Plasma citrulline is a biomarker of enterocyte mass and an indicator of parenteral nutrition in HIV-infected patients

Pascal Crenn, Pierre De Truchis, Nathalie Neveux, Tatiana Galpérine, Luc Cynober, and Jean Claude Melchior

ABSTRACT
Background: Plasma citrulline is a biomarker of enterocyte mass and function in humans.
Objective: We evaluated citrulline in the reemerging context of diarrhea in HIV-infected patients receiving highly active antiretroviral therapy.
Design: This study prospectively measured citrulline in 6 groups of HIV-1 patients (n = 115): 1) undetectable viral load without chronic diarrhea (a; n = 40) and with protease inhibitor–associated toxic chronic diarrhea (b; n = 26), 2) detectable viral load and CD4 > 200/mm3 without (a; n = 6) and with (b; n = 11) chronic diarrhea, and 3) detectable viral load and CD4 <200/mm3 without chronic diarrhea (a; n = 7) and with opportunistic intestinal infections or HIV enteropathy (b; n = 25). The influence of diarrhea on citrulline was assessed by comparing the a and b subgroups with healthy control subjects (n = 100).
Results: Citrulline was slightly decreased (22–30 μmol/L) in groups 1b and 2b and was <22 μmol/L in 19 of 25 patients in group 3b. In group 3b, a citrulline concentration <10 μmol/L was associated with a clinical indication for parenteral nutrition (n = 6 of 8 compared with 2 of 17 if the citrulline concentration was >10 μmol/L; P < 0.05). Citrulline correlated positively with albumin (P < 0.01) and BMI (P < 0.05) and negatively with C-reactive protein (P < 0.01). When antifungal and nutritional therapies were successful (n = 18 of 25), citrulline normalized in 2–12 wk. Neither chronic hepatic or pancreatic disease nor lipodystrophy and the metabolic syndrome affected citrulline. Compared with control subjects (38 ± 8 μmol/L), patients without chronic diarrhea (groups 1a, 2a, and 3a) had normal citrulline concentrations (36 ± 6 μmol/L).
Conclusions: Plasma citrulline is a reliable biomarker of enterocyte functional mass in HIV patients. Citrulline does not allow the etiologic diagnosis of enteropathy, but it can discriminate between protease inhibitor toxic diarrhea and infectious enteropathy and quantify the functional consequences, which makes it an objective tool for indicating the need for parenteral nutrition. Am J Clin Nutr 2009;90:587–94.

INTRODUCTION
Since the advent of highly active antiretroviral therapy (HAART), digestive opportunistic infections or HIV-specific enteropathy and wasting have decreased dramatically (1), consequently improving prognosis (2). However, there has recently been a trend toward a recurrence of digestive symptoms, such as chronic or intermittent diarrhea, which is estimated to affect ≥9% of patients in Western countries (3), along with wasting syndrome against a background of HIV infection. These symptoms are due to 2 main situations: 1) intestinal opportunistic infections and/or HIV enteropathy—due to late diagnosis, poor compliance, and/or antiretroviral resistance, especially among active drug-users (4), among socially deprived patients, or in developing countries (5)—associated with, for example, protozoan infections (6), mycobacteria, or immune-related HIV small-bowel involvement (7); and 2) the adverse toxic effects of HAART, especially protease inhibitors such as lopinavir and/or ritonavir, on intestinal mucosa (8). These digestive involvements can contribute to weight loss and/or malnutrition and related complications.

In these situations, a biomarker of intestinal involvement would be a valuable tool, especially for monitoring digestive symptoms related to toxicity of the antiviral treatment and the efficacy of antifungal treatments. It could also provide an objective indicator for nutritive assistance and usable route. Plasma or serum citrulline assays have recently emerged as the best candidate tool, regardless of the etiology of the intestinal mucosal disease (9, 10). Citrulline is the metabolic product of glutamine and its related amino acids, and of arginine (11), and it is specifically synthesized by small-bowel enterocytes (12). Since our pioneering work on short-bowel syndrome (13), there have been numerous reports validating this biomarker in various clinical settings. Citrulline has been validated for quantitative enterocyte mass assessment in villous atrophy disease (14), Crohn disease (15), digestive toxicity of chemotherapy (15) and radiotherapy (16), and for follow-up on small bowel transplantation (17), notably but nonspecifically for the diagnosis of cellular rejection (18). Citrulline remains uninfluenced by nutritional status (13), level of albuminemia (14), or inflammatory status (15). The only limitation is significant renal failure (creatinine clearance <30 mL/min), because citrulline is metabolized into arginine in the proximal convoluted tubule (10, 19). It seems logical that this marker will give valuable results in HIV-

