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Clin Transplant.

Abstract

The aim of the present study was to investigate association of serum amino (AA) acids and metabolites of AAs with post-transplant outcome in liver transplant recipients.

Eighty-nine patients with end-stage liver diseases and available pre- and early post-transplant serum were characterised as patients with (GI) and without one-year mortality (GII) and patients with and without early graft dysfunction (EAD). A panel of pre- and early post-transplant serum levels of AAs and early and metabolites of tryptophan were measured using tandem mass spectrometry.

Patient groups had significantly higher pre-transplant serum levels of phenylalanine, tryptophan, and tryptophan metabolites than healthy controls (for all $p<0.001$). Pre-transplant serum levels of all these parameters were significantly higher in GI than in GII (for all $p<0.001$). GI had a higher MELD score and re-transplantation number than GII ($p\leq0.005$ for both investigations). Serum bilirubin on day 5 and serum phenylalanine on day 10 post-transplant were associated parameters of mortality, whereas day 1post-transplant phenylalanine and kynurenine and female gender were associated parameters of EAD.

Our results indicate that pre- and early post-transplant levels of phenylalanine, tryptophan and metabolites of tryptophan are increased in patients and are associated with EAD and one-year mortality in liver transplant recipients.

Key words: tryptophan, kynurenine, kynurenic acid, phenylalanine, MELD

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ABREVIATIONS

CMV: Cytomegalovirus

CRP: C-reactive protein

EAD: Early graft dysfunction

ESLD: End-stage liver diseases

HBV: Hepatitis B virus

HCV: Hepatitis C virus

HIV: Human immunodeficiency virus

IDO: indoleamine 2,3-dioxygenase

IFN: Interferon

MELD: Model for End-Stage Liver Disease

NPV: Negative predictive value

PPV: Positive predictive value

ROC: Receiver operating characteristic

Authorship

Hani Oweira participated in the study design, operated the patients, gathered the samples and assisted in writing the manuscript. Imad Lahdou assisted in the design of the study and measured the parameters. Volker Daniel participated in writing the manuscript. Gerhard Opelz and Peter Terness assisted in writing the manuscript. Arianeb Mehrabi and Jan Schmidt operated the patients. Gerhard Fusch and Joerg Schefold measured tryptophan and tryptophan metabolites. Mahmoud Sadeghi assisted in developing the design of the study, analyzed the data, and wrote the manuscript.

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Introduction

Post-transplant outcome in adult liver transplant recipients is associated with pre-, peri- and post-transplant risk factors including Model for End-Stage Liver Disease (MELD) score (1), pre-transplant nutritional status (2), pre- (3) and post-transplant kidney (4) and heart (4) disease, post-transplant infection (5), and venous thrombosis (6). A multivariate analysis by McDiarmid et al. indicated that vascular thrombosis, bowel perforation, septicemia, and re-transplantation, each independently increased the risk of patient and graft loss by 3 to 4 fold during the first 6 post-transplant months (7). Sun et al. identified MELD score >30, Intensive Care Unit stay >48 h prior to transplantation, intra-operative transfusion ≥15 units, re-transplantation, post-transplant dialysis, or reoperation as risk factors for infection during the first 3 post-transplant months (8).

AAs have direct or indirect inflammatory, anti-inflammatory and immunomodulatory effects. Recently, we reported on an association of phenylalanine and tryptophan metabolites with activated cytomegalovirus (CMV) infection in kidney transplant recipients (9) and pre-transplant plasma kynurenine as a predictor of acute rejection in kidney transplant recipients (10). Almost all published research on the kynurenine pathway is restricted to inflammatory responses to nervous system (11) diseases. Indoleamine 2,3-dioxygenase (IDO) degrades the essential AA tryptophan into kynurenine and other downstream metabolites that suppress effector T-cell function (12) and favor the differentiation of regulatory T cells (13). IDO is widely distributed in mammals and is inducible preferentially by interferon (IFN)-γ (14). IDO is traditionally regarded as a general suppressor of T-cell activation and mediator of immune escape in cancer (13). Recently, evidence has emerged to support a greater functional complexity of IDO1 as modifier of pathogenic inflammation (13). For instance, IDO1 activity may sustain autoantibody production by B cells, and elicit the development of cancer in the context of chronic inflammation (13). Studying an old population, Capuron et al. showed that increased inflammation was related to reduced tryptophan concentrations and increased kynurenine levels. In the study by Capuron, inflammation was associated with increases in phenylalanine concentrations (15). In a recent study we reported on association of plasma quinolinic acid and severity of hepatic dysfunction in patients with liver cirrhosis (16). The results showed that quinolinic acid and neopterin are more sensitive markers for severity of liver disease than established markers of inflammation such as C-reactive protein (CRP), and IL-6 and that quinolinic acid provided the most sensitive index with regard to the identification of patients with hepatic encephalopathy (16). We also reported on an association of early post-transplant neopterin (IFN-γ-dependent response) and one-year patient survival and bacteremia in liver transplant recipients (17). Associations of pre- and early post-transplant serum AAs and their metabolites with recipient outcome in liver transplantation have not been studied. In the present study, we investigated whether pre-transplant serum AA and AA metabolites -especially the IFN-γ-dependent kynurenine pathway, among other risk factors, are associated with recipient survival in liver transplant recipients.

