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Mass spectrometric measurement of urinary kynurenine-to-tryptophan ratio in children with and without urinary tract infection

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Abstract

Background—Indoleamine-2,3-dioxygenase (IDO) catalyzes the first step of tryptophan (Trp) catabolism, yielding kynurenine (Kyn) metabolites. The kynurenine-to-tryptophan (K/T) ratio is used as a surrogate for biological IDO enzyme activity. IDO expression is increased during *Escherichia coli* urinary tract infection (UTI). Thus, our objective was to develop a method for measurement of Kyn/Trp ratio in human blood and urine and evaluate its use as a biomarker of UTI.

Methods—A mass spectrometric method was developed to measure Trp and Kyn in serum and urine specimens. The method was applied to clinical urine specimens from symptomatic pediatric patients with laboratory-confirmed UTI or other acute conditions and from healthy controls.

Results—The liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was linear to 500 $\mu\text{mol/L}$ for both Trp and Kyn. Imprecision ranged from 5–15% for Trp and 6–20% for Kyn. Analytical recoveries of Trp and Kyn ranged from 96–119% in serum and 90–97% in urine. No correlation was found between the K/T ratio and circulating IDO mass ($r = 0.110$) in serum. Urinary Kyn and Trp in the pediatric test cohort demonstrated elevations in the K/T ratio in symptomatic patients with UTI (median 13.08) and without UTI (median 14.38) compared to

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DECLARATION OF INTEREST

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healthy controls (median 4.93; $p < 0.001$ for both comparisons). No significant difference in K/T ratio was noted between symptomatic patients with and without UTI ($p = 0.84$).

Conclusions—Measurement of Trp and Kyn by LC-MS/MS is accurate and precise in serum and urine specimens. While urinary K/T ratio is not a specific biomarker for UTI, it may represent a general indicator of a systemic inflammatory process.

Graphical abstract

Keywords

indoleamine 2,3-dioxygenase; mass spectrometry; urine; K/T ratio

1. INTRODUCTION

Indoleamine 2,3-dioxygenase (IDO) is a mammalian enzyme that catalyzes the enzymatic conversion of tryptophan to its first stable metabolite, L-kynurenine. The best recognized physiologic role of IDO is in regulatory T lymphocytes, where consumption of tryptophan limits local proliferation of effector T cells in the settings of maternal-fetal tolerance, autoimmunity, and tumorigenesis [1, 2]. Expression of IDO in many cell types is low in the basal state but is upregulated in a variety of inflammatory conditions, and several groups have investigated the clinical utility of measuring IDO activity as a marker of disease burden [3–7]. This has typically been accomplished by measurement of kynurenine and tryptophan in serum samples, with the so-called “K/T ratio” serving as a surrogate for IDO enzymatic activity [8, 9]. Mass spectrometry (MS) has been used to define the urine metabolome, first in a diabetic murine model and subsequently applied clinically in the search for bladder cancer markers [10–12]. Measurement of urine kynurenine has been reported previously with high-performance liquid chromatography (HPLC) [13]. Here, we sought to establish methods for measurement of urine kynurenine and tryptophan utilizing liquid chromatography-tandem mass spectrometry (LC-MS/MS).

The diagnosis of urinary tract infection (UTI) in children remains challenging, as current methodologies provide suboptimal sensitivity and specificity, and clinical presentations may feature only nonspecific symptoms (*e.g.*, fever), especially in preverbal children [14]. Infections of the bladder (cystitis) and kidney (pyelonephritis) are caused primarily by uropathogenic strains of *Escherichia coli* (UPEC). Current guidelines for UTI diagnosis require thoughtful interpretation of the dipstick urinalysis in conjunction with clinical features and the results of urine culture [14]. To reduce contamination, appropriate urine cultures in young children are typically obtained via an invasive procedure, either urethral

catheterization or suprapubic aspiration. Finally, urine culture and antimicrobial susceptibility results may require 24–48 h of incubation, making timely treatment decisions difficult.