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2 Address correspondence to P Crenn, Raymond Poincaré Hospital, 104 Boulevard Raymond Poincaré, 92380 Garches, France. E-mail: pascal.crenn@rpc.aphp.fr.
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positive patients as well as in other small-bowel diseases associated with villous atrophy and enterocyte alterations, because HIV or its associated opportunistic pathogens create an immunologic situation fostering a significant risk of small-bowel mucosa involvement (7, 20, 21). In 52 HIV-negative patients, citrulline was lowered by <20 μmol/L in patients with proximal-only subtotal villous atrophy and by <10 μmol in patients with extensive (proximal and distal) villous atrophy, irrespective of the underlying etiology (14). In these patients, a citrulline concentration <10 μmol/L was highly associated with the need for parenteral nutrition (PN), reflecting intestinal failure due to a reduced enterocyte mass. Patients with only mild enterocyte involvement, ie, partial proximal villous atrophy, presented normal or only moderately lowered citrulline concentrations (14, 15, 22).

There are few data on citrulline as an intestinal biomarker in HIV patients. To the best of our knowledge, only Schon et al (23) studied HIV patients, but in a small group without associated intestinal disease. In HIV infection, there is a close correspondence between intestinal structure and function (24). Thus, in this study, we prospectively studied citrulline and its modifications during antinfectious, antiretroviral, and nutritional therapies in a cohort of patients followed up or referred to the same specialized center for HIV disease, gastroenterology, and nutrition.

SUBJECTS AND METHODS

Patients

Consecutive adult patients infected by HIV-1 were prospectively included and studied between January 2005 and December 2007. Antiretroviral treatment was managed in compliance with International AIDS Society recommendations (25). Patients with chronic diarrhea were investigated according to published management guidelines (5, 26). All patients with diarrhea underwent repeated stool examination and specific coloration investigation for pathogens. Patients with previous intestinal resection (n = 2), renal failure with creatinine clearance <30 mL/min (n = 5), or celiac disease (n = 0); patients unable to intake food orally because of occluding stenoses (n = 0); or patients with any other known intestinal disease not linked to HIV infection were not included in the study. Informed consent for participation was obtained from all patients in compliance with national legislation on observational studies.

The patients included were classified into 6 groups based on a composite index of immunovirologic status and clinical, ie, digestive (diarrhea), symptoms (Table 1): 1) patients with an undetectable plasma viral load (ie, <50 copies/mL) receiving antiretroviral treatment (HAART) (n = 66) without chronic diarrhea (a; n = 40) or with protease inhibitor–associated toxic chronic diarrhea (b; n = 26), which was defined as a temporal association between drug initiation and diarrhea, mild-to-moderate severity, and negative stool studies (5); 2) patients with a detectable viral load and blood lymphocyte CD4 count >200/mm³ (n = 17) whether under HAART (n = 12) or not (n = 5) and without (a; n = 6) or with (b; n = 11) chronic or intermittent diarrhea (5); and 3) patients with a detectable viral load and blood lymphocyte CD4 count <200/mm³ (n = 32) and without chronic diarrhea (a; n = 7) or with small-bowel chronic enteropathy (b; n = 25) [idiopathic HIV enteropathy (11), defined as diarrhea in a context of immunodepression without detectable pathogens despite intensive investigation with endoscopy and biopsies (5) or microbiologically documented opportunistic digestive infection: Mycobacterium infection (8) including 2 cases of intestinal tuberculosis or protozoan infection (6) including 4 cryptosporidiosis and 2 isosporiasis]. In this small-bowel chronic enteropathy group, 18 of the 25 patients had histologically documented enteropathy and included all HIV enteropathy patients.