Material and Methods

Characteristics of the study population

The retrospective study was conducted in accordance with local ethical guidelines and all individuals gave informed consent for the analysis of their plasma samples. Between January 2008 and April 2010, 178 patients underwent liver transplantation at the university hospital Heidelberg, Germany. Pre- and early post-transplant concentrations of all essential amino acids including tryptophan and phenylalanine and metabolites of tryptophan such as kynurenine, kynurenic acid and quinolinic acid were measured in available serum samples of 89 adult (age 52.2 ± 10.6 years; 23 female) patients. Patients showed different disease severity based on MELD staging. Fifty patients experienced first bacterial infections including urinary tract infection, blood stream infection, pneumonia, wound infection and cholangitis during 29±31 days post-transplant. Sixteen patients (8.9% of all patients and 18% of the study group, GI) died during one-year post-transplant due to graft failure and sepsis, whereas 73 patients survived more than one year and were considered as age- and gender-matched patients (GII). Patients who died due to sepsis had similar demographic and mortality risk factors as patients who died by graft failure. Twenty-one patients experienced early allograft dysfunction (EAD).

The transplantation was due to liver failure caused by chronic viral hepatitis C and/or B in 28 patients, alcohol abuse in 28, congenital or autoimmune disease including cryptogenic cirrhosis, biliary disease, metabolic liver disease, autoimmune hepatitis and amyloidosis in 28 patients. Five patients experienced acute toxic hepatitis.

We examined the association of one-year post-transplant mortality and EAD with re-transplantation, pre-transplant serum amino acid profiles, metabolites of tryptophan, albumin, neopterin and the severity of diseases (determined by MELD score) and post-transplant serum amino acids, metabolites of amino acids, neopterin, CRP and bacterial infections. Proportion of re-transplants was higher in non-survivors and patients with EAD (Table 1) whereas proportion of patients with hepatic encephalopathy was similar in the study groups (Table 1).

Post-transplant anti-infection prophylaxis included 3 days cefuroxime and metronidazole, 3 months cotrimoxazole, 10 days itraconazol, voriconazole or caspofugin. For recipients of CMV-positive donors, oral prophylaxis with valganciclovir was performed for a period of 3 months.

Fifty healthy volunteers (HCs) (24 females, age: mean±SD 40.5±11.2 years) served as controls to establish references for the studied parameters such as neopterin, tryptophan, kynurenine, kynurenic acid, quinolinic acid and phenylalanine. Controls were free of infectious and other inflammatory illnesses.

Demographic data including age, gender, original liver diseases, pre-transplant CMV, HBV, and HCV IgG status as well as kidney function were similar in survivors and non-survivors (Table 1). The number of females was significantly higher in patients with EAD (Table 1).

Serum Separation

Serum separator tubes were centrifuged at 4000 rpm for 15 min at 4°C. Serum was collected after the blood clotting process. The serum was snap frozen and stored at -20°C until testing. All serum samples were thawed only once before testing.

Determination of serum phenylalanine, tryptophan and tryptophan metabolites (kynurenine, kynurenic acid and quinolinic acid)

Serum amino acid levels were measured by tandem mass spectrometry. For analysis of tryptophan catabolism, heparinized serum samples were drawn from peripheral veins in all patients. 100 µL of serum was analysed after addition of 10 µl trichloroacetic acid (50%) (FLUKA, Germany), 60 µl water, 100 µl methanol (JT Baker, Deventer, The Netherlands) and 10 µl deuterated standard solutions each (phenylalanine (d5-Phe), kynurenine (d6-Kyn) and kynurenic acid (d5-Kyna), [Cambridge Isotope Laboratories, Andover, MA, USA]. Respective serum samples were mixed, stored at 4°C over night and centrifuged (20,000 g for 15 minutes) after thawing. For recording, a Wallac MS2 tandem mass spectrometer (Perkin Elmer, Rodgau, Germany) equipped with an electrospray ion source was used. All ions were detected in a positive ion mode using multiple reaction monitoring. The first quadrupole selected the protonated ions at mass-to-charge ratios (m/z) of 205, 171, 166, 209, 215, 168, 190 and 195 for Trp, d5-Phe, Phe, Kyn, d6-Kyn, Quin, Kyna and d5-Kyna, respectively. Nitrogen was chosen as a collision gas. Fractioned ions m/z 159 for Trp, 125 for d5-Phe, 120 for Phe, 192 for Kyn, 198 for d6-Kyn, 78 for Quin, 144 for Kyna and 149 for d5-Kyna were detected in quadrupole Q3 (Q3) (flow solvent: 0.02% formic acid in 80% aqueous acetonitrile, flow rate 50 µl/min). For quantification, serum samples were spiked with standards. Calibration curves were fitted by linear least square regression and correlated with the concentration of d5-Trp and d5-Kyna. Estimated IDO-activity was assessed as previously reported (18).