These clinical conundrums have prompted calls for research to identify new UTI biomarkers, ideally in samples collected noninvasively [14–18]. Recent efforts include evaluations of urinary small peptides for rapid diagnosis in the clinical setting, which have not yet proven successful [19, 20]. Preclinical studies have demonstrated that UPEC induce local IDO expression in the urinary bladder during acute cystitis *in vivo* [21, 22]. We therefore aimed to develop mass spectrometric methods to measure K/T ratio in human urine as well as serum, and to evaluate the potential utility of the urinary K/T ratio in the diagnosis of UTI in young children.

2. MATERIALS AND METHODS

2.1 Materials and hardware

Tryptophan, kynurenine, acetonitrile, methanol, and formic acid were purchased from Sigma-Aldrich (St. Louis, MO). Tryptophan (indole-D5) and kynurenine (ring-D4, 3,3-D2) were obtained from Cambridge Isotope Laboratories (Andover, MA). Human indoleamine-2,3-dioxygenase (IDO) ELISA was purchased from Kamiya Biomedical (Seattle, WA). Metabolite assays were performed using an AB-Sciex API 3200 tandem mass spectrometer (Foster City, CA) equipped with an electrospray ion source coupled to an Agilent 1200 HPLC system (Santa Clara, CA) in positive ionization mode.

2.2 Analytical methods

Serum or urine was diluted 10-fold in mobile phase (80% acetonitrile/0.1% formic acid) containing 25 $\mu\text{mol/L}$ D5-tryptophan and 2.5 $\mu\text{mol/L}$ D6-kynurenine, then centrifuged at $13,000 \times g$ for 5 min at room temperature. 1.0 μL of supernatant was injected into mobile phase at a flow rate of 350 $\mu\text{L/min}$, and ion current was monitored for 1.5 min. Precursor/product pairs monitored were 205/146, 210/150, 209/94, and 215/98 for tryptophan, D5-tryptophan, kynurenine, and D6-kynurenine, respectively. Declustering, entrance, collision cell entry, and collision cell exit potentials were 26.0/31.0 V, 7.0/6.5 V, 7.0/14.0 V, 4.0/4.0 V, respectively for tryptophan/kynurenine. Collision energies were 23 eV and 19 eV for tryptophan and kynurenine, respectively. Desolvation temperature was 350°C, and dwell time was 100 msec. A seven-point, linear, unweighted calibration curve was employed for quantitation. Circulating immunoreactive IDO was determined in serum from patients with a broad range of K/T ratios using a two-site ELISA method performed according to manufacturer instructions. Samples were diluted 20-fold in PBS prior to analysis, and concentrations were determined in 2–4 replicates.

2.3 Study subjects

All activities involving human subjects received review and approval in advance from the Human Research Protection Office at Washington University. Analytic development was supported with residual serum and urine specimens submitted to the St. Louis Children's Hospital (SLCH) Core Laboratory for clinical indications. Serum and urine reference

intervals were determined in specimens with negative serologic testing and normal urinalysis that were frozen at -20°C until analysis. Prior studies have indicated that there is no significant change in either kynurenine or tryptophan concentration after prolonged specimen storage at freezing temperatures in serum and urine [23, 24]. For the UTI test cohort, children < 2 years of age were enrolled from the Emergency Department (ED) and inpatient units at SLCH from March 2013 through June 2014. Written consent was obtained in all cases from the parent or legal guardian; enrolled children were not of appropriate age to provide assent. Children were selected for screening if there was clinical concern for UTI and if urine and blood sampling had been ordered for routine clinical purposes. Children were excluded from the study if they had a history of urinary tract instrumentation (surgery, cystoscopy, stenting), if there was no English-speaking caregiver, or if consent was not obtained. After full laboratory results and clinical follow-up were available, subjects were then categorized into one of two groups. Children with “Confirmed UTI” had urinalysis compatible with UTI (positive leukocyte esterase, nitrites, and/or pyuria by microscopy) plus a catheter-obtained urine culture yielding greater than 50,000 colony-forming units (CFU) of a typical uropathogen [14]. Enrolled patients not meeting these UTI criteria, and therefore having other final diagnoses, were termed “Symptomatic.” Separately, we contemporaneously enrolled healthy control children < 2 years of age from the Same Day Surgery unit at SLCH. This “Healthy” cohort included children presenting for elective ambulatory surgery procedures, those receiving sedation in the ambulatory procedure center for a scheduled outpatient procedure, or those visiting SLCH outpatient areas who were having blood drawn for clinical testing.