All 25 patients in the 3b group were regularly reevaluated at varying frequencies during the weeks or months after the first evaluation and were followed-up for ≥6 mo (median: 15 mo; maximum: 36 mo). Furthermore, 28 patients from the other groups, including 6 from group 1b and 6 from group 2b in which protease inhibitor switch or discontinuation was a possible option, were also studied longitudinally (Table 2). In addition, in 4 of the 40 patients with neither HAART-toxic chronic diarrhea nor opportunistic or HIV infection (all of group 1a), citrulline was later measured in the clinical setting of acute diarrhea considered as a common epidemic clinical “gastroenteritis.”

Studied variables

All clinical and biological investigations were performed in the same week of hospitalization for each and every patient.

Clinical nutritional status

Clinical nutritional status was assessed by body mass index (BMI; in kg/m²) and graded by Subjective Global Assessment (SGA) into grades A (well-nourished), B (moderately malnourished), or C (severely malnourished) according to Detsky et al (27). Nutritive assistance, including oral nutritional supplements, was prescribed according to routine practice by the clinician in charge of the patient. All patients were fed orally. Eleven patients (10 in group 3b and 1 in group 3a) received standard cyclic polymeric or continuous enteral nutrition, and 8

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**TABLE 1**

Composite index of immunovirologic status and chronic diarrhea for the individual breakdown into 6 HIV patient groups (n = 115)

<table>
<thead>
<tr>
<th>Subgroup a: absence of chronic diarrhea (n = 53)</th>
<th>Group 1: undetectable HIV plasma viral load (n = 66)</th>
<th>Group 2: detectable HIV plasma viral load and CD4 count &gt;200/mm³ (n = 17)</th>
<th>Group 3: detectable HIV plasma viral load and CD4 count &lt;200/mm³ (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1a: absence of chronic diarrhea (n = 53)</td>
<td>40</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Group 1b: presence of chronic diarrhea (n = 62)</td>
<td>26</td>
<td>11</td>
<td>25</td>
</tr>
<tr>
<td>(toxic enteropathy)</td>
<td>(toxic enteropathy)</td>
<td>(opportunistic digestive infection or HIV enteropathy)</td>
<td></td>
</tr>
</tbody>
</table>

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patients (all in group 3b) received cyclic or continuous PN in compliance with technical guidelines (28). PN was based on standard (ie, glutamine- and citrulline-free) amino acid solutions containing 5–18% nitrogen from arginine.

**Routine biological tests**

Prothrombin time, hemoglobin, albumin, prealbumin, creatinine, calcium, phosphorus, and magnesium were measured as nonspecific markers of biological deficiencies (14). Creatinine clearance was calculated according to Cockcroft and Gault equations. C-reactive protein (CRP) was used as a marker of inflammation. All of these examinations were run by using automatic analyzers: RxL Max Dade Behring (Brookfield, CT) for biochemistry, Star (Stago, Franconville, France) for prothrombin time, and LH780 (Beckman Coulter, Villepinte, France) for hemoglobin.

**Immunovirologic tests**

The serum CD4 count was assessed by Facscount flow cytometry (Beckman Coulter FC500). The HIV viral load was measured by polymerase chain reaction (Amplicor Roche version 1.5) with a threshold set at 50 copies/mL.

**Plasma amino acids**

Plasma citrulline and arginine were measured after an overnight fast in oral and enteral nutrition patients and ≥8 h after infusion cessation in PN patients (postabsorptive conditions). Sampling was performed under stable conditions, without obvious clinical evidence of acute dehydration. Citrulline and arginine were measured by automated ion-exchange chromatography as previously described (13, 14). The laboratory in charge of the assays is a member of the European Quality Control scheme, thus guaranteeing the accuracy of the amino acid determinations. One hundred healthy subjects, laboratory volunteers (51 men and 49 women) with no evidence of metabolic, kidney, or digestive diseases, were studied as control subjects. They were sampled after an overnight fast. Control subjects had a median age of 36 y (range: 20–64 y), a BMI of 21.7 (18.5–28.2) and a serum albumin concentration of 43 g/L (35–56 g/L). It should be noted that healthy subjects were slightly younger than the patients, but it is well established (9, 10) that plasma citrulline in adults does not vary with age.