Determination of serum neopterin, CRP and albumin

Serum neopterin was measured with the Neopterin ELISA kit (Brahms, Berlin, Germany). Based on control measurements in 70 healthy individuals, serum level of >15 nmol/L was considered abnormally high. The protocol provided by the assay manufacturer was strictly followed. Serum CRP and albumin were assessed in a certified laboratory at Heidelberg University Hospital.

Statistical analysis

Group comparison was performed using Fisher's exact test, χ^2 and Mann-Whitney-U test. For all tests, two-sided significance levels of p≤0.05 after Bonferroni correction were considered significant and are bold printed in tables and figures. Univariate analysis of variance (ANOVA) for interference analysis and Receiver Operating Characteristic (ROC) curve analyses for determination of diagnostic sensitivity and specificity of parameters were carried out. Data were analyzed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

Results

Correlation between MELD score, phenylalanine, tryptophan, tryptophan metabolites, inflammatory markers and serum albumin

Our results showed that MELD score was positively correlated with phenylalanine (r=0.370: p<0.001), tryptophan (r=0.300: p=0.004), kynurenine (r=0.625: p<0.001), kynurenic acid (r=0.609: p<0.001), quinolinic acid (r=0.778: p<0.001), kynurenine/tryptophan ratio (r=0.544: p<0.001), CRP (r=0.558: p<0.001), neopterin (r=0.744: p<0.001), and negatively with albumin (r=-484: p<0.001). Inflammatory markers were likewise found to correlate with serum levels of phenylalanine and tryptophan metabolites (kynurenine, kynurenic acid and quinolinic acid): phenylalanine was found to be positively correlated with serum levels of CRP (r=0.288: p=0.005), neopterin (r=0.441: p<0.001), Kynurenine, kynurenic acid and quinolinic acid correlated positively with CRP (r=0.496, r= 0.418 and r=0.557, respectively: p<0.001) and neopterin (r=0.642, r=0.601 and r=0.746, respectively: p<0.001). The results suggest an association of MELD score, phenylalanine and tryptophan metabolites with inflammation and immune responses.

Increased pre-transplant serum levels of phenylalanine, tryptophan and tryptophan-metabolites in patients with ESLD compared to healthy volunteers

Patients who died during the first year post-transplant due to graft failure or sepsis (group-I, n=15) and patients who survived more than one year (group-II, n=74) had significantly higher pre-transplant serum levels of phenylalanine, kynurenine, kynurenic acid, and a higher kynurenine/tryptophan ratio than HCs ($p \leq 0.001$ for all measurements) (Figure 1a, 1b, 1d and 1f). Serum levels of pre-transplant tryptophan and quinolinic acid were significantly higher in group-I than in HCs ($p \leq 0.001$ for all measurements) and were similar in group-II and HCs (p=0.92 and 0.48, respectively) (Figure 1c and 1e). The results indicate increased serum levels of phenylalanine, tryptophan and tryptophan-metabolites in patients with ESLD

Increased pre- and post-transplant serum levels of neopterin, phenylalanine, tryptophan and tryptophan metabolites in patients with <one-year vs ≥one-year survival

GI had significantly higher pre-transplant serum levels of phenylalanine, tryptophan, kynurenine, kynurenic acid, quinolinic acid and neopterin than GII ($p \leq 0.005$ for all measurements) (Figure 1a-c, 1e and 1f). Moreover, group-I had increased MELD score and decreased serum albumin as compared to group-II ($p \leq 0.005$ for both investigations). Survivors had steadily increased serum levels of phenylalanine, metabolites of tryptophan and serum bilirubin than non-survivors from pre transplant to day 10 post-transplant (Figure 2). Serum neopterin from day 5 post-transplant was significantly increased in non-survivors and was significantly higher on day 10 than in survivors (p=0.002) (Figure 1). The results suggest that pre- and early post-transplant serum levels of phenylalanine, metabolites of tryptophan and serum neopterin might indicate risk of post-transplant mortality.

