

# Tryptophan pathway catabolites (serotonin, 5-hydroxyindolacetic acid, kynurenine) and enzymes (monoamine oxidase and indole amine 2,3 dioxygenase) in patients with septic shock

## A prospective observational study versus healthy controls

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### Abstract

Septic shock is associated with a strong inflammatory response that induces vasodilation and vascular hyporeactivity. We investigated the role for tryptophan-pathway catabolites of proinflammatory cytokines in septic shock.

We prospectively included 30 patients with very recent-onset septic shock and 30 healthy volunteers. The following were assayed once in the controls and on days 1, 2, 3, 7, and 14 in each patient: plasma free and total tryptophan, platelet and plasma serotonin, total blood serotonin, urinary serotonin, plasma and urinary 5-hydroxyindolacetic acid, plasma kynurenine, monoamine oxidase activity, and total indole amine 2,3-dioxygenase activity. Organ-system failure and mortality were recorded.

Compared with the healthy controls, the patients with septic shock had 2-fold to 3-fold lower total tryptophan levels throughout the 14-day study period. Platelet serotonin was substantially lower, while monoamine oxidase activity and 5-hydroxyindolacetic acid were markedly higher in the patients than in the controls, consistent with the known conversion of tryptophan to serotonin, which is then promptly and largely degraded to 5-hydroxyindolacetic acid. Plasma kynurenine was moderately increased and indole amine 2,3-dioxygenase activity markedly increased in the patients versus the volunteers, reflecting conversion of tryptophan to kynurenine. Changes over time in tryptophan metabolites were not associated with survival in the patients but were associated with the Sequential Organ Failure Assessment score and hemodynamic variables including hypotension and norepinephrine requirements.

Our results demonstrate major tryptophan pathway alterations in septic shock. Marked alterations were found compared with healthy volunteers, and tryptophan metabolite levels were associated with organ failure and hemodynamic alterations. Tryptophan metabolite levels were not associated with surviving septic shock, although this result might be ascribable to the small sample size.

Trial registration: ClinicalTrials.gov; No: NCT00684736; URL: [www.clinicaltrials.gov](http://www.clinicaltrials.gov).

**Abbreviations:** 5HIAA = 5-hydroxyindoleacetic acid, 5HIAAp = plasma 5HIAA, 5HIAAu = urinary 5HIAA, 5-HT = 5-hydroxytryptamine (serotonin), 5HTp = plasma 5HT, 5HTpt = platelet 5HT, 5HTtot = total 5HT, 5HTu = urinary 5HT, AU = arbitrary unit, ICU = intensive care unit, IDO = indoleamine 2,3-dioxygenase, KYN = kynurenine, KYNp = plasma KYN, MAO = monoamine oxidase, SOFA = Sequential Organ Failure Assessment score, SS = septic shock, TRP = tryptophan, TRPpf = plasma free TRP, TRPtot = total TRP.

**Keywords:** human septic shock, mortality, organ failure, serotonin, tryptophan catabolites

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## 1. Introduction

Septic shock (SS) is associated with an excessive inflammatory response that involves a variety of pathways and induces vasodilatation and vascular hyporeactivity with hypotension, tissue hypoxia, lactate production, and potentially fatal multi-organ failure. The levels of proinflammatory cytokines and their metabolites are elevated and correlate with the outcome.<sup>[1–3]</sup> The essential amino acid tryptophan is metabolized to the serotonin (5HT) or kynurenine pathway (Fig. 1).<sup>[4,5]</sup> Conversion of tryptophan to kynurenine is regulated by the enzyme indole amine 2,3-dioxygenase (IDO). During sepsis, IDO gene transcription is modulated either via various cytokines, notably interferon  $\gamma$ , or directly by lipopolysaccharides.<sup>[4]</sup> Serotonin is primarily found in platelets (10%), the gastrointestinal tract (90%), and the central nervous system. Lipopolysaccharides induce serotonin release from platelets, platelet aggregation, vasodilation, and the production of reactive oxygen species in the lungs and central nervous system. Serotonin is either rapidly metabolized to 5-hydroxyindolacetic acid (5HIAA) by monoamine oxidase (MAO) or excreted by the kidneys. 5HIAA has little clinical effect but serves as a mechanism for eliminating released serotonin.

The aims of this study were to evaluate the potential changes in tryptophan pathways, serotonin, kynurenine, 5HIAA, IDO, and MAO levels or activities during SS compared with healthy

controls and to look for associations linking these compounds to clinical complications, laboratory parameters, and patient outcomes.

## 2. Materials and methods

### 2.1. Methods

We conducted a prospective, observational, single-center study that compared 30 patients with septic shock to healthy volunteers included between June 2004 and April 2007. All individuals who performed the laboratory assays and the statistical analyses were blinded to the study group.

The study was approved by the ethics committee of the Saint Germain-en-Laye Hospital and registered on ClinicalTrials.gov (NCT00684736). Written informed consent was obtained from the patients or relatives and from the healthy volunteers before study inclusion.

The laboratory assays done to assess tryptophan pathways were the only procedures performed specifically for the study.