2.4 Statistical analysis

Correlations of analytes with age, and of K/T ratio with measured IDO mass, were studied using Pearson’s coefficient in SPSS version 25 (IBM Analytics, Armonk, NY). To compare the K/T ratios in healthy controls vs. UTI or symptomatic non-UTI groups, Mann-Whitney U test was performed using Prism version 7 for Windows (GraphPad, La Jolla, CA). Proportions of true positive (TP), false positive (FP), true negative (TN), and false negative (FN) test results were determined using urine culture as the gold standard for comparison. Sensitivity and specificity were calculated as follows: sensitivity = $\text{TP}/(\text{TP}+\text{FN})$ and specificity = $\text{TN}/(\text{TN}+\text{FP})$.

3. RESULTS

3.1 Analytical performance of Trp and Kyn measurement by mass spectrometry

After correcting for endogenous concentrations, Trp and Kyn were measured in serially diluted serum and urine pools supplemented with Trp and Kyn to a starting concentration of $500\text{ }\mu\text{mol/L}$. Measurements of both analytes were linear to at least $500\text{ }\mu\text{mol/L}$ (Figure 1). Accuracy of both measurements was established by assessing recovery at two concentrations in both serum and urine. Mean recovery of Trp and Kyn ranged from 97–101% in serum and from 90–97% in urine (Table 1). Imprecision of Trp and Kyn was assessed at two concentrations in both sample matrices (Table 1) by performing 5 replicate measurements in 5 analytic runs over 5 d. At concentrations of 51 and $224\text{ }\mu\text{mol/L}$ in serum, imprecision in Trp measurement was 5.1% and 6.2%, respectively; in urine, imprecision was 15% and 9.2%

at 85 and 1150 $\mu\text{mol/L}$, respectively. For Kyn measurement, imprecision was 14.4% and 6.7% in serum at 1.6 and 6.6 $\mu\text{mol/L}$, respectively, and 20.8% and 9.0% in urine at 3.2 and 18 $\mu\text{mol/L}$, respectively. Within-run imprecision ($n=10$) at the lowest measured concentration of Trp and Kyn (0.5 $\mu\text{mol/L}$) was 12% and 3.1%, respectively (data not shown).

Endogenous Kyn and Trp concentrations were measured in residual serum samples ($n = 53$) from general pediatric patients (1–21 years of age). Non-parametric reference intervals were calculated as 14–97 $\mu\text{mol/L}$ for Trp and <3.5 $\mu\text{mol/L}$ for Kyn (Figure 2). The reference interval for K/T ratio was calculated as 1.6–8.0. No age-dependent correlation with serum Trp ($r = -0.0496$, $p = 0.72$) or Kyn ($r = -0.2075$, $p = 0.13$) concentration was found. Age was significantly correlated with serum K/T ratio ($r = -0.2807$, $p = 0.04$).

Kyn and Trp measurement in residual urine specimens ($n = 60$) from general pediatric patients (aged 2 months–22 years) with normal urinalysis demonstrated reference intervals of 1.3–34 $\mu\text{mol/mmol}$ creatinine for Trp and 0.03–4.1 $\mu\text{mol/mmol}$ creatinine for Kyn (Figure 3). In these same specimens, the reference interval for K/T ratio was 0.9–27. Urine tryptophan ($r = -0.4062$, $p = 0.001$) and kynurenine ($r = -0.3448$, $p = 0.007$) concentrations were negatively correlated with age. No age-dependent trend was observed in urine K/T ratio ($r = 0.0842$, $p = 0.52$).