Sensitivity and specificity of risk factors in the two patients groups

We performed ROC curve analysis of pre- and post-transplant significant parameters to calculate cut-off values. The sensitivity and specificity of all significant parameters were calculated and depicted in Table 2. Day 10 post-transplant serum phenylalanine >68 µmol/l showed a sensitivity of 69%, specificity of 93%, positive predictive value (PPV) of 56% and negative predictive value (NPV) of 90%. Serum bilirubin >6 mg/dl on day 5 post-transplant had a sensitivity of 75%, specificity of 81%, PPV of 46% and NPV of 94%.

Regression analysis of significant parameters

We calculated odds ratios (ORs) and 95% confidence intervals (CIs) to evaluate the association between significant parameters and mortality using uni- and multi-variate logistic regression models. Univariate regression analyses showed that day 5 post-transplant serum bilirubin, day 5 and 10 posttransplant phenylalanine and pretrans-plant serum kynurenine ($p<0.001$ for all investigations) are most significant associated factors of one-year mortality (Table 3). Multivariate regression analysis showed that day 10 post-transplant serum phenylalanine > 68 µmol/L ($p<0.001$: OR=4.045 CI 1.250-13.095) and day 5 post-transplant serum bilirubin> 6 mg/dl ($p=0.005$: OR=7.121 CI 2.263-22.403) are significant associated factors of one-year mortality (Table 3).

Discussion

The aim of this study was to evaluate the pre- and early post-transplant measurement of phenylalanine, tryptophan and tryptophan metabolites (kynurenine, kynurenic acid and quinolinic acid) in liver transplant recipients in relation to the one-year post-transplant patient survival. High phenylalanine plasma levels or phenylalanine/tyrosine ratios were reported in patients with HIV-1, Dengue fever, HCV, CMV infection (9, 19-21) and inflammatory responses (15, 22-24). The reason for this phenomenon is unclear. However, all these clinical conditions are known to be linked with inflammation and immune activation (24).

IDO degrades the essential amino acid tryptophan into kynurenine and other downstream metabolites (13). Acquired immunity is impaired in hemodialysis patients and Eleftheriadis et al. suggested that this is the result of increased IDO activity which is inducible by inflammation (12). In a new study involving trauma and sepsis patients, Ploder et al. showed that, compared to healthy controls, patients exhibited increased kynurenine concentrations, kynurenine/tryptophan ratios, and TNF-α, IL-6 and neopterin plasma levels (25). Compared to the survivors, the non-survivors showed higher concentrations of kynurenine, neopterin, TNF-α and IL-6, as well as a higher kynurenine/tryptophan ratio (25). IDO has previously been shown to be associated with outcomes of organ transplantation (9, 10, 26-30). Weng et al. indicated that the expression of the IDO gene in peripheral blood tightly correlated with the severity of acute rejection in a rat liver transplant model (30). Tryptophan metabolites in the kynurenine pathway induce immunosuppression (31-34). These results imply that increased tryptophan degradation in patients is due to activated IDO, which most probably is a consequence of a host defense response. These findings support a possible role of IDO in the development of immunodeficiency that predisposes to death in transplanted patients (25).

In this study, we showed for the first time the association of increased amino acid and amino acid metabolites serum levels with mortality from sepsis and/or graft failure during the first year post-transplant. Patients at risk of death showed significantly increased serum levels of phenylalanine and tryptophan metabolites pre- and early post-transplant. Statistical analysis indicated the closest association of the rate of death with pre- and early post-transplant phenylalanine, kynurenine and kynurenic acid serum levels.

We found the optimal cut-off value of pre-transplant kynurenine at 4. 8 µmol/L, kynurenic acid at 1.8 µmol/L and phenylalanine at 70 µmol/L. These cut-off values showed a sensitivity and specificity of 68% and 77% for kynurenine, 63% and 73% for kynurenic acid and 69% and 70% for phenylalanine for the association with the one year post-transplant mortality rate. As expected, post-transplant parameters especially phenylalanine and serum bilirubin were stronger associated with one year mortality than pre-transplant parameters.

Prediction of post-transplant mortality following organ transplantation is essential to save the patient and the organ, and would initiate more care to rescue patients at risk. Sensitivity and specificity of the analyzed parameters were not excellent but acceptable. However, the high negative predictive values (92%, 90%, and 91%, respectively) reliably allow the identification of patients likely not to experience mortality.

