Dynamics and Diagnostic Relevance of Kynurenine Serum Level after Kidney Transplantation

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Inflammatory events after kidney transplantation (Tx) may lead to activation of the tryptophane-catabolizing enzyme indoleamine 2,3-dioxygenase followed by the formation of kynurenine (KYN). Post-transplant KYN serum levels in kidney allograft recipients were analyzed for their diagnostic value.

Material/Methods: This was a retrospective analysis of KYN levels (normal value: 2.7±0.6 nmol/ml) measured in 4083 blood samples collected from 355 kidney graft recipients in connection with uncomplicated courses, acute rejections (ARs), infections, and type of immunosuppression. We performed descriptive data analysis and analysis of variance.

Results: In 212 recipients with immediately functioning grafts, the KYN levels dropped from pre-Tx 13.3±5.9 nmol/ml to nearly normal values at day 5 (5.8±3.0 nmol/ml). In patients with delayed graft function, the KYN reduction started only after the last hemodialysis treatment. With respect to ARs in recipients with creatinine values <300 µmol/l pre-AR, the increase of KYN levels depended on the severity of ARs (steroid-sensitive ARs: from 4.5±1.4 to 6.0±6.1 nmol/ml; steroid-resistant ARs: from 6.1±3.1 to 12.9±7.1 nmol/ml; vascular rejections: from 5.8±3.0 to 16.9±9.1 nmol/ml). In patients with creatinine values ≥300 µmol/l pre-AR, a further increase of the KYN level (from 10.1 to 13.2 nmol/ml) was only observed in severe, steroid-resistant ARs. With respect to infections evaluated, the KYN levels before diagnosis/start of treatment were 5.7±3.4 nmol/ml in asymptomatic CMV infections, 7.5±4.4 nmol/ml in CMV diseases, 8.3±3.3 nmol/ml in pneumonia, and 10.4±6.5 nmol/ml in bacterial sepsis.

Conclusions: Serum KYN seems to be a reliable diagnostic tool for the assessment of post-transplant inflammatory complications, already in an early stage, and for monitoring the efficacy of therapeutic interventions. Prospective studies are recommended.

MeSH Keywords: Cytomegalovirus Infections • Graft Rejection • Immunosuppression • Kidney Transplantation • Kynurenine

Abbreviations: **AR** – acute rejection; **ARs** – acute rejections; **ATG-F** – anti-T-lymphocyte globulin-Fresenius; **ALGs** – anti-lymphocyte globulins; **ATN** – acute tubular necrosis; **AZA** – azathioprine; **b.w.** – body weight; **CMV** –Cytomegalovirus; **CRP** – C-reactive protein; **CyA** – Cyclosporine A; **e.g.** – for example; **ELISA** – enzyme-linked immunosorbent assay; **HDI** – high-dose induction; **IFT** – immunoglobulin class M; **IgG** – immunoglobulin class G; **IDO** – indoleamine 2,3-dioxygenase; **IL** – interleukin; **ID** – identity number; **IFN-γ** – interferon-γ; **KYN** – kynurenine; **Mab** – monoclonal antibody; **MDI** – multiple-dose induction; **MP** – methylprednisolone; **PN** – pyelonephritis; **sIL-2R** – soluble interleukin-2 receptor; **TDT** – triple drug therapy; **TNF-α** – tumour necrosis factor-α; **TRP** – tryptophan; **Tx** – transplant; **UTI** – urinary tract infection

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Background

In 1984 Pfefferkorn [1] reported that treatment of human fibroblasts with interferon-γ [IFN-γ] blocked growth of the intracellular protozoan parasite *Toxoplasma gondii* by degradation of the amino acid tryptophan (TRP). In 1986 Byrne et al. [2] were able to inhibit the intracellular *Chlamydia psittaci* replication in continuous cell lines by IFN-γ, and Carlin et al. [3] reported that the initiation of TRP metabolism along the kynurenine pathway depends on the IFN-γ induced increase of the indoleamine 2,3-dioxygenase (IDO) activity. In contrast to the liver-specific enzyme tryptophan 2,3-dioxygenase, which is not inducible by immune signals, IDO is expressed in many tissues and cells (e.g., macrophages, dendritic cells, endothelia, and placenta) and is strongly activated by immune signals (e.g., IFN-γ, tumour necrosis factor-α, lipopolysaccharide toll-like-receptor ligation) [4–8], and inflammation. This activation of IDO leads to the formation of kynurenine (KYN) and other metabolites which have essential functions during immune activation, e.g., in down-regulating of the immune responses of T cells, dendritic cells, and macrophages [9], resulting in restoration of immune homeostasis. However, in chronic immune activation, the immunosuppressive feedback mechanisms may continue as indicated by an enhanced IDO activity [rev. by 10]. The interest of transplantologists in this control circuit rose sharply after it was shown that IDO activity is of critical importance for immunologic acceptance of semi-allogeneic fetuses in a mouse model [11]. In experimental transplant systems it was shown that over-expression of IDO in corneal grafts [12] or pancreatic islets [13] prolonged the graft survival. Further experimental data led to the hypothesis that regulatory T-cells exert their immunosuppressive function by initiation of IDO activity [14].