3.2 Comparison of serum K/T ratio and circulating IDO mass

The circulating IDO mass as measured by ELISA was compared to Kyn and Trp as measured by LC-MS/MS in residual samples from general pediatric patients ($n = 69$). Measured IDO mass ranged from 173–1951 ng/mL (mean 671 ng/mL) in samples with K/T ratios ranging from 1.9 to 56 (mean 8.5). There was no correlation between the serum K/T ratio and measured IDO mass ($r = 0.110$, $p = 0.37$; Figure 4).

3.3 Clinical utility of the K/T ratio as a biomarker in UTI

With the intention to develop improved noninvasive testing for young children, we enrolled subjects who were between 1 and 14 months of age (Table 2). Urinalysis from patients with confirmed UTI included a majority with positive leukocyte esterase by dipstick and white blood cells visible by microscopy. The majority of microbial isolates recovered from patients with confirmed UTI were *E. coli* (Table 2). Urine from healthy controls, symptomatic subjects without UTI, and confirmed UTI subjects underwent Kyn and Trp measurement, from which the urinary K/T ratio was obtained; data from the three groups were compared using the Mann-Whitney U test. The urinary K/T ratio was significantly higher in both symptomatic non-UTI subjects (median, 14.38) and confirmed UTI subjects (median, 13.08) as compared to healthy controls (median, 4.93; $p<0.0001$ for each comparison; Figure 5). However, there was no statistically significant difference in urinary K/T ratio between subjects with UTI and those who were symptomatic with other acute conditions ($p=0.84$; Figure 5). Using the K/T ratio reference ranges determined for urine specimens (0.9–27), the K/T ratio was 30% sensitive and 61% specific in patients with culture-confirmed UTI.

4. DISCUSSION

Serum IDO activity has been investigated as a means to monitor disease progression or response to therapy in tuberculosis, community acquired pneumonia, solid-organ transplantation, and inflammatory bowel disease [3–9, 25, 26]. In the absence of readily available methods to directly measure IDO enzymatic activity, measurement of IDO mass by immunoassay or of Kyn and Trp concentrations by high-performance liquid chromatography has served as a surrogate for IDO activity. However, these methods can be cumbersome to perform, and the analytical performance of these methods is not well characterized. Here, we developed a rapid and accurate method for measurement of Trp and Kyn using LC-MS/MS in clinical serum and urine samples. This method is sufficiently sensitive to measure biomarker concentrations of Trp and Kyn in biological matrices including serum and urine, with acceptable assay characteristics. Previous validation studies have reported mass spectrometric methods for measurement of Trp and Kyn [27–30] but did not include urine as a validated biological specimen matrix or assess the clinical utility of the method. Advantages to this method include simultaneous measurement of multiple analytes with low sample volume, adaptability to measure additional metabolites of the Trp pathway, rapid performance, and ease of sample preparation.

Reliable measurement of Kyn and Trp, in turn, should facilitate calculation of more precise K/T ratios. Interestingly, our measurements of IDO mass by immunoassay to estimate IDO activity in unselected serum specimens representing a broad range of K/T ratios found no correlation between circulating IDO mass and serum K/T ratio. This finding may reflect a lack of correlation between circulating IDO mass and IDO enzymatic activity, though mechanisms regulating IDO activity in this manner have not been described. If IDO enzymatic activity is enhanced during inflammatory states, this might lead to a tighter correlation between IDO mass and K/T ratio. In addition, such correlation might be confounded by activity of tryptophan dioxygenase (TDO), another heme-dependent enzyme that converts tryptophan to kynurenine but whose mammalian tissue expression is much more limited (liver and epidermis) compared with the ubiquitously expressed IDO [31].

Given that experimental acute cystitis in mice provokes local IDO expression in the urinary tract [21], we sought to demonstrate a relationship between the K/T ratio and the presence of UTI in a cohort of young children. The K/T ratio was elevated in human UTI, as would be suspected from the aforementioned preclinical data, but did not exhibit specificity for UTI. Indeed, the performance characteristics we observed for urine K/T by LC-MS/MS are substantially lower than existing point-of-care and laboratory indicators of UTI (reviewed in [14]; e.g., leukocyte esterase, nitrite, and urine microscopy for pyuria). Despite this, establishing a means to accurately measure the K/T ratio in urine may be useful in monitoring other inflammatory disease processes, as an easier alternative to serum measurements.