As surrogate tests in combination with other parameters such as re-transplantation and MELD score, phenylalanine, kynurenine and kynurenic acid help to predict mortality in liver transplant recipients and may be used as an additional criterion to select and allocate patients for transplantation. The combination of pre-transplant phenylalanine and tryptophan metabolites with the MELD score substantially enhances outcome prediction of liver transplant recipients and may help to identify subsets of patients that are more or less likely to benefit from transplantation. We suggest that pre- and early post-transplant measurement of phenylalanine and tryptophan metabolites of the kynurenine pathway beside MELD score are very helpful tests to identify patients at risk of post-transplant mortality.

When correlation analyses were performed, phenylalanine, tryptophan and its metabolites correlated with inflammatory markers with the highest correlation for kynurenine, kynurenic acid and quinolinic acid. Our results are in agreement with previous findings on activation of the tryptophan metabolic pathway by pro-inflammatory stimuli (35-37). These results also indicate that liver dysfunction is associated with a strongly activated catabolic pathway of tryptophan degradation.

In summary, disturbance in pre- and early post-transplant metabolism of amino acids and their metabolites correlate with one-year mortality rate in liver allograft recipients. In conclusion, we have shown that pre-and early post-transplant serum levels of phenylalanine and metabolites of tryptophan are significantly sensitive and specific parameters with high NPV for post-transplant mortality in liver transplant recipients.

Ethical approval

This study was approved by the Ethics Committee of the Faculty of Medicine, University of Heidelberg. Written consent was obtained from patients and healthy controls.

Competing interest: The authors declare that they have no competing interests.

Table 1: Demographic and clinical characteristics of the study population

Parameters	non-survivor (n=16)	survivor (n=73)	EAD+ (n=21)	EAD- (n=68)
age [mean±SD years]	52±10	52±11	52±12	53±11
female gender (n)	5	18	10**	13**
Child-pugh category A/BA/C (n)	6/4/6	28/21/24	4/7/10	27/19/22
donor age [mean±SD years]	59±16	57±18	56±18	55±19
anti-viral IgG status				
HBV-Ab+ (n)	1	9	1	9
HCV-Ab+ (n)	6	16	7	15
CMV-Ab+ (n)	10	44	18	36
immunosuppressive drugs				
cyclosporine A (n)	9	41	15	35
Tacrolimus (n)	5	31	9	27
Prednisolone (n)	15	72	24	62
MMF (n)	6	27	9	24
original liver disease				
congenital/autoimmune/toxic (n)	8	25	10	23
alcoholic (n)	4	24	8	23
viral hepatitis (n)	4	24	5	23
risk factors for EAD and post-transplant mortality				
MELD score (mean±SD)	24.3±8.7**	17.3±8.1**	22.0±9.4*	17.0±8.2*
pre-Tx albumin (g/L)	27.0±6.0**	32.0±7.0**	31.0±7.0	31.0±7.0
1-10 days post-Tx SIRS (n)	7	17	9	15
post-Tx bacterial infection (n)	13*	37*		
re-transplant (n)	8***	10***	8*	10*
pre-Tx bilirubin (mg/dL)	12.9±8.8*	7.4±8.6*	11.6±10.8	6.9±8.0
pre-Tx neopterin (nmol/L)	43.0±27.0* (Median=34)	42.0±67.0* (Median=18)	38.0±34.0 (Median=33)	43.0±66.0 (Median=21)
pre-Tx creatinine (mg/dL)	1.5±1.0	1.3±1.0	1.4±0.9	1.3±1.0
pre-Tx CRP (mg/L)	32.0±24.0*	21.0±24.0*	19.0±18.0	22.0±25.0
pre-Tx encephalopathy (n)	8	35	10	33
intra-operative transfusion (n)	29.0±25.0	27.0±22.0	35.0±28.0	24.0±21.0
rejection (n)	2	9	2	9

Mann-Whitney U-test, χ^2 or Fisher exact test were used. *p≤0.05, **p≤0.01, ***p≤0.001