These basic findings also made the tryptophan metabolism very interesting for clinical transplantation. After kidney transplantation, about half of recipients experience inflammatory complications (e.g., T-cell- or/and antibody-mediated rejections as well as viral, bacterial, or mycotic infections) which are associated with an increase of pro-inflammatory stimuli [15], leading to IDO induction followed by an increase of the KYN serum level. More recently, it has been observed that persistent inflammation is also associated with fibrosis progression and chronic humoral rejection, which are 2 histological conditions associated with poor allograft survival. Importantly, subclinical inflammation following acute rejections occurs in more than 20% of patients and constitutes a risk factor for the development of interstitial fibrosis [16]. Therefore, several authors investigated whether changes in serum KYN level could be a reliable marker for the detection of post-transplant (post-Tx) complications preceding the appearance of clinical signs and symptoms. Homes et al. [17] reported significantly increased serum concentration of KYN at 5–7 days prior to biopsy-confirmed rejections and also in viral or bacterial infections.

Brandacher et al. [18,19] and Lahdou et al. [20] confirmed these results in a small cohort of patients after kidney transplantation. Abendroth et al. [21,22] found significantly elevated serum KYN levels in connection with 8 irreversible rejections. In contrast, low post-Tx KYN levels reflected a status of immune homeostasis and were associated with good graft survival, confirmed by Kaden et al. [23]. Dharnidharka et al. [24] evaluated the KYN data of 25 kidney-grafted children and found significantly elevated KYN/TRP ratios in rejecting recipients compared to the stable and the infections group. Analyzing only the KYN data, no significant differences between the 3 groups were found.

The data reported so far are not conclusive, and some are even contradictory, and were obtained from a small number of patients. This study presents the results of KYN serum levels measured in 4083 post-transplant serum samples from 355 kidney graft recipients, the largest cohort evaluated to date, to help clarify their diagnostic relevance.

Material and Methods

This retrospective analysis investigated potential associations between the patients' post-transplant KYN levels and post-transplant graft function, rejections, CMV-infection or disease, pneumonia, sepsis, and the type of immunosuppression.

Study population

A total of 355 recipients of kidneys from deceased donors were included in this retrospective analysis. Written informed consent was obtained from all patients regarding serum storage and scientific evaluation of all data.

Immunosuppression

The patients received ATG-Fresenius (ATG-F, n=267) or other polyclonal ALGs (n=32, details in Table 1) for induction, in addition to standard triple-drug therapy (TDT), or TDT alone (n=51) consisting of cyclosporine (CyA), azathioprine (AZA), and steroids. Only 5 recipients received other drug combinations. The immunosuppressive protocols are already described in detail [25].

Patients of the *high-dose ATG-F induction cohort* (*HDI,* n=186) received 9 mg/kg body weight (b.w.) of ATG-F before completion of anastomoses. The initial CyA level was adjusted to a lower level as compared to the solely TDT cohort (median, 1st week post-Tx: 162 *vs.* 196 ng/ml, 2nd week: 197 *vs.* 240 ng/ml).

Patients of the *multiple-dose ATG-F induction cohort* (*MDI*, n=81) received 7 or 8 infusions of 3 mg/kg b.w. ATG-F in addition to

Table 1. Characteristics of the population.

	TDT+ATG-F HDI	TDT+ATG-F MDI	TDT	Other*	Total	p
Number of recipients	186	81	51	37	355	
Age, years	43.4±11.8	40.5±10.5	41.4±12.1	43.2±11.6	42.4±11.6	=0.253
Gender						
Male	115	40	29	22	206 (58%)	=0.302
Female	71	41	22	15	149 (42%)	
Number of sera, total	2092	962	662	366	4082	
Number of sera per patient, mean, other*	11.2	11.9	13.0	9.9	11.5	
Kynurenine preTx nmol/ml	13.4±5.8 (177/186)	16.4±6.4 (77/81)	12.7±4.4 (50/51)	11.8±4.9 (36/37)	13.8±5.9 (340/355)	

* Other immunosuppressive regimens (Aza+Pred: n=3, CyA+Pred: n=1, TDT + intra-operative high-dose induction with Lymphoglobulin Merieux [30 mg/kg; n=17], with Pressimmun Behring [60 mg/kg; n=6], with ATG Biotest [1.5 mg/kg; n=7], with ATGAM Upjohn [45 mg/kg; n=2], incomplete data [n=1]).