**Small-bowel mucosal assessment and intestinal microbiological analysis**

Multiple perendoscopic, duodenal, duodenoejejunral, or distal ileal biopsies were performed through duodenoscopy, enteroscopy, or retrograde colo-ileoscopy in patients with the clinical indication. None of the patients not in group 3b (other than 2) were investigated by collecting small-bowel biopsy samples. Proximal biopsy samples were collected from the second and fourth parts of the duodenum of the 20 studied patients and from the jejunum in 7 of these patients. Ileal biopsy samples were investigated by collecting small-bowel biopsy samples.

**TABLE 2**

Clinical and biological characteristics of the 115 HIV patients

<table>
<thead>
<tr>
<th></th>
<th>Healthy control subjects</th>
<th>No digestive symptoms</th>
<th>Groups 1b + 2b: diarrhea without immunodeficiency</th>
<th>Groups 3b: enteropathy with immunodeficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Groups 1a + 2a (n = 46)</td>
<td>Group 3a (n = 7)</td>
<td>(n = 37)</td>
<td>(n = 25)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>36 ± 2</td>
<td>44 ± 9</td>
<td>47 ± 8</td>
<td>42 ± 9</td>
</tr>
<tr>
<td>CD4 count (cells/mm³)</td>
<td>ND</td>
<td>300 (200–700)</td>
<td>89 (14–103)</td>
<td>300 (200–870)</td>
</tr>
<tr>
<td>HIV viral load (log/mL)</td>
<td>ND</td>
<td>0 (group 1a)</td>
<td>4.6 (2.7–4.15)</td>
<td>0 (group 1b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(group 2a)</td>
<td>(group 2b)</td>
<td>(group 3b)</td>
</tr>
<tr>
<td>Plasma citrulline (µmol/L)</td>
<td>38 ± 8</td>
<td>36 ± 6</td>
<td>34 ± 8</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>Plasma arginine (µmol/L)</td>
<td>72 ± 16</td>
<td>66 ± 24</td>
<td>73 ± 14</td>
<td>60 ± 25</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>43 ± 3</td>
<td>41 ± 3</td>
<td>32 ± 4</td>
<td>42 ± 3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.7 ± 2</td>
<td>23.7 ± 4</td>
<td>22.9 ± 4</td>
<td>21.2 ± 4</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>ND</td>
<td>2 ± 1</td>
<td>10 ± 4</td>
<td>3 ± 3</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>105 ± 15</td>
<td>87 ± 17</td>
<td>63 ± 27</td>
<td>90 ± 15</td>
</tr>
<tr>
<td>Chronic pancreatitis and diabetes (n)</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Chronic hepatitis (n)</td>
<td>0</td>
<td>9</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Lipodystrophy and/or metabolic syndrome (n)</td>
<td>0</td>
<td>18</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Geographic origin (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europe and North Africa</td>
<td>100</td>
<td>41</td>
<td>3</td>
<td>34</td>
</tr>
<tr>
<td>Sub-Saharan Africa</td>
<td>0</td>
<td>5</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Patients studied longitudinally (n)</td>
<td>14</td>
<td>2</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>Time between initial and final assessment (mo)</td>
<td>6–33</td>
<td>9–19</td>
<td>1–24</td>
<td>6–36</td>
</tr>
</tbody>
</table>

1 ND, not determined. Values in a row with different superscript letters are significantly different, P < 0.05 (ANOV A and Tukey’s test).

2 Mean ± SD (all such values).

3 Median; range in parentheses (all such values).
HIV enteropathy was considered as the diagnosis when no pathogenic agent was identified in patients with HIV infection with immunodeficiency and when the histology was compatible, ie, total, subtotal, or partial villous atrophy with infected lymphocytes in the lamina propria (7).

### Statistical methods

Results are expressed as means ± SDs or as medians (ranges). Plasma amino acid concentrations were compared between patient groups and control subjects by the Student’s t test. Quantitative variables were compared between subgroups of patients by using analysis of variance and Tukey’s post hoc test, a t test, or a paired t test where appropriate, according to the immunovirologic and digestive situation. Nonparametric tests were used for small-sized patient groups where appropriate. The specific overall influence of diarrhea was studied by comparing recomputed subgroups a (1a, 2a, and 3a) compared with b (1b, 2b, and 3b). Simple regression analyses were performed to test associations between citrulline concentrations, BMI, CRP, and albuminemia. The ability of citrulline to discriminate patients with or without chronic infectious enteropathy was assessed by receiver operating characteristic (ROC) curves (sensitivity versus 1 – specificity), while varying the value of the citrulline cutoff. SPSS software version 11.5 (SPSS Inc, Chicago, IL) was used for the statistical analysis. Statistical significance was set at P < 0.05.