Table 2: Sensitivity and specificity of significant parameters

Variables	Sensitivity%	Specificity%	AUC%	p
mortality				
pre-Tx kynurenine	75	77	**77**	**0.001**
pre-Tx phenylalanine	69	71	**77**	**0.001**
day 5 post-Tx bilirubin	75	80	**77**	**0.001**
pre-Tx kynurenic acid	63	76	**74**	**0.002**
day 10 post-Tx neopterin	82	77	**81**	**0.002**
MELD score	75	71	**74**	**0.003**
day 10 post-Tx phenylalanine	69	80	**77**	**0.004**
day 1 post-Tx phenylalanine	60	79	**73**	**0.005**
day 1 post-Tx bilirubin	63	75	**73**	**0.005**
day 5 post-Tx kynurenine	73	67	**73**	**0.005**
day 1 post-Tx kynurenic acid	60	82	**73**	**0.006**
pre-Tx qunolinic acid	69	64	**72**	**0.006**
day 5 post-Tx kynurenic acid	60	76	**71**	**0.011**
day 5 post-Tx phenylalanine	68	72	**71**	**0.011**
pre-Tx tryptophan	69	76	**70**	**0.013**
pre-Tx S_albumin	63	63	**69**	**0.019**
early allograft dysfunction				
day 5 post-Tx CRP	61	80	**75**	**<0.001**
day 5 post-Tx kynurenine	70	66	**74**	**0.002**
day 10 post-Tx kynurenine	64	80	**77**	**0.003**
day 1 post-Tx phenylalanine	62	82	**71**	**0.004**
day 3 post-Tx kynurenine	70	67	**69**	**0.009**
day 5 post-Tx phenylalanine	65	72	**69**	**0.017**
MELD score	61	71	**66**	**0.020**
pre-Tx S_kynurenine	61	62	**64**	**0.047**

Table 3: regression analysis of significant parameters			
Variables	**exponentiated coef-ficients**	**CI**	**p**
univariate Cox-regression analysis of mortality risk factors			
day 10 post-Tx phenylal.>68 µmol/L	**10.812**	3.456-33.832	**<0.001**
day 5 post-Tx bilirubin>6 mg/dl	**10.177**	3.270-31.673	**<0.001**
day 5 post-Tx phenylal.>84 µmol/L	**7.296**	2.583-20.609	**<0.001**
Re-Tx	**4.210**	1.838-9.645	**0.001**
day 5 post-Tx Kyn/Trp ratios>0.15	**5.434**	1.533-19.265	**0.003**
day 1 post-Tx phenylal.>70 µmol/L	**4.471**	1.586-12.604	**0.004**
pre-Tx Kyn≥4.0 µmol/L	**4.448**	1.434-13.799	**0.005**
day 1 post-Tx kynurenic acid>3 µmol/L	**4.426**	1.572-12.463	**0.005**
pre-Tx tryptophan>23 µmol/L	**4.200**	1.525-11.565	**0.005**
day 1 post-Tx bilirubin>5 mg/dl	**4.025**	1.461-11.078	**0.006**
day 10 post-Tx neopterin>43 nmol/L	**8.781**	1.892-40.754	**0.006**
pre-Tx phenylal.≥70 µmol/L	**4.063**	1.410-11.706	**0.009**
pre-Tx kynurenic acid≥1.8 µmol/L	**3.480**	1.264-9.582	**0.014**
day 5 post-Tx kynurenic acid>2 µmol/L	**3.415**	1.236-9.435	**0.018**
pre-Tx qunolinic acid>0.77 µmol/L	**3.236**	1.124-9.317	**0.022**
pre-Tx Kyn/Trp ratios>0.25	**3.153**	1.174-8.471	**0.023**
post-Tx bacteremia	**3.428**	1.091-10.778	**0.024**
day 1 post-Tx Kyn/Trp ratios>0.16	**3.178**	1.134-8.961	**0.028**
day 5 post-Tx kynurenine 3.0 µmol/l	**3.070**	1.112-8.479	**0.030**
MELD score≥20	**2.893**	1.077-7.772	**0.035**
multivariate Cox-regression analysis significant parameters of mortality			
day 10 post-Tx phenylal.>68 µmol/L	**13.833**	4.007-47.805	**<0.0001**
day 1 post-Tx Kyn/Trp ratios>0.16	**4.704**	1.424-15.554	**0.011**
univariate regression analysis of EAD risk factors			
day 1 post-Tx phenylal. ≥ 70 µmol/l	**9.652**	2.003-31.024	**<0.001**
day 5 post-Tx kynurenine 3.0 µmol/l	**8.905**	2.272-29.075	**<0.001**
day 5 post-Tx CRP≥ 27mg/l	**6.000**	2.708-17.325	**0.001**
day 10 post-Tx kynurenine 3.0 µmol/l	**9.900**	2.240-43.747	**0.002**
day 1 post-Tx kynurenine ≥5 µmol/l	**6.296**	2.0002-19.804	**0.002**
day 3 post-Tx phenylal. ≥ 65 µmol/l	**4.952**	1.554-15.778	**0.007**
MELD score ≥20	**3.704**	1.338-10.255	**0.012**
female gender	**3.508**	1.241-9.908	**0.018**
Re-Tx	**3.569**	1.179-10.801	**0.024**
day 3 post-Tx kynurenine 3.5 µmol/l	**3.235**	1.093-9.576	**0.034**
multivariate regression analysis significant parameters of EAD			
day 5 post-Tx kynurenine ≥ 5 µmol/l	**6.926**	2.236-21.453	**0.001**
female gender	**5.312**	1.455-19.395	**0.011**
day 1 post-Tx phenylal. ≥ 70 µmol/l	**4.588**	1.231-17.099	**0.023**

Figure Legends:

Figure 1a-f: a) phenylalanine, b) kynurenine, c) kynurenic acid, d) quinolinic acid, e) neopterin and f) bilirubin at different pre- and posttransplant intervals in survivors (S) and non-survivors N.S.).