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References

1. Mellor AL, Munn DH. IDO expression by dendritic cells: Tolerance and tryptophan catabolism. *Nat Rev Immunol.* 2004; 4:762–774. [PubMed: 15459668]
2. Zelante T, Fallarino F, Bistoni F, Puccetti P, Romani L. Indoleamine 2,3-dioxygenase in infection: the paradox of an evasive strategy that benefits the host. *Microbes Infect.* 2009; 11:133–141. [PubMed: 19007906]
3. Adu-Gyamfi CG, Snyman T, Hoffmann CJ, Martinson NA, Chaisson RE, George JA, Suchard MS. Plasma indoleamine 2, 3-dioxygenase, a biomarker for tuberculosis in human immunodeficiency virus-infected patients. *Clin Infect Dis.* 2017; 65:1356–1358. [PubMed: 29017244]
4. Suzuki Y, Suda T, Asada K, Miwa S, Suzuki M, Fujie M, Furuhashi K, Nakamura Y, Inui N, Shirai T, Hayakawa H, Nakamura H, Chida K. Serum indoleamine 2,3-dioxygenase activity predicts prognosis of pulmonary tuberculosis. *Clin Vaccine Immunol.* 2012; 19:436–442. [PubMed: 22219312]
5. Suzuki Y, Suda T, Furuhashi K, Suzuki M, Fujie M, Hahimoto D, Nakamura Y, Inui N, Nakamura H, Chida K. Increased serum kynurenine/tryptophan ratio correlates with disease progression in lung cancer. *Lung Cancer.* 2010; 67:361–365. [PubMed: 19487045]
6. Suzuki Y, Suda T, Yokomura K, Suzuki M, Fujie M, Furuhashi K, Hahimoto D, Enomoto N, Fujisawa T, Nakamura Y, Inui N, Nakano Y, Nakamura H, Chida K. Serum activity of indoleamine 2,3-dioxygenase predicts prognosis of community-acquired pneumonia. *J Infect.* 2011; 63:215–222. [PubMed: 21784100]
7. Suarez-Fuentetaja N, Domenech-Garcia N, Paniagua-Martin MJ, Marzoa-Rivas R, Barge-Caballero E, Grille-Cancela Z, Pombo-Otero J, Muniz-Garcia J, Castro-Beiras A, Crespo-Leiro MG. Indoleamine 2-3 dioxygenase activity could be an early marker of graft rejection in heart transplantation. *Transplant Proc.* 2012; 44:2645–2648. [PubMed: 23146482]
8. Brandacher G, Cakar F, Winkler C, Schneeberger S, Obrist P, Bosmuller C, Werner-Felmayer G, Werner ER, Bonatti H, Margreiter R, Fuchs D. Non-invasive monitoring of kidney allograft rejection through IDO metabolism evaluation. *Kidney Int.* 2007; 71:60–67. [PubMed: 17136028]
9. Dharnidharka VR, Al Khasawneh E, Gupta S, Shuster JJ, Theriaque DW, Shahlaee AH, Garrett TJ. Verification of association of elevated serum IDO enzyme activity with acute rejection and low CD4-ATP levels with infection. *Transplantation.* 2013; 96:567–572. [PubMed: 23823655]
10. Godzien J, Ciborowski M, Angulo S, Ruperez FJ, Martinez MP, Senorans FJ, Cifuentes A, Ibanez E, Barbas C. Metabolomic approach with LC-QTOF to study the effect of a nutraceutical treatment on urine of diabetic rats. *J Proteome Res.* 2011; 10:837–844. [PubMed: 21087057]
11. Liu X, Cheng X, Liu X, He L, Zhang W, Wang Y, Sun W, Ji Z. Investigation of the urinary metabolic variations and the application in bladder cancer biomarker discovery. *Int J Cancer.* 2018; epub ahead of print. doi: 10.1002/ijc.31323
12. Alberice JV, Amaral AF, Armitage EG, Lorente JA, Algaba F, Carrilho E, Marquez M, Garcia A, Malats N, Barbas C. Searching for urine biomarkers of bladder cancer recurrence using a liquid chromatography-mass spectrometry and capillary electrophoresis-mass spectrometry metabolomics approach. *J Chromatogr A.* 2013; 1318:163–170. [PubMed: 24139504]
13. Marfella A, Polese C, d'Alessio P, Beneduce G, Rossi F, Perna M. An HPLC method for the simultaneous analysis of urinary neopterin and kynurenine. *Pharmacol Res.* 1992; 26(Suppl 2): 174–175. [PubMed: 1409297]
14. Roberts KB, Downs SM, Finnell SME, Hellerstein S, Shortliffe LD, Wald ER, Zerlin JM. Urinary tract infection: clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. *Pediatrics.* 2011; 128:595–610. [PubMed: 21873693]
15. Chaudhari PP, Monuteaux MC, Bachur RG. Urine concentration and pyuria for identifying UTI in infants. *Pediatrics.* 2016; 138 pii: e20162370.
16. Shaikh N, Shope TR, Hoberman A, Vigliotti A, Kurs-Lasky M, Martin JM. Association between uropathogen and pyuria. *Pediatrics.* 2016; 138doi: 10.1542/peds.2016-0087
17. Krzemien G, Panczyk-Tomaszewska M, Adamczuk D, Kotula I, Demkow U, Szmigielska A. Neutrophil gelatinase-associated lipocalin: a biomarker for early diagnosis of urinary tract infections in infants. *Adv Exp Med Biol.* 2017; doi: 10.1007/5584_2017_107