### 2.2. Selection criteria for the group of patients with septic shock

We included adults ( $\geq 18$  years) admitted to our intensive care unit (ICU) with a strong presumption of septic shock defined as

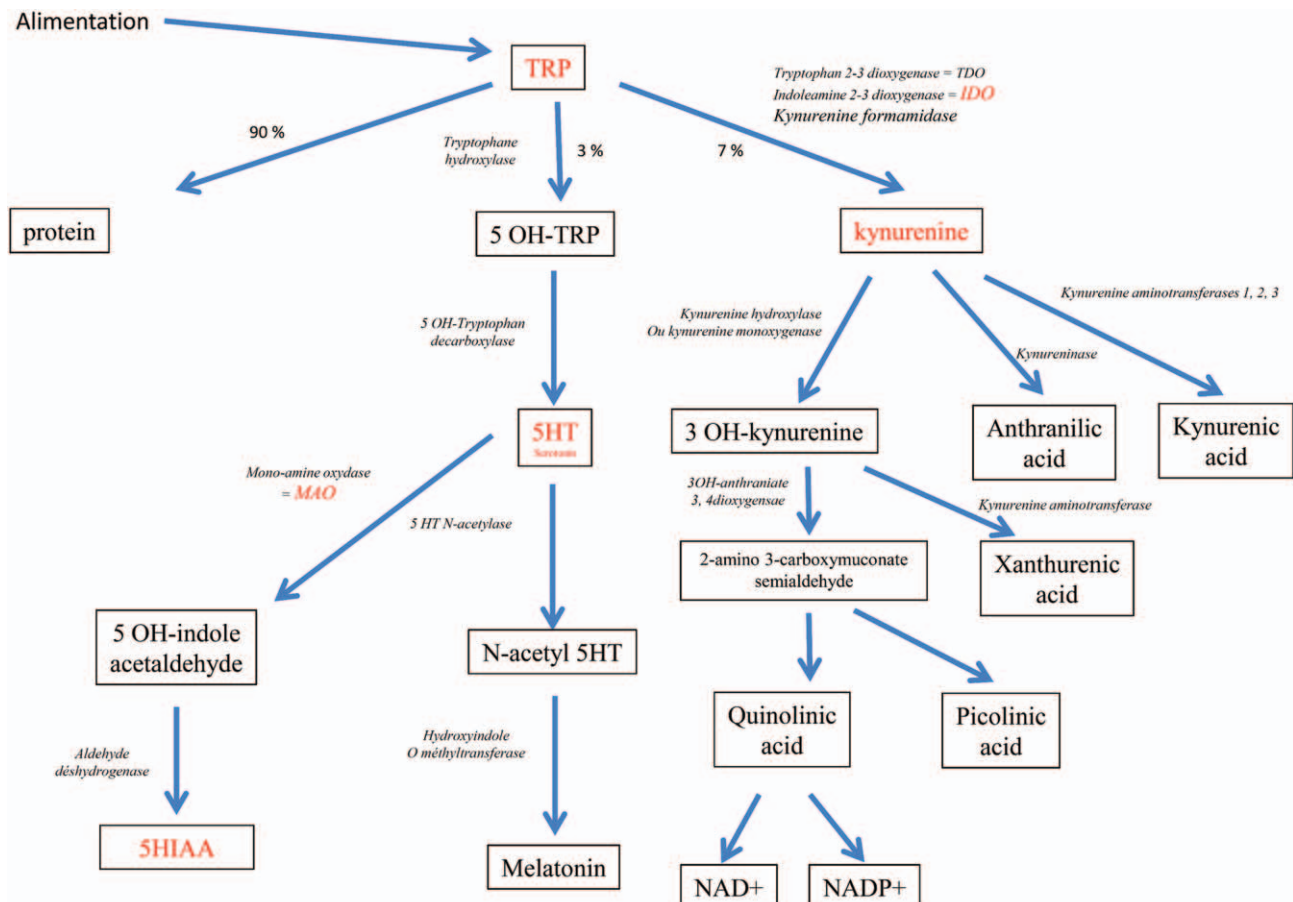


Figure 1. Metabolism of tryptophan.

**Table 1****Treatments known to modify serotonin level.**

Almotriptan	Fluvoxamine	Olanzapine
Amitriptyline	Granisetron	Ondansetron
Amoxapine	Imipramine	Oxetorone
Citalopram	Indoramin	Paroxetine
Clomipramine	Interferon alfa	Pizotifen
Clozapine	Interferon alfacon-1	Risperidone
Desipramine	Interferon beta	Sertraline
Dihydroergotamine	Iproniazid	Sumatriptan
Dolasetron	Maprotiline	Tianeptine
Dosulepin	Methysergide	Trimipramine
Doxepin	Mianserin	Tropisetron
Eletriptan	Milnacipran	Venlafaxine
Ergotamine	Mirtazapine	Viloxazine
Flunarizine	Moclobemide	Zolmitriptan
Fluoxetine	Naratriptan	

body temperature  $>38^{\circ}\text{C}$  or  $<36^{\circ}\text{C}$ , heart rate  $>90$  bpm, systolic blood pressure  $<90$  mmHg despite adequate fluid replacement or need for vasopressor therapy initiation within the last 3 hours, need for mechanical ventilation, and presence of at least one of the following:  $\text{PaO}_2/\text{FiO}_2 < 300$  mmHg, urine output  $< 0.5$  mL/kg/h or  $< 30$  mL/h for at least 1 hour, and/or arterial lactate  $> 2$  mmol/L. We did not include patients with any of the following: age  $< 18$  years, pregnancy, underlying disease expected to be fatal within 24 hours, do-not-resuscitate order, psychopathology (e.g., depression or psychosis), seizures, migraine, drug addiction, neuroendocrine tumor, obstructive cardiomyopathy or acute myocardial ischemia, pulmonary embolism, advanced malignancy or hematological malignancy, acquired immunodeficiency syndrome with a decision to withhold or withdraw aggressive treatments, inclusion in another clinical study, exposure to medications known to modify serotonin levels (Table 1), shock not due to sepsis, and/or septic shock onset at night, during the weekend, or on a weekday outside the laboratory opening hours.