TDT. The initial CyA level was also adjusted to a lower level as compared to the solely TDT cohort (median, 1st week post-Tx: 92 ng/ml; 2nd week: 136 ng/ml) [26].

Rejections

For diagnosis of rejection, the following clinical and laboratory signs were decisive: enlargement and tenderness of the graft, increase in serum creatinine and serum C-reactive protein (CRP), concomitant change in blood urea nitrogen, oliguria, albuminuria, immunoglobulinuria, CRP-uria, sonographic changes, core biopsies, and fine-needle aspiration cytology [27,28].

The quantitative determination of urinary albumin and IgG was done by nephelometry (BN 100, fixed-time method, Behring, Marburg, Germany) using urine collected over 24 hours. Urinary CRP was measured by a luminescence immunoassay as described by Steinhoff et al. [29].The cut-off of this test is 6 µg/L; therefore, the assay is about 1000 times more sensitive than nephelometry.

All clinically suspected and/or biopsy-proven rejections were included in this analysis.

The first-line treatment consisted of 5 mg/kg MP for 5 consecutive days. Biopsy-proven steroid-resistant rejections received second-line treatment with ATG-F for 8–10 days using a dose-by-T-cell protocol (target T cell values: 50–150/µl). OKT3 (10 days, 2.5 mg/d; Cilag, Sulzbach, Germany) was given as third-line rescue therapy or primarily in cases of biopsy-proven

vascular rejections or humoral rejections proven by the detection of donor-reactive complement-dependent lymphocytotoxic antibodies, then combined with 3–5 sessions of plasmaphereses on alternate days.

CMV

The laboratory diagnostics of CMV infections was done by antibody determination (CMV-IgG and CMV-IgM: IFT [in-house] and ELISA [ENZYGNOST-CMV, ELISA-Behringwerke AG, Marburg, Germany; ABBOTT, IMx, Wiesbaden, Germany]) and CMV-pp65-antigen detection (CLONAB-CMV, IFT using Mak H11 as mab, Biotest, Germany and Argene Biosoft, France, IFT CINAkit using 1C3 and AYM-1 as mab) [30].

CMV infection was characterized by CMV-IgM seroconversion without any clinical signs.

CMV disease was characterized by laboratory findings and/or symptoms like leukocytopenia, spike-like fever, elevation of aminotransferases, and deterioration of graft function or pneumonia solely or in combination.

Prophylaxis of CMV infection/disease was done only in the Donor-CMV-IgG+/Recipient-CMV-IgG– combination. All recipients received 2 ml/kg Cytotect® (Biotest, Dreieich, Germany) pre-operatively and post-operatively on day 18 and 1 mg/kg on day 38.

CMV therapy was managed as pre-emptive therapy starting at the day of pp65 detection and consisted of Cytotect®
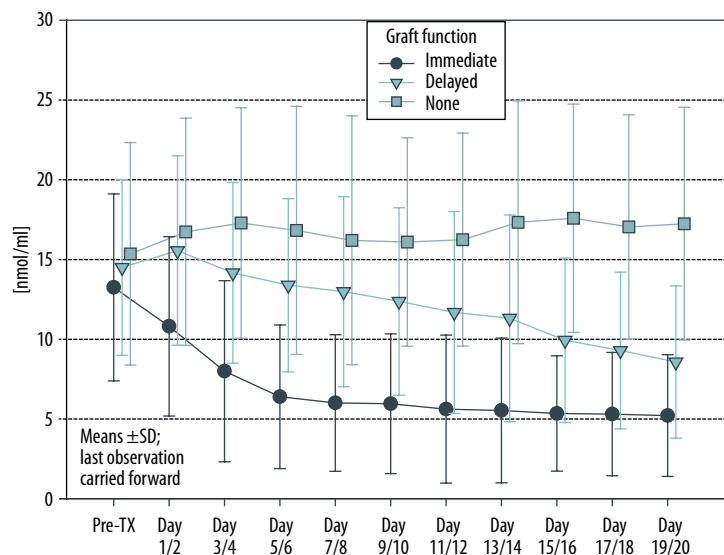
Figure 1. Kynurenine serum levels after renal transplantation depending on graft function.

(2×2 mg/kg) and ganciclovir (until disappearance of pp65) or as early therapy starting at the first day of leukocytopenia below 4000/µl and consisted also of Cytotect® (2×2 mg/kg) and ganciclovir (till improvement).

Determination of kynurenine serum levels

Blood for routine monitoring of the recipients was normally withdrawn every Monday, Wednesday, and Friday between 7:00 and 8:00 o'clock a.m. After measurement of routine parameters, the remaining serum was stored at −30°C and served as a pool for longitudinal en bloc KYN measurements. In this study, all serum samples available in our serum bank were tested. There were no exclusions.