18. Valdimarsson S, Jodal U, Barregard L, Hansson S. Urine neutrophil gelatinase-associated lipocalin and other biomarkers in infants with urinary tract infection and in febrile controls. *Pediatr Nephrol*. 2017; 32:2079–2087. [PubMed: 28756475]
19. Gill K, Horsley H, Kupelian AS, Baio G, De Iorio M, Sathiananamoorthy S, Khasriya R, Rohn JL, Wildman SS, Malone-Lee J. Urinary ATP as an indicator of infection and inflammation of the urinary tract in patients with lower urinary tract symptoms. *BMC Urol*. 2015; 15:7. [PubMed: 25886951]
20. Watson JR, Hains DS, Cohen DM, Spencer JD, Kline JM, Yin H, Schwaderer AL. Evaluation of novel urinary tract infection biomarkers in children. *Pediatr Res*. 2016; 79:934–939. [PubMed: 26885759]
21. Loughman JA, Hunstad DA. Induction of indoleamine 2,3-dioxygenase by uropathogenic bacteria attenuates innate responses to epithelial infection. *J Infect Dis*. 2012; 205:1830–1839. [PubMed: 22474038]
22. Loughman JA, Yarbrough ML, Tiemann KM, Hunstad DA. Local generation of kynurenines mediates inhibition of neutrophil chemotaxis by uropathogenic *Escherichia coli*. *Infect Immun*. 2016; 84:1176–1183. [PubMed: 26857571]
23. Geeraerts F, Schimpfessel L, Crokaert R. The stability of tryptophan metabolites prior to urine analysis. *Clin Chim Acta*. 1980; 102:247–251. [PubMed: 6154548]
24. Middtun O, Townsend MK, Nygard O, Tworoger SS, Brennan P, Johansson M, Ueland PM. Most blood biomarkers related to vitamin status, one-carbon metabolism, and the kynurenine pathway show adequate preanalytical stability and within-person reproducibility to allow assessment of exposure or nutritional status in healthy women and cardiovascular patients. *J Nutr*. 2014; 144:784–790. [PubMed: 24647388]
25. Ciorba MA. Indoleamine 2,3 dioxygenase in intestinal disease. *Curr Opin Gastroenterol*. 2013; 29:146–152. [PubMed: 23283180]
26. Gupta NK, Thaker AI, Kanuri N, Riehl TE, Rowley CW, Stenson WF, Ciorba MA. Serum analysis of tryptophan catabolism pathway: correlation with Crohn's disease activity. *Inflam Bowel Dis*. 2012; 18:1214–1220.
27. Moller M, Du Preez JL, Harvey BH. Development and validation of a single analytical method for the determination of tryptophan, and its kynurenine metabolites in rat plasma. *J Chromatogr B*. 2012; 898:121–129.
28. Huang Y, Louie A, Yang Q, Massenkoff N, Xu C, Hunt PW, Gee W. A simple LC-MS/MS method for determination of kynurenine and tryptophan concentrations in human plasma from HIV-infected patients. *Bioanalysis*. 2013; 5(11):1397–1407. [PubMed: 23742309]
29. Fuertig R, Ceci A, Camus SM, Bezard E, Luippold AH, Hengerer B. LC-MS/MS-based quantification of kynurenine metabolites, tryptophan, monoamines and neopterin in plasma, cerebrospinal fluid and brain. *Bioanalysis*. 2016; 8(18):1903–1917. [PubMed: 27524289]
30. Yamada K, Miyazaki T, Shibata T, Hara N, Tsuchiya M. Simultaneous measurement of tryptophan and related compounds by liquid chromatography/electrospray ionization tandem mass spectrometry. *J Chromatogr B*. 2008; 867:57–61.
31. Thackray SJ, Mowat CG, Chapman SK. Exploring the mechanism of tryptophan 2,3-dioxygenase. *Biochem Soc Trans*. 2008; 36:1120–1123. [PubMed: 19021508]