### 2.3. Selection criteria for the control group of healthy volunteers

For each patient, we recruited a healthy volunteer among the staff members and students of our hospital. Only volunteers who were found upon detailed questioning to be free of exposure to compounds known to affect the serotonin or kynurenine pathway (Table 1) were eligible. Each volunteer was matched to a patient on age ( $\pm 3$  years), sex, smoking history, and season of the year.

### 2.4. Laboratory assays

In the patients, blood into EDTA tubes and urine samples was drawn between 8:30 and 9:00 am on the day of inclusion (D1), D2, D3, D7, and D14. In the volunteers, blood and urine samples were obtained once.

Blinded technicians used 3 different high performance liquid chromatography techniques with colorimetric electrochemical detection as previously described<sup>16,71</sup> to assay plasma free tryptophan (TRPpf) and total tryptophan (TRPtot); plasma serotonin (5HTp), platelet serotonin (5HTpt), total blood serotonin (5HTtot), and urinary serotonin (5HTu); and plasma 5HIAA (5HIAAp) and urinary 5HIAA (5HIAAu). Plasma

kynurenine (KYNp) was assayed using KYN ELISA (Cusabio Biotech, Wuhan Hubei, China). The results of the 5HTu and 5HIAAu assays were adjusted to renal function. MAO activity was measured in arbitrary units (AUs) as the ratio of 5HIAAp over 5HTp and IDO activity, also in AU, as the ratio of KYNp over TRPtot.

### 2.5. Follow-up of the patients

On D1, D2, D3, D7, and D14, in the morning at the same time as the blood sample collection, we recorded the vital signs, Sequential Organ Failure Assessment (SOFA) score, treatments, laboratory test results, and microbiological findings from any samples taken from new sites of infection. Organ-system failure was defined for each of the 6 major organ systems (respiration, coagulation, liver, cardiovascular, central nervous system, renal) as a score of 3 or 4 on a 0 to 4 scale for each organ system; thus, the total score could range from 0 to 24, with higher scores indicating greater organ dysfunction severity.<sup>181</sup> The corticotropin test was defined as non-responsive if the cortisol level rose by less than  $9 \mu\text{g/dL}$  ( $248 \text{ nmol/L}$ ).<sup>191</sup> Mortality was recorded.

### 2.6. Endpoints

The primary endpoints were the differences in TRPpf, TRPtot, 5HTp, 5HTpt, 5HTtot, 5HTu, 5HIAAp, 5HIAAu, KYNp, MAO, IDO, and platelet count values between the patients and controls on D1. In the patients, we also evaluated the changes in the same variables across the study period, looked for differences in these variables between survivors and nonsurvivors, and looked for correlations between these variables and the criteria for SS.

### 2.7. Statistics

The statistical analysis was carried out by a consultant statistician (FS) who had no role in patient care and was independent from the study ICU. Quantitative variables are variables not following normal distribution and described as number of observed and missing data, median and interquartile. Qualitative variables were described as number (%) of observed data by category. Differences between patients and controls and differences between survivors and nonsurvivors were assessed using the Wilcoxon signed-rank test for matched pairs. The Kendall tau rank correlation coefficient was computed to evaluate associations between 2 quantitative variables. Bonferroni correction for repeated measures was applied. So, two-tailed  $P$  value of  $\leq 0.01$  was considered statistically significant. SAS 9.1 software (SAS Institute, Inc., Cary, NC) was used for the statistical analysis.

## 3. Results

Figure 2 is the patient flow diagram. During the 3-year period, 255 patients with septic shock who required mechanical ventilation and had  $> 2$  organ failures were screened at ICU admission. Among them, 34 patients were included then 4 subsequently excluded.

Table 2 reports the main patient characteristics. The initial infection was pulmonary (16/30), septicemia (12/30), abdominal (6/30), urinary (3/30), or a central venous catheter (3/30); 11 patients had  $> 1$  initial infection site (septicemia and intra-abdominal infection,  $n=3$ ; septicemia and central venous