### RESULTS

#### Routine clinical and biological results

A total of 115 HIV-1-infected adult patients (24 women and 91 men aged 24–68 y, mean age: 44 y) were prospectively included. Twenty-one patients (18%) originated from sub-Saharan Africa.

#### Citrulline concentrations according to immunovirologic and clinical group

In comparison with healthy controls subjects (citrulline concentration = 38 ± 8 μmol/L), all HIV patients in groups 1a, 2a, and 3a had a citrulline concentration >22 μmol/L, ie, 36 ± 6 μmol/L (Figure 1); citrulline was not significantly different between controls and patients 1a, 2a, and 3a; and was significantly higher in patients with chronic diarrhea (patients 1b, 2b, and 3b) (Table 2). When retested in some (n = 16) of these patients without diarrhea, citrulline remained within the normal range. The citrulline concentration was significantly (analysis of variance and Tukey’s post hoc test) different between the 3 clinical status–based patient groups: 36 ± 6 μmol/L for patients 1a, 2a, and 3a; 25 ± 3 μmol/L for patients 1b and 2b; and 16 ± 8 μmol/L for patient 3b (P < 0.01) in Figure 1.

Citrulline was slightly but significantly lower (22–30 μmol/L) in comparison with control subjects in all 37 patients in groups 1b and 2b (25 ± 3 μmol/L) (Figure 1), except in 5 patients (<22 μmol/L). There were no differences in citrulline concentrations or in other tested variables between patients 1b and 2b (Table 2), ie, patients with diarrhea without immunodeficiency, notably because of the toxic action of HAART on intestinal mucosa. For patients studied longitudinally (12 of 37 of patients 1b and 2b), citrulline evolved toward a significant (22 ± 4 compared with 42 ± 14 μmol/L; P < 0.05) increase associated with an improvement or cessation of patient–reported diarrhea after protease inhibitor switch or discontinuation (n = 6), whereas citrulline and digestive symptoms did not change in patients with no HAART modifications (n = 6).

In the 4 patients initially presenting no digestive symptoms, but who later developed acute “gastroenteritis,” plasma citrulline decreased significantly (34 ± 7 compared with 19 ± 5 μmol/L; P < 0.01) and returned to normal values at the next evaluation performed a few weeks later after symptom resolution.

#### Nutritional and other possible confounding factors on citrulline

The percentage of SGA grade B and C (22 ± 3 μmol/L) (P < 0.05). There was a significant positive correlation (r = 0.49, P < 0.01) between citrulline and albuminemia and between citrulline and BMI (P < 0.05). We also noted a negative correlation between CRP and citrulline (r = −0.45, P < 0.01). As expected, albuminemia was highly negatively correlated to CRP (r = −0.73, P < 0.0001).

There was no significant difference in citrulline concentrations between patients with hepatic and/or pancreatic disease and the other patients. Moreover, citrulline was not significantly different between patients with or without lipodystrophy and the metabolic syndrome. Furthermore, the citrulline concentration was not statistically different between patients originating from sub-Saharan Africa and other patients (26 ± 11 compared with 29 ± 9 μmol/L, respectively; P = 0.17). There was no significant difference (P = 0.09) in arginine concentrations between the different subgroups of patients (64 ± 23 μmol/L) and control subjects (72 ± 16 μmol/L) (Table 2).
enteropathy (group 3b) are presented in Table 3. Patients with histologically documented enteropathy (n = 18) showed ducodenal (and, when tested, jejunal) subtotal villous atrophy in 5 cases and partial villous atrophy or intramucosal lymphocytosis with or without enteric pathogens in 13 cases. Nineteen of the 25 patients (76%) had a citrulline concentration <22 μmol/L. Only 3 patients had a citrulline concentration ≥30 μmol/L; these 3 patients exhibited localized small-bowel stenoses due to mycobacterial infection, including intestinal tuberculosis in 2 cases. All of the other patients had low citrulline values. There was no significant difference in the tested variables (ie, BMI, albumin, CRP, and citrulline) between the 3 main etiologic categories of HIV-associated enteropathy (Table 3).