HIGHLIGHTS

- An LC-MS/MS method accurately measures urinary kynurenine and tryptophan
- Urinary K/T ratio does not correlate with circulating IDO mass by ELISA
- Urinary K/T ratio is elevated in UTI and other acute febrile illnesses in children

Figure 1.

Linearity of TRP (top) and KYN (bottom) determined in a serum (closed circles) and urine (open circles) matrix. Each data point represents the mean of at least two determinations at each indicated concentration. Matrix-independent regression statistics for TRP are: Pearson r , 0.998; slope, 1.03, intercept, 1.1; S_{yx} , 11.2. Matrix-independent regression statistics for KYN are: Pearson r , 0.996; slope, 0.997; intercept, 3.5; S_{yx} , 13.1.

Figure 2.

Determination of reference intervals for tryptophan (upper), kynurenine (middle), and K/T ratio (lower) in 53 residual serum specimens from patients with negative serology results. All residual specimens were stored at -20°C for less than 1 month prior to analysis. There was no significant correlation of Trp ($r = -0.0496$, $p = 0.72$) or Kyn ($r = -0.2075$, $p = 0.13$) concentration with age. Serum K/T ratio was significantly correlated with age ($r = -0.2807$, $p = 0.04$).

Figure 3.

Determination of reference intervals for tryptophan (upper), kynurenine (middle), and K/T ratio in 60 residual urine specimens from patients with normal urinalysis. Specimens were frozen within 6 h of collection and stored at -20°C prior to analysis. Urine Trp ($r = -0.4062$, $p = 0.001$) and Kyn ($r = -0.3448$, $p = 0.007$) concentrations were negatively correlated with age. There was no age-dependent trend in urine K/T ratio ($r = 0.0842$, $p = 0.52$).

Figure 4. Circulating IDO-1 mass as measured by ELISA does not correlate with serum K/T ratio as measured by LC-MS/MS. Circulating concentrations of Trp, Kyn, and IDO-1 were determined in 69 unselected, residual serum specimens from pediatric subjects ($r = 0.110$, $p = 0.37$).

Figure 5.

Measurement of urinary kynurenine-to-tryptophan (K/T) ratio in three cohorts of pediatric human subjects. Each dot represents the mean of multiple measurements on the enrollment sample from each subject in the indicated groups, and the horizontal line represents the median for each group. Those with confirmed urinary tract infection (UTI; $n = 33$) and those who were symptomatic with other acute illnesses ($n = 30$) showed significantly higher urine K/T ratios than healthy controls ($n = 15$; $p < 0.0001$ for each comparison). However, the test was not specific for UTI, as there was no difference in median, range, or ranks of the K/T ratio between UTI and symptomatic non-UTI patients.