Figure 2a-e: a) phenylalanine, b) kynurenine, c) CRP, d) bilirubin at different pre- and posttransplant intervals in patients with and without EAD.

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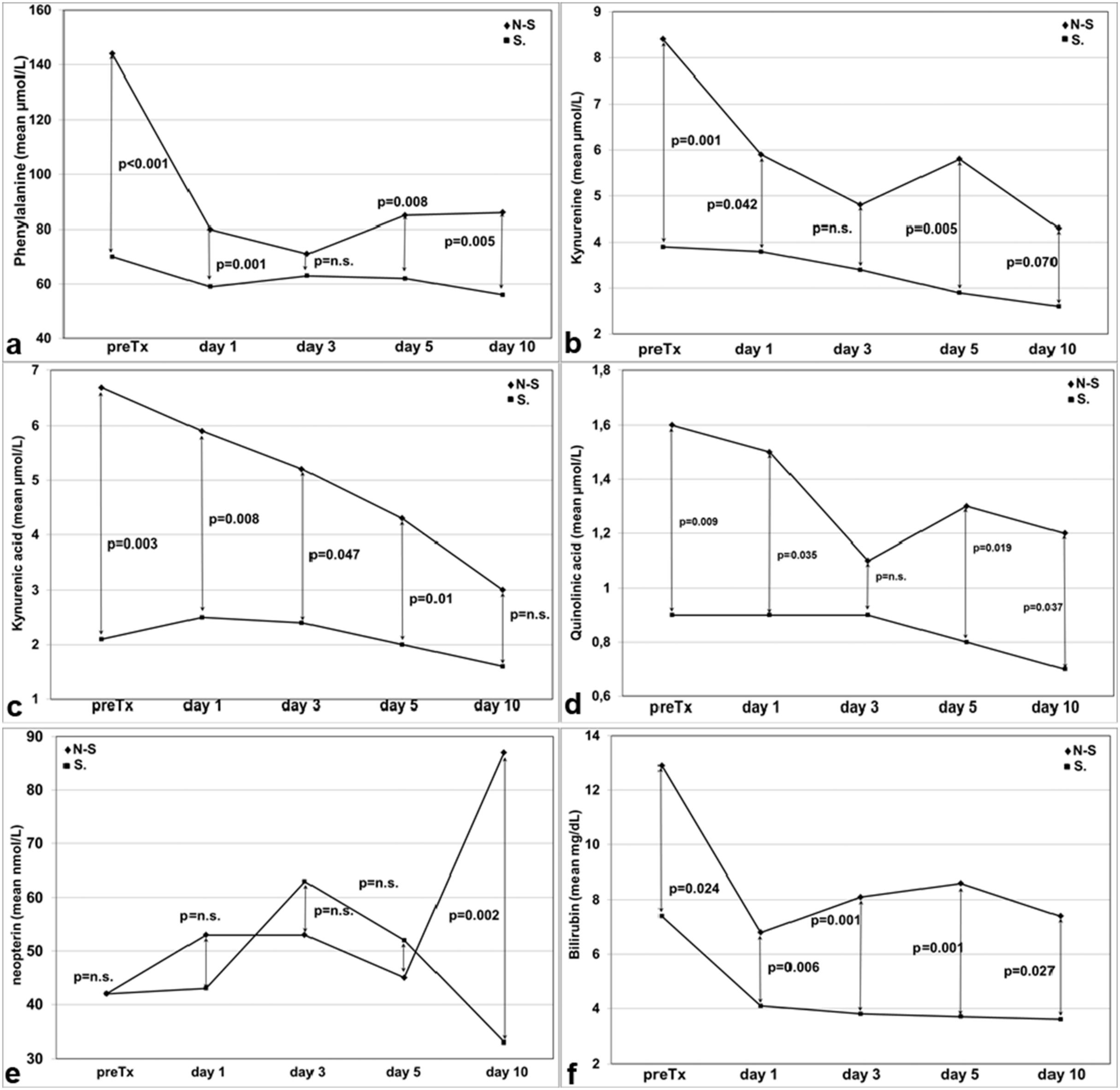
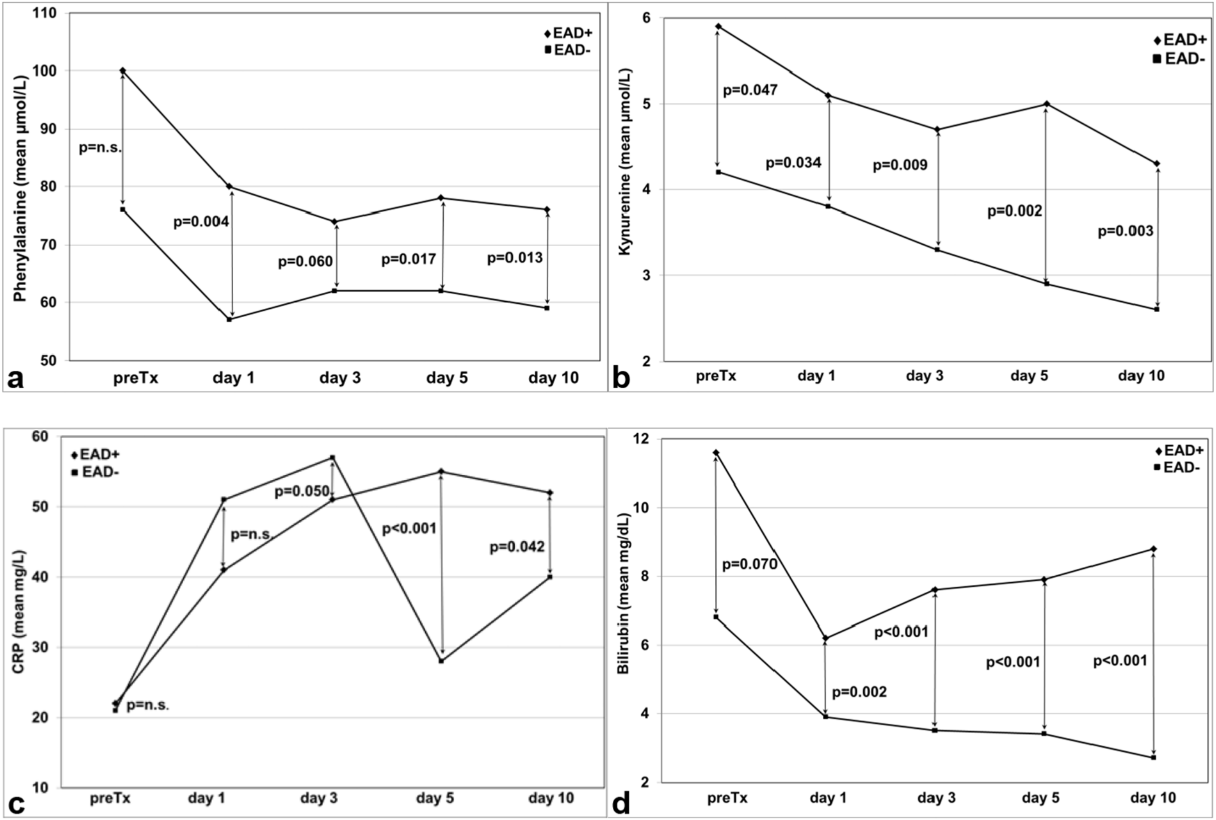