After deproteinization of the serum with acetic acid trichloride and consecutive proton-dominant hydrolysis, the stable metabolite KYN is reacting under the use of 4-dimethylamino-benzaldehyde (Ehrlich's reagent) into a yellow product. Absorption was measured with 492 nm wave length in a linear sector from 0.5–100 µM of the concentration of N-formyl-kynurenine, proportional to the activity of the enzyme indoleamine-2,3-dioxygenase. The intra-assay variance was 1.53% and the inter-assay-variance was 2.77%.

Statistical analyses

Descriptive data analysis and analysis of variance methods were used to characterize the data. All p-values are 2-sided and considered to be descriptive. For a formal statement of descriptive significance, a nominal type I error level of $\alpha=0.05$ (2-sided) was assumed.
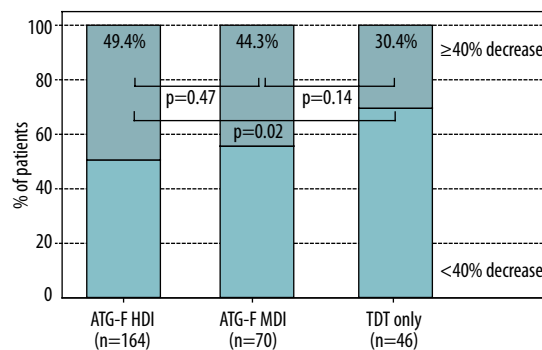

Figure 2. Kynurenine serum level reduction by ≥40% of the pre-transplant level depending on the immunosuppressive therapy.

Results

Kynurenine level in healthy volunteers

KYN levels in 292 healthy adults (age 18-81 years) were determined to be 2.7±0.6 nmol/ml. All values >x+ 3SD of this healthy reference population (i.e., >4.5 nmol/ml) were considered to be significantly elevated.

Relationship between post-transplant dynamics of KYN and graft function

Figure 1 shows the time course of the post-transplant KYN levels in 212 recipients with immediately functioning grafts, in 120 recipients with DGF, and in 23 recipients with never-functioning grafts (NF) during the first 3 weeks after transplantation.
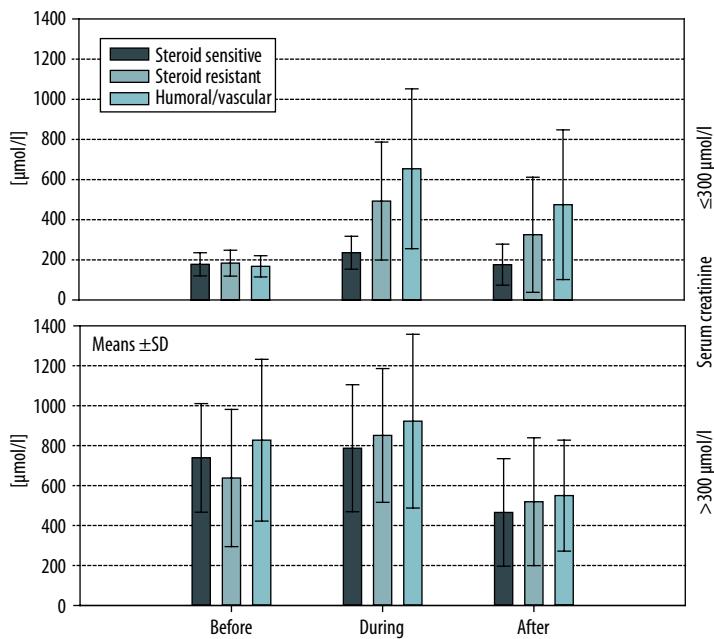
Figure 3. Kynurenine serum levels before, during, and after rejection episodes depending on the pre-episode serum creatinine concentration and type of rejection.

While the mean KYN levels of recipients with immediately functioning grafts decreased from pre-Tx 13.3±5.9 to 5.8±3.0 nmol/ml within 5 days (followed by a further reduction), a decrease from pre-Tx 14.5±5.5 to 8.6±4.8 nmol/ml at day 19/20 post-Tx was observed in recipients with DGF. Recipients with NF never showed mean KYN levels below the pre-Tx level of 15.4±7.0 nmol/ml during the first 3 post-operative weeks.

In order to exclude the influence of dialysis in patients with DGF on KYN, we calculated the post-Tx KYN level beginning at the time point of the last hemodialysis in a sensitivity analysis. Using this mode of analysis, the observed KYN decrease was comparable to that found in patients with immediate graft function.

