.403, p<0.05). Moreover, both urine tryptophan and kynurenine levels at week 2 correlated with the albuminuria at year 2 after transplantation (Figure 5E, R = 0.285, Figure 5F, R = 0.365, p<0.05, respectively). Additionally, urine kyn/trp ratio at month 6 correlated with the albuminuria at year 2 after transplantation (Figure 5G, R = 0.342, p<0.05). Multiple regression analyses identified urine tryptophan level at 2 weeks as the only independent predictor for serum creatinine at month 6 and for estimated creatinine clearance at year 2 (best fitting model: serum creatinine 6 months = 116.2 - 0.49 x tryptophan 2 weeks; R = 0.530; p<0.05; best fitting model: estimated creatinine clearance 2 years = 52.4 + 0.36 x tryptophan 2 weeks; R = 0.497; p<0.05), when urine tryptophan 2 weeks, kyn/trp ratio 2 weeks, tryptophan 6 months and kyn/trp ratio 6 months were included as independent factors.

Thus, the urine tryptophan levels at 2 weeks significantly predicted the higher serum creatinine at 6 months.
tryptophan 2 wk (µmol/l)

serum creatinine 2 yrs (µmol/l)

R = -0.319
p < 0.05

Ckyn/trp ratio 2 wk

A

B

R = 0.326
p < 0.05

albuminuria 2 yrs (mg/24 hrs)

tryptophan 2 wk (µmol/l)

R = -0.281
p < 0.05

serum creatinine 6 mo (µmol/l)

R = -0.281
p < 0.05

D

R = 0.341
p < 0.05

C

D

kynurenine 2 wk (µmol/l)

R = 0.365
p < 0.05

F

E

R = 0.285
p < 0.05

R = 0.285
p < 0.05

R = 0.365
p < 0.05

R = 0.365
p < 0.05
Figure 5. The correlations between 2 weeks urine tryptophan, kyn/trp ratio and serum creatinine and albuminuria. The urine tryptophan level 2 weeks after transplantation negatively correlated with the serum creatinine 6 months after transplantation (A). The urine kyn/trp ratio at week 2 after transplantation correlated with the serum creatinine at month 6 after transplantation (B). The urine tryptophan level 2 weeks after transplantation negatively correlated with the serum creatinine 2 years after transplantation (C). The urine kyn/trp ratio at week 2 after transplantation correlated with the serum creatinine at year 2 after transplantation (D). The urine tryptophan level 2 weeks after transplantation correlated with the albuminuria 2 years after transplantation (E), as well as the urine kynurenine level 2 weeks after transplantation (F). The urine kyn/trp ratio 6 months after transplantation correlated with albuminuria 2 years after transplantation (G). p<0.05; trp, tryptophan; kyn, kynurenine; wk, weeks; mo, months; yrs, years; hrs, hours
Discussion

The current study demonstrates that the serum and urine levels of tryptophan and kynurenine measured early after renal transplantation (i.e. at 2 weeks and 6 months) predict long-term renal outcome after transplantation, as assessed by serum creatinine and albuminuria in patients without overt CTD. The progressive decline in the renal function and the development of proteinuria and hypertension, are indicators alerting the clinician to the presence of CTD. A biomarker which could predict these changes years before may allow early identification of patients at high risk for development of CTD. Our data indicate that the analysis of tryptophan metabolism early after transplantation might contribute to early detection of CTD.

Several studies suggested the changes in tryptophan, kynurenine and IDO activity to reflect the short-term outcome of kidney transplantation. Serum kyn/trp ratio is higher in non-rejecting renal allograft recipients in comparison with healthy volunteers. Moreover, kyn/trp is rapidly increasing in recipients with acute rejection compared to non-rejectors as early as by day one post-transplant. Additionally, IDO-positive cells were detected in renal biopsy of rejecting patients but not of those who did not reject the graft. It has also been shown that serum tryptophan and kynurenine levels predict acute rejection of human kidney transplant. The pre-transplant levels of serum kynurenine and tryptophan were increased in patients who went on to develop acute renal rejection compared to those who did not. These and other findings indicate that IDO activity is induced during acute rejection. Up till now, there is a lack of information regarding IDO activity, expression and tryptophan catabolism in long-term post-transplant outcome. Schefold et al. analyzed tryptophan catabolism and IDO activity in a cohort of forty patients with chronic kidney disease with various backgrounds, such as diabetic and hypertensive nephropathy and glomerulonephritis. The serum tryptophan level was relatively unchanged, but IDO activity and serum levels of tryptophan catabolites (such as kynurenine, kynurenic acid and quinolinic acid) increased with the severity of chronic kidney disease. Furthermore, the levels of kynurenic and quinolinic acid correlated with serum creatinine, creatinine clearance and in the case of kynurenic acid, also with eGFR. The induction of IDO in chronic kidney diseases may primarily be a consequence of chronic inflammation.

In our study, urine IDO activity 2 years after transplantation was increased as compared to 2 week values, whereas serum IDO activity decreased. Previously, little IDO expression was found in kidney biopsies without acute rejection approximately 2 weeks after transplantation, whereas strong IDO expression was found in our study at 2 years after grafting. It may thus be possible that the increased IDO activity in urine at 2 years
reflects the increased expression of IDO in the transplanted kidney. However, no correlation was found between the amount of IDO staining and the kyn/trp ratio in urine, possible because not all the IDO protein in the kidney is enzymatically active. Earlier, Brandacher et al. \textsuperscript{19} documented IDO expression in acutely rejected renal grafts. IDO positive cells were identified in the mononuclear cell infiltrates and the tubular epithelial cells. In our study, the strongest expression was also found in the interstitial inflammatory infiltrates. Only weak expression was found in tubular epithelial cells and, as opposed to the study of Brandacher et al., glomerular staining in (mostly) endothelial cells was also documented. The reason for this difference is unclear; it may rely for instance on the time to biopsy (6 months in our study vs. weeks in the study of Brandacher), the differences in the anti-IDO antibody or the differences in the (induction) immunosuppressive medication. In the 2 year biopsies IDO expression followed a similar pattern, however stronger expression was noticed in (both endothelial and mesangial) glomerular cells. This pattern of IDO expression is not unexpected. IDO is known to be up-regulated in immunologically active cells (i.e., infiltrating mononuclear cells). Also, expression of IDO in endothelial cells at the foeto-maternal interface has been earlier documented\textsuperscript{23}. Expression of IDO in mesangial cells has, to the best of our knowledge, not been reported so far. What role does exactly IDO play in these cells remains to be elucidated.

In this study we describe for the first time the kinetics of the tryptophan metabolism during a 2 year follow up after kidney transplantation in humans. Moreover, we document that the levels of tryptophan and its metabolite kynurenine at week 2 and month 6 can predict the long-term changes in serum creatinine and albuminuria. Hence, analyzing the combination of tryptophan, kynurenine and kyn/trp ratio early after the transplantation may assist the clinician to define the subgroup of the patients more likely to develop CTD.
Reference List