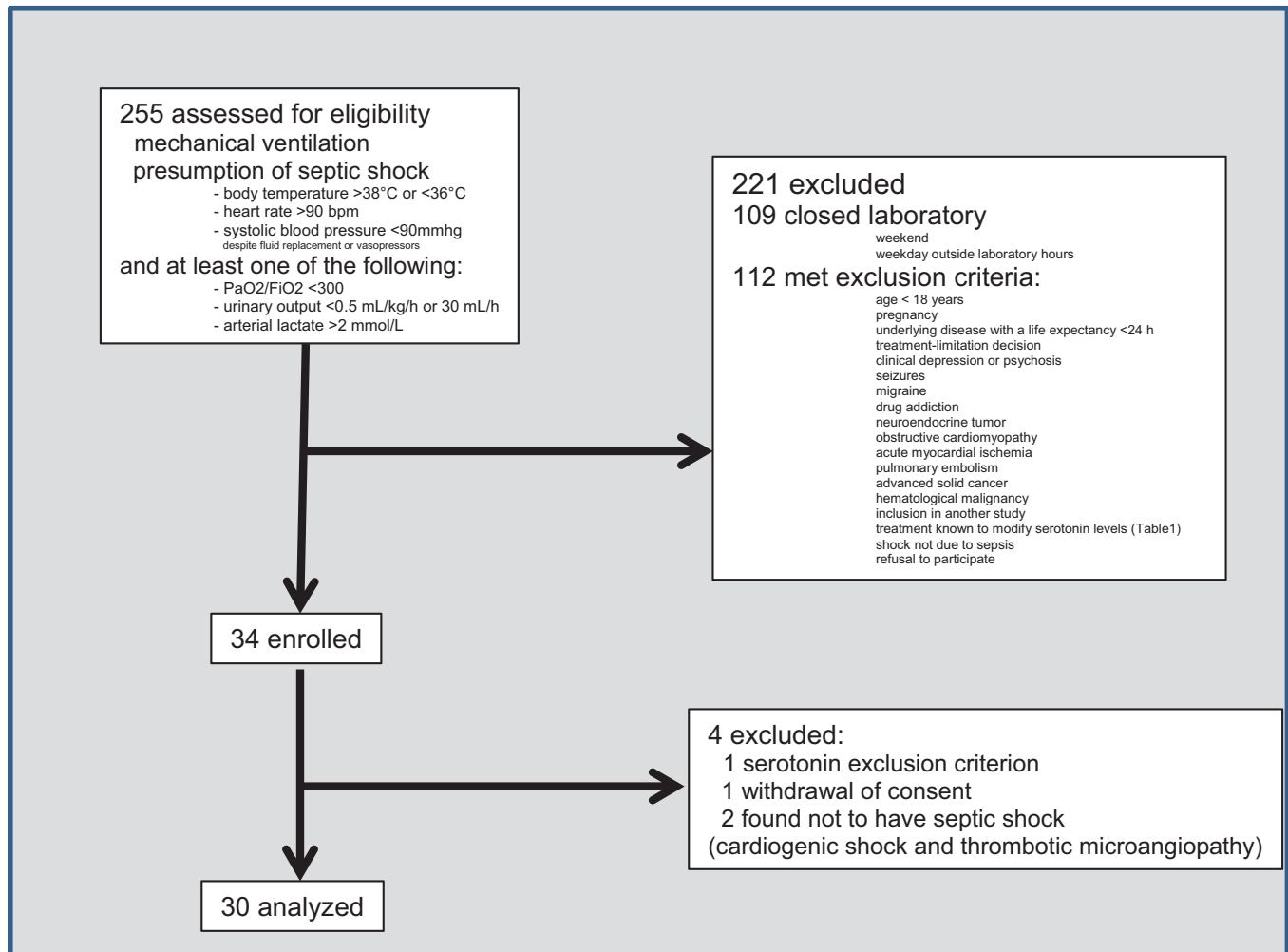


Figure 2. Patient flow diagram.

catheter infection,  $n=3$ ; septicemia and urinary tract infection,  $n=2$ ; septicemia and pulmonary infection,  $n=2$ ; and septicemia, intra-abdominal, and urinary tract infection,  $n=1$ ). The microbiological studies recovered a Gram-positive agent in 19 patients, a Gram-negative agent in 19 patients, and a yeast in 6 patients. No patient received granulocyte-macrophage colony-stimulating factor therapy.

The number of patients with available data decreased over time, due to death or ICU discharge: data were available from all 30 patients on D1, 27 patients on D2, 26 patients on D3, 24 patients on D7, and 20 patients on D14. The cumulative number of patients who died was 0 on D1, 2 on D2, 3 on D3, 4 on D7, and 7 on D14. The SOFA score was 8.5 [8.0–10.5] on D1, 8.0 [6.5–11.5] on D2, 8.0 [5.0–10.0] on D3, 4.0 [2.7–7.5] on D7, and 3.0 [2.0–4.0] on D14.

Table 3 shows the changes over time in tryptophan, serotonin, 5HIAA, kynurenine (KYN), MAO, IDO, and platelet counts. The total tryptophan level was lower in the patients than in the controls throughout the 14-day study period. In the patients, platelet serotonin decreased markedly until D7, while MAO activity and 5HIAA<sub>p</sub> increased throughout the 14-day period, reflecting the fact that tryptophan is metabolized to serotonin, which is promptly degraded to 5HIAA. The major increase in

plasma kynurenine and IDO activity throughout the 14 days in the patients is consistent with the known conversion of tryptophan to KYN. The 5HIAA/5HT ratio, reflecting MAO activity, fluctuated over time between 1.8 and 4.2 and the KYN/TRP ratio, reflecting IDO activity, between 2.7 and 4.8, indicating a very high level of tryptophan and serotonin metabolism in the patients with septic shock compared with control patients.

Table 4 reports the changes over time in tryptophan, serotonin, 5HIAA, MAO, KYN, MAO and IDO levels, as well as the comparison of survivors and nonsurvivors. No laboratory parameter was significantly associated with survival.

Table 5 shows the analysis of correlations linking the hemodynamic and laboratory parameters to the SOFA score. The epinephrine dose and the 5HIAA level were positively correlated, and the platelet serotonin level negatively correlated, with the SOFA score throughout the study period.

Table 6 reports the analysis of correlations linking norepinephrine dose, blood pressure values, and the SOFA score to platelet serotonin. Platelet serotonin showed significant positive correlations with blood pressure values and negative correlations with the norepinephrine dose and SOFA score throughout the 14-day period.