Relationship between dynamics of KYN and immunosuppressive regimen

This analysis was based on 164 patients treated with TDT+ATG-F-HDI, 70 Patients treated with TDT+ATG-F-MDI, and 46 patients who received TDT alone. At day 4–6 after transplantation, KYN decreased by ≥40% of the pre-Tx level in 49.4% (81/164) for the HDI cohort, in 44.3% (31/70) for MDI, and in 30.4% (14/46) for TDT (Figure 2). The difference between the HDI cohort and the TDT cohort was significant (p=0.023).

Kynurenine and rejections

Because of the known influence of hemodialysis on the KYN serum level [26], recipients were divided in 2 cohorts: recipients with serum creatinine ≤300 µmol/l (no hemodialysis)

and recipients with serum creatinine >300 µmol/l (hemodialysis treatment). Figure 3 shows the minimum KYN serum level within 7 days before, maximum KYN level during, and the last measured KYN level within 7 days after the rejection crises. In non-dialyzed recipients (serum creatinine ≤300 µmol/l; upper panel), the weakest KYN increase was observed in steroid-sensitive rejections (n=37: from 4.5±1.4 to 6.0±6.1 nmol/l), followed by steroid-resistant rejections (n=22: from 6.1±3.2 to 11.0±5.6 nmol/l), and by exclusively biopsy proven, vascular rejections (n=10; from 5.8±3.0 to 16.9±9.1 nmol/l). The median relative KYN increase between the minimum pre-episode level and the peak within-episode level was 111.8% in steroid-sensitive rejections, 171% in steroid-resistant rejections, and 189.5% in vascular rejections.

A completely different result was obtained in dialyzed recipients (Figure 3, lower panel). The respective changes of the KYN levels were from 11.0±3.8 to 10.7±4.2 nmol/l in steroid-sensitive rejections (n=50), from 10.1±4.4 to 13.2±6.6 nmol/l in steroid-resistant rejections (n=17), and from 12.8±6.9 to 12.2±6.5 nmol/l in biopsy proven vascular rejections (n=11).

Thus, while the rejection-associated elevations of the KYN serum levels in non-dialyzed recipients showed a strong dependence on the severity of rejections, the elevations were confounded with dialysis-associated KYN increases in dialyzed recipients.

The behavior of the serum creatinine concentrations before, during, and after rejection episodes was comparable to that of the KYN levels (not shown in the figure). In non-dialyzed
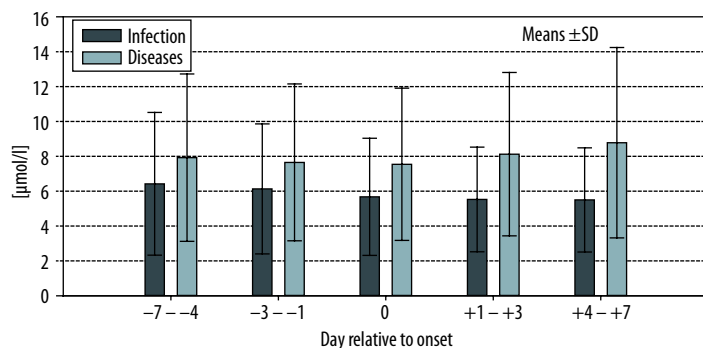
Figure 4. Kynurenine serum levels before and after onset of CMV infection or disease.
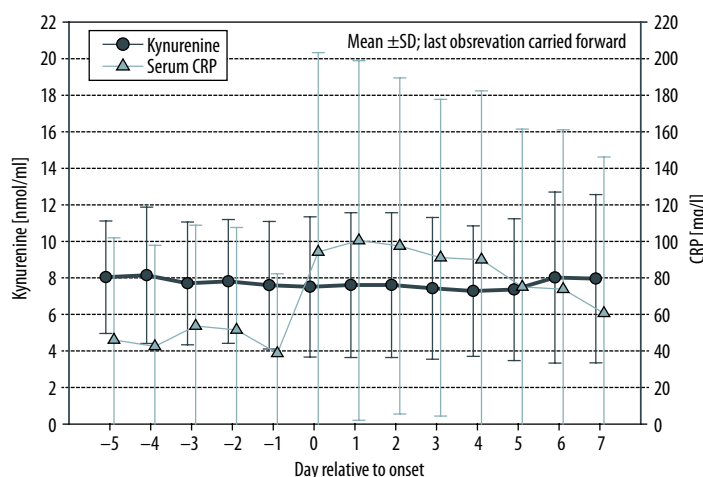

Figure 5. Kynurenine and CRP serum levels relative to onset of pneumonia.

recipients, the weakest creatinine elevation was observed in steroid-sensitive rejections (n=37: from 177.9±57.4 to 235.5±82.0 µmol/l), followed by steroid-resistant rejections (n=22: from 183.7±64.3 to 493.2±294.1 µmol/l), and by exclusively biopsy proven vascular rejections (n=10; from 167.7±52.8 to 653.8±398.2 µmol/l). The median relative serum creatinine increase between the minimum pre-episode level and the peak within episode level was 121.1% in steroid-sensitive rejections, 270.1% in steroid-resistant rejections, and 279.5% in vascular rejections.