Plasma citrulline <10 μmol/L (n = 8) lasting >2 wk after the diagnosis of infectious enteropathy was associated with clinical indication of PN support (n = 6 of 8 patients compared with 2 of 17 patients if citrulline >10 μmol/L; P < 0.05, Fisher’s exact test). No patients of other immunologic and clinical status groups outside 3b had a citrulline concentration <10 μmol/L and/or clinical indication of PN. Except for one patient with a citrulline concentration <10 μmol/L, none of the patients could tolerate enteral nutrition because of severe diarrhea, discomfort, and/or pain, whereas enteral nutrition was clinically indicated and tolerated in 9 of 17 patients with a citrulline concentration >10 μmol/L. Only one patient in groups other than 3b required enteral nutrition because of severe malnutrition due to low food intake. The selected citrulline threshold of 22 μmol/L (ie, mean minus 2 SD) was able to differentiate patients with infectious or immunologic HIV enteropathy from other patients with 76% sensitivity and 94% specificity (Table 4). The best sensitivity + specificity pairing was obtained at this citrulline cutoff (1.70 for a maximum of 2.00). A serum albumin concentration of 30 g/L was able to discriminate HIV patients with enteropathy from other patients with 95% sensitivity and 77% specificity. Moreover, the ROC curve of citrulline for discriminating patients with and without chronic infectious enteropathy indicated an area under the curve of 0.89 (95% CI: 0.81, 0.97), ie, nonsignificantly different from the ROC curve corresponding to albuminemia (area under the curve: 0.95; 95% CI: 0.89, 1). The selected threshold of 10 μmol/L discriminated patients needing PN from patients with enteropathy (Table 4), whereas albuminemia was unable to discriminate between these 2 patient categories. Citrulline concentrations were not significantly different between patients in whom enteral nutrition was indicated and in patients in whom normal feeding or oral supplementation was used.

Evolution of citrulline in patients with chronic infectious enteropathy

Antifungal therapy, associated if necessary with nutritional support [including PN in 8 cases (Figure 2) and enteral nutrition in 10] was virologically (HIV viral load becoming undetectable) and immunologically (CD4 count >200/mm³) successful in 18 of 25 (72%) patients. In patients who needed PN, Wilcoxon’s test of the comparison of plasma citrulline concentrations was nonsignificant for patients who could not be weaned from PN and was nearly statistically significant (P = 0.068) for the PN-weaned patients (Figure 2). Citrulline normalized in weeks to months remained in the normal range (>22 μmol/L) in 15 patients with previous diffuse enteropathy (9 ± 3 compared with 26 ± 6 μmol/L; P < 0.01) and remained normal throughout follow-up in the 3 patients with localized small-bowel stenoses due to mycobacterial infection. Of these 18 patients, all except one who remained under home enteral nutrition were successfully weaned from nutritional support. Monitoring of citrulline assays confirmed that the cutoff previously published for weaning from PN support (13), ie, mean minus 2 SDs (22 μmol/L in this study), was also sensitive and specific for HIV patients (Figure 2). All patients with citrulline above this threshold were able to be PN-weaned, whereas other patients were not.

Five of the 7 patients with no clinical, biological (plasma citrulline remaining <22 μmol/L), histologic, or microbiological improvement in enteropathy died, whereas 1 patient remained under home PN and 1 remained chronically malnourished.

DISCUSSION

The intestinal architecture supports a complex absorptive function. This function interrelates with other complex gut functions, notably defensive functions, with an important CD4 lymphocyte content in the lamina propria of intestinal mucosa,
HIV enteropathy or associated intestinal infectious opportunistic diseases were observed between the 3 etiological categories. In addition, the enteropathy or associated intestinal infectious opportunistic diseases and enterocyte loss in HIV-associated enteropathy linked to protozoan or mycobacterial infection or even HIV itself, the decrease in citrullinemia can be considered as a biomarker of the extent of villous atrophy and enterocyte loss and its repair under specific treatments, as is now strongly documented in both medical and postsurgical non-HIV situations.