**Table 2****Main patient features at baseline.**

Features	Patients (n = 30)
Age, y, median (IQ)	63 (56.0–72.7)
Sex, n (%)	
Male	18 (60)
Female	12 (40)
Body mass index, median (IQ)	27.0 (23.4–31.5)
Smokers, n (%)	7 (23)
White, n (%)	29 (97)
Black, n (%)	1 (3)
Comorbidities, n (%)	
Hypertension	20 (67)
Coronary artery disease	3 (10)
Congestive heart failure	5 (17)
Neurological disease	3 (10)
Chronic obstructive pulmonary disease	5 (17)
Other pulmonary disorder	1 (3)
Cancer	6 (20)
Diabetes	8 (27)
Liver	6 (20)
Chronic renal failure	2 (7)
Admission category, n (%)	
Medical	24 (80)
Emergent surgery	3 (10)
Elective surgery	3 (10)
Cortisol, µg/dL	
Before corticotropin, median (IQ)	26.0 (21.4–43.2)
30 min after corticotropin, median (IQ)	30.0 (22.8–56.7)
60 min after corticotropin, median (IQ)	32.0 (25.4–51.7)
No response to corticotropin test, n (%)	22 (79)
Body temperature, °C, median (IQ)	38.4 (36.7–39.0)
Heart rate, bpm, median (IQ)	116 (105–134)
Systolic blood pressure, mmHg, median (IQ)	85 (75–104)
Mean blood pressure, mmHg, median (IQ)	49 (41–58)
SOFA score, median (IQ)	8.5 (8.0–10.8)
Leukocytes, 10 <sup>3</sup> /mm <sup>3</sup> , median (IQ)	10.0 (7.1–14.6)
Platelets, 10 <sup>3</sup> /mm <sup>3</sup> , median (IQ)	165 (128–203)
Glucose, mmol/L, median (IQ)	9.1 (7.1–11.8)
Arterial lactate, mmol/L, median (IQ)	2.5 (2.0–5.1)
Urine output, mL/h, median (IQ)	45 (21–75)
PaO <sub>2</sub> /FI <sub>O</sub> <sub>2</sub> , median (IQ)	163 (127–233)
Vasopressor or inotrope at baseline, n (%)	30 (100)
Type of vasopressor	
Norepinephrine, n (%)	29 (97)
Maximum dose, µg/kg/min, median (IQ)	0.24 (0.16–0.31)
Epinephrine, n (%)	1 (3)
Dobutamine, n (%)	4 (13)
Ventilatory support at baseline:	
Invasive mechanical ventilation, n (%)	30 (100)
FI <sub>O</sub> <sub>2</sub> , %, median (IQ)	60 (50–100)
Positive end-expiratory pressure, cm H <sub>2</sub> O, median (IQ)	5 (5–6)
Initial effective antimicrobial therapy, n (%)	28 (93)
Cumulative fluid dose on D1, mL/kg, median (IQ)	34.3 (27.8–58.4)

D1 = day 1 (first day after study inclusion), FI<sub>O</sub><sub>2</sub> = fraction of inspired oxygen, IQ = interquartile, PaO<sub>2</sub> = arterial blood oxygen pressure, SOFA = Sequential Organ Failure Assessment score.

Moreover, platelet serotonin showed a negative correlation with the lactate concentration on D1 ( $r = -0.44$ ;  $P = .0145$ ) and D3 ( $r = -0.58$ ;  $P = .0022$ ). MAO activity exhibited a significant negative correlation with the mean arterial pressure on D2 ( $r = -0.45$ ;  $P = .0218$ ), a positive correlation with the norepinephrine dose on D7 ( $r = 0.65$ ;  $P = .0006$ ), and positive correlations with the lactate concentration on D1 ( $r = 0.48$ ;  $P = .0091$ ) and D3 ( $r = 0.56$ ;  $P = .0039$ ).

#### 4. Discussion

Tryptophan is an essential amino acid that is normally metabolized to kynurenine, kynurenine acid, and quinolinic acid via IDO activation and to serotonin, melatonin, and 5HIAA via MAO activation (Fig. 1).<sup>[4]</sup> To the best of our knowledge, our study is the first investigation of these 2 pathways in patients with septic shock versus healthy volunteers. We found major differences in tryptophan metabolites during the first 14 days after the onset of septic shock compared with controls. In addition, tryptophan metabolite levels correlated significantly with septic shock severity, although not with mortality. Compared with healthy volunteers, tryptophan levels in the patients were substantially lower; kynurenine pathway metabolites were elevated, with an increase in IDO activity and a 2-fold rise in kynurenine levels; and serotonin pathway activity was also increased, although serotonin was promptly metabolized to HIAA, which was 1.9-fold to 2.5-fold higher than in the controls, concomitantly with an increase in MAO activity. Renal excretion of 5HT and 5HIAA was not marked and was clearly not the main mechanism of tryptophan clearance. Quantitatively, the serotonin pathway compounds showed major changes that were significantly associated with septic shock severity. Worse SOFA score values correlated with lower serotonin and higher 5HIAA values.

Serotonin is chiefly stored in gastrointestinal tract cells (95%). The remainder is within the brain and platelets. Platelets capture but do not produce serotonin. The half-life of plasma serotonin is equal to the half-life of platelets, that is, 4 to 5 days. The effects of serotonin are mediated by numerous receptors belonging to 7 different families. Serotonin exerts major effects at many sites including the central nervous system, heart, vessels, platelet aggregation, and smooth muscles.

In particular, septic shock is associated with hemodynamic variations that can be fatal.<sup>[10]</sup> The hemodynamic alterations seen in septic shock are related to increased endothelial barrier permeability with microvascular leakage. Serotonin plays a major role in these abnormalities.<sup>[11]</sup> However, endothelial cell activation by various mediators or cytokines induces the release of serotonin. Thus, the interactions between serotonin and endothelial cells are complex and bidirectional.