As expected in post-Tx dialyzed recipients, the 'gold standard' marker creatinine was found to be an unreliable diagnostic parameter for detecting rejection crises.

Kynurenine and infections

KYN and CMV

The behavior of the KYN level in the week before CMV-IgM seroconversion or before the start of CMV-therapy, and in the week after the diagnosis is presented in Figure 4. Compared to the upper reference limit of 4.5 nmol/l derived from the healthy control cohort, the mean KYN levels during asymptomatic CMV infections were slightly increased already at 1 week before seroconversion (mean levels between 6.1 and 6.4 nmol/ml), and only marginally increased values were observed during the week after seroconversion (mean: 5.5 nmol/ml). In contrast, the mean KYN levels were noticeable higher 1 week before clinical manifestation of CMV disease (mean levels between 7.7 and 7.9 nmol/ml) and increased from 8.1 to 8.4 nmol/ml during the week after clinical manifestation and start of therapy. At the onset of CMV infection/or disease (start of treatment), there was a significant difference of the KYN level (5.68±3.36 [n=65] *vs.* 7.54±4.37 [n=45] nmol/ml, p=0.013) between both cohorts.

KYN and pneumonia

Figure 5 shows the serum concentrations of KYN and CRP determined in a total of 21 patients with pneumonia, comparing the values during the last 5 days before and the initial 7 days after start of antibiotic treatment. While the average KYN levels constantly increased (compared to the upper reference limit) during the entire period (indicated by mean values between 7.3 and 8.0 nmol/ml), the CRP levels increased from an
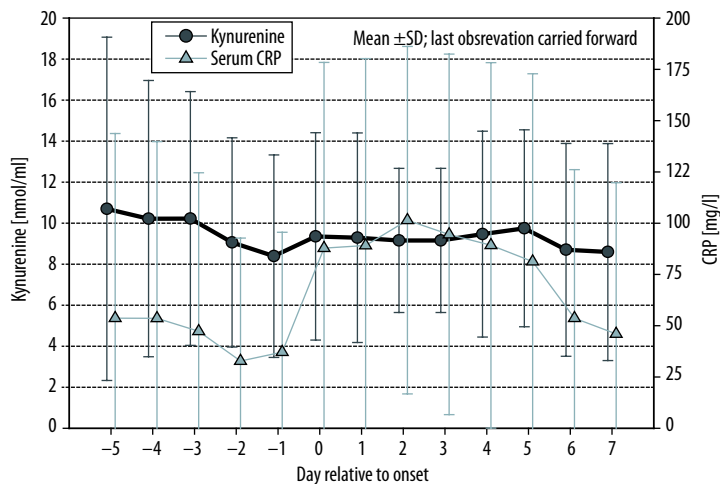
Figure 6. Kynurenine and CRP serum levels relative to the onset of sepsis.

average of 46.0 at day −5 to 94.1 mg/l at day 0 (start of treatment). Within 7 days after beginning of treatment, the CRP level decreased again to an average of 60.7 mg/l.

KYN and sepsis

KYN and CRP serum levels during the time interval from 5 days before to 7 days after the onset of bacterial sepsis in 12 cases are depicted in Figure 6. While the KYN levels constantly increased during the pre-onset period (mean values between 8.4 and 10.7 nmol/ml), the CRP levels increased during the pre-onset period from an average of 53.7 mg/l at day −5 to 87.8 mg/l at day 0, and further to 101.5 mg/l at day +2. Under therapy, a reduction of both parameters was observed, but with a more pronounced decrease for CRP (mean: 46 mg/l at day +7). The comparatively large standard deviation reflects the fact that the clinical manifestations were of different severities. The highest KYN levels were determined in a patient (ID 1529) who had a mycotic sepsis (aspergillus) associated with a central aspergilloma. During the last week of his life, the KYN level increased from 62 to 79 and finally to 92 nmol/ml.

Discussion

The TRP metabolism is part of a broad range of immune processes, including anti-infective [1,2,31–34] and anti-neoplastic [9,35–39] immunity, as well as autoimmunity [40–42], transplant tolerance [4,12,13,43–45], and tolerance during pregnancy [rev. by 46]. Combining all these effects, IDO constitute a very potent immunoregulatory mechanism. *In vitro* there is a strong correlation between inflammation, TNF-ℵ, and IDO expression, but LPS, IL-1, and TNF-α are also able to enhance IDO. *In vivo* experiments clearly show that the modulation of TRP metabolism by local IDO expression has a strong effect in protecting semi-allogeneic fetuses [11] as well as allogeneic

and xenogeneic graft against rejections [12,13,47,48]. From a clinical point of view, it would be very interesting to determine if there is a systemic detectable correlate of the local event within the graft, in particular, KYN as breakdown product of TRP catalyzed by IDO.