Table 1

Imprecision and Recovery

IMPRECISION				
Sample	Analyte	Concentration (μM)	%CV	
Serum	Tryptophan	51	5.1	
Serum	Tryptophan	224	6.2	
Serum	Kynurenine	1.7	6.7	
Serum	Kynurenine	6.6	8.3	
Urine	Tryptophan	85	15	
Urine	Tryptophan	1150 ^a	9.2	
Urine	Kynurenine	3.2	20	
Urine	Kynurenine	18	8.9	
RECOVERY				
Sample	Analyte	Expected (μM)	Measured (μM)	% Recovery (Range)
Serum	Tryptophan	83	81	97 (92–98)
Serum	Tryptophan	258	259	101 (94–105)
Serum	Kynurenine	6.4	7.6	119 (100–132)
Serum	Kynurenine	27	26	96 (88–111)
Urine	Tryptophan	175	157	90 (88–91)
Urine	Tryptophan	350	341	97 (96–98)
Urine	Kynurenine	13	12	95 (94–96)
Urine	Kynurenine	30	28	92 (88–99)

CV, coefficient of variance.

^a Measured on dilution.

Table 2

Patient Characteristics

	Healthy n = 15 (%)	Symptomatic n = 30 (%)	Confirmed UTI n = 33 (%)
Age, mo (mean $\pm$ SD)	8.6 $\pm$ 5.4	7.8 $\pm$ 6.7	7.4 $\pm$ 6.5
Sex (male)	7 (46.7)	15 (50.0)	9 (27.3)
Race			
White	13 (86.7)	15 (50.0)	19 (57.6)
Black	2 (13.3)	14 (46.7)	12 (36.4)
Multiracial	0 (0)	1 (3.3)	0 (0)
Other	0 (0)	0 (0)	2 (6.0)
History of UTI	0 (0)	1 (3.3)	6 (18.2)
Fever			
Reported		23 (76.7)	25 (75.8)
Measured in ED		16 (53.3)	19 (57.6)
Urine culture positive		0 (0)	32 ^a (97.0)
<i>Escherichia coli</i>		0	26
Other bacteria		0	5 ^b
<i>Candida albicans</i>		0	1 ^c
Blood culture positive		0 (0)	4 (12.1)
Other confirmed infection		12 (40.0)	4 (12.1)
Complete blood count			
Number obtained		26 (86.7)	24 (72.7)
WBC/mm ³ $\times$ 1000 (mean $\pm$ SD)		10.7 $\pm$ 6.8	20.0 $\pm$ 8.5
Neutrophil % (mean $\pm$ SD)		42.0 $\pm$ 16.9	50.9 $\pm$ 20.7
Bands/mm ³ $\times$ 1000 (mean $\pm$ SD)		1.1 $\pm$ 2.8	3.0 $\pm$ 7.1
Urinalysis			
Number obtained		29 (96.7)	31 (93.9)
Leukocyte esterase			
Negative or trace		27	3
> 1+		2	28
Nitrite ^e			
Negative		29	16
Positive		0	14
WBC by microscopy ^e			
None or < 5/HPF		27	4
5–10/HPF		2	5
>10/HPF		1	17

UTI, urinary tract infection; ED, emergency department; WBC, white blood cells; HPF, high-power field.

^aIn one patient, urine culture was obtained after antibiotics were given; patient had >20 WBCs/HPF on urine microscopy and was treated for UTI

^b*Enterococcus* spp., *Klebsiella pneumoniae* (n=2), *Citrobacter freundii*, *Klebsiella oxytoca*

^cPatient with history of UTIs, > 20 WBCs/HPF on urine microscopy and was treated for *Candida* UTI

^dNitrite results not available for 1 sample in the Confirmed UTI group

^eMicroscopy results available on all samples from Symptomatic group and on 26 urine samples in the Confirmed UTI group