One of the limitations of our study lies in the nature of the controls, who were healthy volunteers, as opposed to ICU patients without septic shock. However, few ICU patients are free of diseases and/or treatments known to alter the tryptophan pathways. Moreover, no data are available on tryptophan metabolism abnormalities in ICU patients. Another study limitation is the small number of patients. We were only able to include patients admitted on weekdays during the laboratory opening hours. The biologists took the blood and urine samples at the patient's bedside, transported them to the laboratory, and performed the assays immediately, in order to improve the quality of the results. Another major reason for study exclusions was the high prevalence of patients with neuropsychiatric disorders and/or treatments known to modify tryptophan metabolism. Finally, we included only patients who met published criteria for septic shock.<sup>[2]</sup> A study of IDO activity and kynurenine metabolites had a similar number of patients ( $n = 36$ ).<sup>[12]</sup>

We included only patients at the very early stage of septic shock (e.g., with vasopressor initiation within the last 3 hours), in order to ensure comparability of the findings across patients on a given

**Table 3**  
**Time-course of tryptophan, serotonin, 5HIAA, kynurenine, MAO, IDO, and platelet counts in the patients with septic shock patients versus the controls.**

	Healthy controls	Septic shock D1		Septic shock D2		Septic shock D3		Septic shock D7		Septic shock D14	
	Median (IQ)	Median (IQ)	P value	Median (IQ)	P value	Median (IQ)	P value	Median (IQ)	P value	Median (IQ)	P value
TRPpf, $\mu\text{mol/L}$	8.70 (5.52–11.95)	9.60 (5.4–13.0)	.297	7.44 (4.35–11.20)	.307	9.45 (6.77–12.77)	.413	12.40 (10.42–19.45)	.002	12.00 (9.22–15.20)	.058
TRPtot, $\mu\text{mol/L}$	53.4 (48.3–58.7)	18.8 (13.3–25.0)	<.001	24.6 (9.6–27.4)	<.001	26.05 (19.02–32.97)	<.001	34.75 (26.27–46.60)	<.001	35.30 (27.25–42.43)	<.001
5HTp, nmol/L	5.39 (2.65–9.26)	3.41 (1.82–7.60)	.427	3.75 (2.27–7.72)	.674	2.58 (1.64–8.03)	.197	2.16 (1.25–4.21)	.086	2.67 (1.79–4.57)	.096
5HTpt, nmol/L	627 (544–837)	222 (92–409)	<.001	292 (81–407)	<.001	207 (81–422)	<.001	229 (90–421)	<.001	382 (247–635)	.076
5HTtot, nmol/ $10^9\text{plat}$	2.53 (1.95–3.31)	1.45 (0.72–2.23)	<.001	1.48 (0.88–2.76)	.012	1.52 (0.93–2.53)	<.001	1.31 (0.84–1.77)	<.001	1.22 (0.84–1.42)	.001
5HTu, nmol/ mmolCreat	54.4 (37.5–59.5)	31.0 (16.3–40.3)	.052	15.4 (11.8–29.7)	<.001	25.1 (17.4–53.4)	.133	37.0 (23.9–51.1)	.229	53.7 (28.7–67.1)	.980
5HIAAp, nmol/L	36.5 (33.5–44.7)	91.0 (55.0–181.0)	<.001	90.0 (51.7–127.5)	<.001	87.0 (50.0–112.0)	<.001	69.0 (45.3–128.5)	<.001	72.5 (36.7–105.0)	.005
5HIAAu, $\mu\text{mol}/$ mmolCreat	2.09 (1.70–3.28)	2.38 (1.65–3.38)	.407	2.75 (1.84–4.14)	.065	2.92 (2.11–3.46)	.023	2.91 (1.87–3.92)	<.001	3.42 (2.58–4.63)	<.001
KYNp, $\mu\text{mol/L}$	1.89 (1.63–2.37)	2.78 (2.30–4.19)	<.001	3.19 (2.42–4.62)	<.001	3.18 (2.37–4.60)	<.001	3.61 (2.95–6.02)	<.001	3.84 (2.01–4.92)	<.001
MAO, AU	7.25 (3.95–15.29)	18.03 (7.85–59.35)	.003	12.95 (4.38–53.29)	.016	17.62 (7.42–60.40)	.002	30.43 (16.81–80.98)	<.001	22.91 (11.86–54.70)	<.001
IDO, AU	0.037 (0.029–0.045)	0.150 (0.119–0.209)	<.001	0.179 (0.106–0.217)	<.001	0.138 (0.074–0.196)	<.001	0.106 (0.078–0.160)	<.001	0.100 (0.082–0.127)	<.001
Plat (G/L)	256 (217–293)	165 (128–203)	.002	164 (116–262)	.001	151 (99–249)	.001	187 (114–317)	.350	304 (198–462)	.014

5HIAAp = plasma 5-hydroxyindolacetic acid, 5HIAAu = urinary plasma 5-hydroxyindolacetic acid, 5HTp = plasma serotonin, 5HTpt = platelet serotonin, 5HTtot = total serotonin, 5HTu = urinary serotonin, AU = arbitrary unit, IDO = indole amine 2,3-dioxygenase, KYNp = plasma kynurenine, MAO = monoamine oxidase activity, Plat = platelet count, TRPpf = plasma free tryptophan, TRPtot = total tryptophan.

day, since tryptophan metabolites might fluctuate over time. We believe that including patients several days after the onset of septic shock may produce unreliable results.<sup>[12]</sup> However, we found that the values changed only very slowly, at least during the first week. Septic shock severity and the microbiological findings were consistent with other studies and, therefore, cannot explain differences in results across studies.<sup>[12]</sup> Those differences may be related to variations in assay methods, in assay compartments

(e.g., free vs total plasma, urine, platelets), and in time since septic shock onset. Details on these points are not always available in study reports.