In our large study we found that KYN serum levels showed clear dynamics coinciding with chronic kidney diseases, hemodialysis treatment, and successful kidney transplantation. Importantly, the normal course of post-Tx KYN decrease was modified by events leading to a re-activation of the IDO/KYN system.

In 2009, Schefold et al. [49] reported on significantly increased KYN levels in patients with chronic kidney diseases already in the pre-dialysis stadium. In dialyzed patients, elevated KYN serum or plasma levels have been reported by several authors [18,19,21,50–56] and this might be a reason for the high cardiovascular death rate in hemodialysis patients.

These pre-transplant elevated KYN levels are initially caused by an inflammatory process leading to end-stage renal disease, but subsequently they much more the result of hemodialysis-induced vascular endothelial irritation followed by IDO activation and KYN formation. This hypothesis implies that the removal of the cause of elevated KYN levels immediately leads to their normalization.

First results of our pilot study [51] suggested the correctness of this hypothesis in 16 recipients, now confirmed by our extended study. In 212 recipients with immediately functioning kidney grafts, the mean KYN levels dropped within 5 days from pre-Tx 13.3±5.9 to 5.8±3.0 nmol/ml with subsequent further reduction, independently of the type of the initial immunosuppressive regimen. However, when analyzing all recipients with a KYN decrease by ≥40% of the pre-Tx level at day 4–6 after transplantation, there was a significant difference between the

ATG-F and the TDT cohort in favor of the single intraoperative high-dose ATG-F-induction. This was strongly associated with a significantly higher percentage of recipients with immediately functioning grafts in patients who received an intraoperative high-dose induction with ATG-F, as shown before [25].

Comparable to KYN, generally elevated pre-Tx levels decreasing rapidly after grafting of immediately functioning kidneys were also observed for thrombomodulin (TM) [57]. Thus, both pre-Tx increased KYN as well as sTM levels could be identified as strong indicators for hemodialysis-induced vascular endothelial irritations.

Corresponding with the pre-Tx increased KYN and sTM levels, the serum concentration of the soluble interleukin-2 receptor (sIL-2R) was also significantly elevated in almost all prospective kidney graft recipients, indicating pre-Tx T-cell activation. In event-free courses, the post-Tx sIL-2R levels decreased (like KYN) continuously towards normal levels within the first 3 weeks [58]. These data indicate the existence of subclinical inflammation and/or immunoactivation related to pre-Tx hemodialysis treatment.

It should be noticed that the peri-transplant dynamics of the KYN level was completely different from those of pro-inflammatory cytokines and the C-reactive protein [59,60]. In contrast to the KYN level, the serum concentrations of all cytokines analyzed (IL-1β, IFN-ɣ, TNF-α, IL-6, and CRP) were within the normal range or only slightly increased [61] immediately prior to kidney transplantation, but rose sharply at post-Tx with peaks between days 1 and 3, after which a rapid normalization could be observed.

Thus, the peri-operative situation is a combination of chronic immunoactivation with IDO activation and KYN formation in the pre-Tx period as well as of trauma-induced acute inflammation with elevated pro-inflammatory cytokines and acute-phase reactants immediately after Tx.

In event-free post-Tx courses, a restoration of the immune homeostasis with down-regulation of pro- and anti-inflammatory factors occurs. Therefore, there was a clinical need to determine the diagnostic potential of re-increased KYN serum levels indicating a renewed disturbed immune homeostasis.

Immediately after Tx, elevated and persistently high KYN level was found in patients with never-functioning grafts as well as in recipients with DGF, indicating progression of inflammation in addition to the effect of hemodialysis. After start of kidney function, the KYN level decreased in a similar manner as seen in patients with immediate graft function.