Phenylalanine (mean µmol/L)
N-S
S.
p<0.001
p=0.001
p=n.s.
p=0.008
p=0.005
preTx
day 1
day 3
day 5
day 10
a
Kynurenine (mean µmol/L)
p=0.001
p=0.042
p=n.s.
p=0.005
p=0.070
b
Kynurenic acid (mean µmol/L)
p=0.003
p=0.008
p=0.047
p=0.01
p=n.s.
c
Quinolinic acid (mean µmol/L)
p=0.009
p=0.035
p=n.s.
p=0.019
p=0.037
d
neopterin (mean nmol/L)
p=n.s.
p=n.s.
p=n.s.
p=n.s.
p=0.002
e
Bilirubin (mean mg/dL)
p=0.024
p=0.006
p=0.001
p=0.001
p=0.027
f

Figure 1



Phenylalanine (mean µmol/L)
EAD+
EAD-
p=n.s.
p=0.004
p=0.060
p=0.017
p=0.013
preTx
day 1
day 3
day 5
day 10
a
Kynurenine (mean µmol/L)
EAD+
EAD-
p=0.047
p=0.034
p=0.009
p=0.002
p=0.003
b
CRP (mean mg/L)
EAD+
EAD-
p=n.s.
p=0.050
p<0.001
p=0.042
c
Bilirubin (mean mg/dL)
EAD+
EAD-
p=0.070
p=0.002
p<0.001
p<0.001
p<0.001
d

Figure 2