IDO elevation may be induced by interleukin 10, whose levels correlate with the severity of sepsis. Tryptophan 2–3, dioxygenase was not tested in our study but may play only a minor role (<1%) in tryptophan metabolism.<sup>[12]</sup> The changes in tryptophan metabolism and enzyme activities induced by the cytokines

**Table 4**  
**Time-course of tryptophan, serotonin, 5HIAA, kynurenine, MAO, IDO, and platelet counts in survivors versus nonsurvivors in the group with septic shock.**

	Day 2			Day 3			Day 7			Day 14		
	Died N=2	Survived N=27	P	Died N=3	Survived N=26	P	Died N=4	Survived N=24	P	Died N=7	Survived N=20	P
TRPtot, $\mu\text{mol/L}$	26.5 (12.8–30.2)	20.7 (7.4–27.4)	.39	33.0 (26.1–41.2)	24.5 (17.7–30.8)	.10	44.5 (38.6–51.4)	33.2 (25.0–42.2)	.15	36.1 (31.0–41.1)	38.6 (27.2–42.4)	.96
5HTpt, nmol/L	93 (20–401)	325 (144–401)	.16	72 (26–451)	253 (133–333)	.20	115 (35–330)	258 (140–463)	.26	562 (479–691)	289 (235–635)	.44
5HIAAp, nmol/L	120 (94–61)	66 (31–98)	.07	99 (88–130)	68 (33–103)	.24	98 (70–239)	67 (44–126)	.28	85 (68–110)	59 (35–104)	.43
KYNp, $\mu\text{mol/L}$	3.28 (2.90–5.42)	3.10 (2.26–4.03)	.32	3.22 (2.70–5.63)	3.14 (2.22–4.35)	.41	4.34 (3.55–7.39)	3.39 (2.66–5.52)	.49	4.49 (3.29–5.13)	3.56 (2.01–4.64)	.74
MAO (AU)	36.0 (4.5–39.5)	12.4 (4.0–27.9)	.60	51.3 (9.7–70.8)	8.4 (6.8–58.9)	.26	37.2 (18.5–89.0)	30.4 (17.1–59.9)	.81	18.1 (16.0–20.9)	30.8 (11.5–56.7)	.67
IDO (AU)	0.176 (0.108–0.230)	0.179 (0.106–0.217)	1.00	0.119 (0.078–0.159)	0.167 (0.077–0.196)	.46	0.132 (0.088–0.176)	0.100 (0.075–0.152)	.66	10.36 (8.58–13.3)	0.102 (0.082–0.127)	.89

5HIAAp = plasma 5-hydroxyindolacetic acid, 5HTpt = platelet serotonin, AU = arbitrary unit, IDO = indole amine 2,3-dioxygenase, KYNp = plasma kynurenine, MAO = monoamine oxidase activity, Median (IQ), TRPtot = total tryptophan.

**Table 5**

**Correlations linking noradrenaline administration rate, arterial pressures, and laboratory parameters to SOFA score in the patients with septic shock.**

	Day 1		Day 2		Day 3		Day 7		Day 14	
	Correlation coefficient	P	Correlation coefficient	P	Correlation coefficient	P	Correlation coefficient	P	Correlation coefficient	P
NOREPINEPHRINE dose, $\mu\text{g}/\text{kg}/\text{min}$	0.161	.245	0.552	<.001	0.519	.003	0.333	.293	1.000	<.001
MBP, mmHg	-0.157	.246	-0.207	.145	-0.315	.028	-0.004	.980	0.010	.951
DBP, mmHg	-0.107	.434	-0.268	.063	-0.245	.091	-0.087	.565	-0.005	.976
5HTpt, nmol/L	-0.456	<.001	-0.462	.001	-0.344	.017	-0.318	.034	-0.078	.645
5HIAAp, nmol/L	0.362	.009	0.384	.008	0.398	.007	0.554	<.001	0.374	.027
MAO (AU)	0.228	.104	0.156	.284	0.252	.086	0.327	.033	0.174	.319
IDO (AU)	0.357	.002	0.193	.207	-0.050	.754	-0.236	.159	0.0266	.126

5HIAAp = plasma 5-hydroxyindolacetic acid, 5HTpt = platelet serotonin, AU = arbitrary unit, DBP = diastolic blood pressure, IDO = indole amine 2,3-dioxygenase, MAO = monoamine oxidase activity, MBP = mean blood pressure, SOFA = Sequential Organ Failure Assessment.

**Table 6**

**Correlations linking norepinephrine dose, blood pressure values, and the SOFA score to platelet serotonin in the patients with septic shock.**

	Day 1		Day 2		Day 3		Day 7		Day 14	
	Correlation coefficient	P	Correlation coefficient	P	Correlation coefficient	P	Correlation coefficient	P	Correlation coefficient	P
Norepinephrine dose, $\mu\text{g}/\text{kg}/\text{min}$	-0.211	.111	-0.434	.002	-0.322	.054	-0.143	.652	1.0	.117
SBP, mmHg	0.169	.192	0.439	.001	0.449	.001	-0.262	.074	0.155	.345
DBP, mmHg	0.145	.268	0.190	.168	0.165	.242	-0.011	.941	0.419	.011
MBP, mmHg	0.154	.232	0.337	.014	0.327	.019	-0.160	.275	0.332	.041
SOFA score	-0.456	<.001	-0.462	.001	-0.344	.017	-0.318	.034	-0.078	.645