With respect to the diagnostic relevance of KYN level to detect ARs, the data reported in the literature to date are not conclusive and are sometimes contradictory. Dharnidharka et al. [62] found significantly elevated serum KYN/TRP ratios in 10 discrete episodes of AR in 7 subjects, but stable and infection groups were not different from each other. When analyzed separately (not as ratio), the mean levels of KYN and TRP did not significantly differ among the 3 groups. In contrast, Brandacher et al. [19], analyzing the data from nine patients with 12 biopsy-confirmed episodes of AR, found significantly elevated KYN/TRP ratios as well as KYN serum levels at the time of AR, permitting an accurate diagnosis of AR but recommended further studies to clarify the influence of ATN, calcineurin toxicity, and infections on tryptophan metabolism. The observed significant difference between the KYN levels measured as early as by day 1 post-Tx in patients with uncomplicated post-operative courses and in patients who subsequently had an AR could, however, not be confirmed by others as yet. Lahdou et al. [20] reported on significantly increased KYN levels between 0 and 4 days before biopsy-confirmed AR compared with a group of non-rejecting recipients. Moreover, Holmes et al. [17] described significantly elevated KYN serum levels at 5–7 days before biopsy-confirmed AR. Thus, in connection with ARs, IDO catalyzed tryptophan degradation and increased KYN production seemed to be accepted by all investigators. In 1993 Merville et al. [63] described in kidney graft in filtrating cells besides IL-6 and IL-10 secreting cells also IFN-ɣ secreting cells during irreversible rejections.

In extension of the data reported in the literature, it was absolutely necessary to classify the severity of AR and to account for additional hemodialysis treatments. This analysis showed a significant association between the severity of ARs and the magnitude of the KYN levels. In non-dialyzed recipients, the weakest KYN elevation was observed in steroid-sensitive ARs, followed by steroid-resistant ARs and biopsy-proven vascular rejections. In these recipients, a similar behavior of the serum creatinine concentration was found, confirming the findings of Brandacher et al. [19] in 34 kidney graft recipients. Holmes et al. [17] also described a high accuracy of AR diagnoses when changes in KYN and creatinine were used in combination. Additionally, it should be pointed out that an efficient anti-rejection therapy was associated with a decrease of the KYN level.

Otherwise, we present clear data that the KYN serum levels like creatinine are not suitable to diagnose ARs in dialyzed recipients because both dialysis and inflammation synergistically cause elevated KYN levels.

Besides immunoactivation by graft antigens resulting in rejections, a variety of microbes induce inflammatory reactions initially associated with an increase of pro-inflammatory stimuli

leading to IDO induction. However, the data published to date are contradictory. Brandacher et al. [19] observed non-significant changes in KYN / TRP, KYN, and TRP concentrations in 6 patients with infectious complication (4×herpes simplex, 1×UTI, 1×sepsis). Dharnidharka et al. [62], however, found a significant difference between children with major infectious events (8×BK viruria, 5×CMV viremia, 1×EBV viremia, 1×CMV+EBV viremia, and 6×transplant PN) and an uneventful control group, but the sample size was not very large (n=36). Holmes et al. [17] listed 5 patients with increased KYN levels in connection with infections (CMV, EBV, 2×UTI, pneumonia caused by CMV+K. pneumoniae). In summary, it can be concluded that published data, mainly based on single cases, do not permit a valid generalization to a broader population of patients.

Our detailed CMV infection/disease analysis shows that the more serious infections were systematically associated with higher KYN levels, which we attribute to an interaction between CMV and the host's immune system. Even in patients who did not develop clinical symptoms, a slight increase of the KYN level was observed in the week before CMV-IgM antibodies and/or pp65 antigen were first detected. In patients who developed a CMV disease, we found increased IL-6 and IFN-ɣ levels 4 days before leukocytopenia [64], as well as an increased mean KYN level above the upper reference limit. At the time of CMV serodiagnosis (infection), respectively start of anti-CMV therapy (disease), there was a significant difference of 1.86 nmol/ml between the KYN levels of the 2 cohorts. Thereafter, the KYN level decreased only in seroconverted patients without any changes in medication, whereas a further increase for at least 1 week was observed depending on the severity of the CMV disease.

Data are very sparse for KYN level in severe bacterial infections after kidney transplantation. We therefore investigated the changes in KYN levels in the course of 2 defined serious infections – pneumonia and sepsis. We recently reported on the incidence of these complications in our recipients [65]. In both complications, characteristic KYN and CRP profiles could be observed. Five days prior to diagnosis and start of antibiotic therapy, the average KYN levels already showed a stable increase by an average of about 2 nmol/ml. In contrast, the CRP level first showed a minor increase and then rose immediately before start of therapy. During therapy a strong CRP decrease was observed, indicating the efficacy of treatment. While successfully treated patients showed a slow decrease in KYN levels, a further increase was associated with a lethal course. Since serious events were thus preceded by a characteristic time course of the KYN level, we strongly recommend careful screening of all patients with exceptional KYN profile.

Conclusions

KYN can show inflammation and rejection in an early stage and can be used as a screening test for risk evaluation in individual patients. Further evaluation and validation in prospective clinical studies is necessary. In addition, the development of a sensitive, inexpensive, and clinically easy to use bed-side test for inflammatory disorders after transplantation is urgently needed.

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