5HTpt = platelet serotonin, DBP = diastolic blood pressure, MBP = mean blood pressure, SBP = systolic blood pressure, SOFA = Sequential Organ Failure Assessment.

released during septic shock may be influenced by genetic factors and by the environment, including the diet.<sup>[13]</sup> These factors were not taken into account in our study and may have contributed to some of the alterations seen. The decrease in tryptophan was due to activation of the serotonin and kynurenine pathways, as demonstrated by the elevations in the corresponding catabolites. However, the gut microbiome may have contributed to diminish the tryptophan levels. A decrease in tryptophan modifies the immune response and alters the homeostasis of the gut, generating a vicious cycle.<sup>[13]</sup> Moreover, serotonin induces manifold responses that are mediated by multiple receptors with different effects. Our study is consistent with experimental data obtained using 5HT-receptor antagonists or tryptophan treatment.<sup>[10,14–16]</sup> However, mortality did not correlate with tryptophan metabolism, in keeping with another study.<sup>[17]</sup> Specific antagonism of serotonin receptors has been reported to improve survival in experimental studies,<sup>[5,10]</sup> but this effect requires evaluation in humans. Oddly enough, despite numerous studies, the role for the tryptophan-serotonin axis is not considered in consensus statements or reviews on sepsis.<sup>[1,2]</sup>

## 5. Conclusion

In ICU patients who had septic shock with hypotension, a requirement for norepinephrine and mechanical ventilation, lactate elevation, and a high SOFA score, the metabolism of tryptophan was severely altered. Tryptophan levels were low compared with healthy controls during the 14-day study period,

with conversion to kynurenine via IDO and conversion to serotonin, which was promptly catabolized to 5HIAA. Overall, the decreases in total tryptophan and platelet serotonin and the increases in MAO activity and plasma 5HIAA were associated with organ failures, hypotension, and higher norepinephrine requirements, but not with mortality. This last result may be related to the small sample size, and larger studies are needed. Our findings suggest that interventions capable of acting on the tryptophan-serotonin axis, notably specific serotonin receptor antagonists, may deserve evaluation as a treatment for septic shock.

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## References

- [1] Annane D, Bellissant E, Cavaillon JC. Septic shock. *Lancet* 2005;365:63–78.
- [2] Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (sepsis-3). *JAMA* 2016;315:801–10.
- [3] Duerschmied D, Suidan GL, Demers M, et al. Platelet serotonin promotes the recruitment of neutrophils to sites of acute inflammation in mice. *Blood* 2013;121:1008–15.
- [4] Schefold JC, Zeden JP, Fotopoulou C, et al. Increased indolamine 2,3-dioxygenase (IDO) activity and elevated serum levels of tryptophan catabolites in patients with chronic kidney disease: a possible link between chronic inflammation and uraemic symptoms. *Nephrol Dial Transplant* 2009;24:1901–8.
- [5] Mauler M, Bode C, Duerschmied D. Platelet serotonin modulates immune functions. *Hämostaseologie* 2016;36:11–6.
- [6] Spreux-Varoquaux O, Gailledreau J, Vanier B, et al. Initial increase of plasma serotonin: a biological predictor for the antidepressant response to clomipramine? *Biol Psychiatry* 1996;40:465–73.
- [7] Spreux-Varoquaux O, Alvarez JC, Berlin I, et al. Differential abnormalities in plasma 5-HIAA and platelet serotonin concentrations in violent suicide attempters: relationships with impulsivity and depression. *Life Sci* 2001;69:647–57.
- [8] Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. *Intensive Care Med* 1996;22:707–10.
- [9] Annane D, Sébille V, Troché G, et al. A 3-level prognostic classification in septic shock based on cortisol levels and cortisol response to corticotropin. *JAMA* 2000;283:1038–45.
- [10] Liu C, Zhang GF, Song SW, et al. Effects of Ketanserin on endotoxic shock and baroreflex function in rodents. *J Infect Dis* 2011;204:1605–12.
- [11] Li Y, Hadden C, Cooper A, et al. Sepsis-induced elevation in plasma serotonin facilitates endothelial hyperpermeability. *Sci Rep* 2016;6:1–3.
- [12] Schefold JC, Zeden JP, Pschowski R, et al. Treatment with granulocyte-macrophage colony-stimulating factor is associated with reduced indoleamine 2,3-dioxygenase activity and kynurenine pathway catabolites in patients with severe sepsis and septic shock. *Scand J Infect Dis* 2010;42:164–71.
- [13] Lamas B, Richard ML, Leducq V, et al. CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. *Nat Med* 2016;22:598–605.
- [14] Nishiyama T. Acute effects of sarpogrelate, a 5-HT<sub>2A</sub> receptor antagonist on cytokine production in endotoxin shock of rats. *Eur J Pharmacol* 2009;614:122–7.
- [15] Del Angel-Meza AR, Dávalos-Marín AJ, Ontiveros-Martinez LL, et al. Protective effects of tryptophan on neuro-inflammation in rats after administering lipopolysaccharide. *Biomed Pharmacother* 2011;65:215–9.
- [16] Fakhfour G, Rahimian R, Ghia JE, et al. Impact of 5-HT<sub>3</sub> receptor antagonists on peripheral and central diseases. *Drug Discov Today* 2012;17:741–7.
- [17] Nagi-Miura N, Shingo Y, Kurihara K, et al. Involvement of platelet activating factor, histamine and serotonin in acute lethal shock induced by candida albicans water-soluble extracellular polysaccharide fraction (CAWS) in mice. *Biol Pharm Bull* 2007;30:1354–7.