In villous atrophy diseases in HIV-negative patients, the ROC curves indicate that the citrulline concentration is more powerful than the albumin concentration in predicting the degree of reduction of enterocyte mass. In our study, despite the apparent relation of citrulline with tested indicators, such as BMI and albuminemia, there was a nonsignificant difference in ROC curves between citrullinemia and albuminemia. Therefore, it is not proven that citrulline is a more specific indicator of enteropathy severity than albuminemia in HIV patients. Nevertheless, enteropathy induces malnutrition and hypoalbuminemia, and the relation between citrulline and nutritional status is dependent on the intestinal disease, whereas hypoalbuminemia is a nonspecific marker that is influenced by systemic inflammation, as shown in this cohort. Previous studies have shown that citrulline has a noncausative correlation with nutritional status.

In HIV patients with a detectable viral load and no intestinal malabsorption, nutritional status impairment is notably due to hypermetabolism, which indicates the need for an increase in oral nutrition. In addition, a very close correlation between citrulline and clinical improvement, PN indication, and weaning-off was observed in this series of HIV patients. Moreover, citrulline did not differ between patients with malmnourished enteropathy, for whom enteral nutrition was indicated, and the other patients.

We showed differences in citrulline concentrations between chronic mucosal enteropathy, such as Crohn disease and HIV-associated enteropathy. Crohn disease is an immunologic disease of the gut linked to abnormal bacterial antigen sampling and processing in intestinal mucosal cells. There is no extensive

### TABLE 3
Clinical and biological characteristics of severely immunocompromised HIV patients with chronic enteropathy (group 3b, n = 25)

<table>
<thead>
<tr>
<th>BMI (kg/m²)</th>
<th>Serum albumin (g/L)</th>
<th>CD4 (cells/mm³)</th>
<th>CRP (mg/L)</th>
<th>Plasma citrulline (µmol/L)</th>
<th>Nutritive assistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV enteropathy (n = 11 of 25)</td>
<td>18.0 ± 3.7</td>
<td>29 ± 6</td>
<td>10</td>
<td>26 ± 28</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>Intestinal protozoan infection (n = 6 of 25)</td>
<td>19.0 ± 4.7</td>
<td>25 ± 5</td>
<td>13</td>
<td>42 ± 34</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>Intestinal mycobacterial infection (n = 8 of 25)</td>
<td>15.7 ± 2.2</td>
<td>24 ± 6</td>
<td>20</td>
<td>40 ± 33</td>
<td>22 ± 5</td>
</tr>
</tbody>
</table>

1 All patients had a CD4 count <200/mm³. EN, enteral nutrition (n = 9); PN, parenteral nutrition (n = 8); CRP, C-reactive protein. No significant differences were observed between the 3 etiological categories, P > 0.05.
2 Mean ± SD (all such values).
3 Median; range in parentheses (all such values).

### TABLE 4
Diagnostic accuracy of plasma citrulline threshold concentrations for the assessment of small-bowel mucosal lesions and indication for parenteral nutrition in HIV patients

<table>
<thead>
<tr>
<th>Plasma citrulline threshold (µmol/L)</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Positive predictive value %</th>
<th>Negative predictive value %</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 µmol/L</td>
<td>76</td>
<td>95</td>
<td>79</td>
<td>93</td>
</tr>
<tr>
<td>10 µmol/L</td>
<td>75</td>
<td>87</td>
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<td>87</td>
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1 This selected threshold concentration discriminated patients with chronic enteropathy (n = 25) from other HIV patients (n = 90).
2 This selected threshold concentration discriminated patients who needed parenteral nutrition (n = 8) from patients treatable without the need for parenteral nutrition (n = 17).
circles indicate patients who were able to be weaned off parenteral nutrition (PN; n = 8). The evaluation was performed at the time that enteropathy was diagnosed and after 6–30 mo (median: 9 mo) of antiretroviral, gastroenterologic, and nutritional therapy. Among patients receiving parenteral nutrition, the open circles indicate patients who were able to be weaned off parenteral nutrition (n = 4), and the filled squares indicate PN-dependent patients (n = 4). Wilcoxon’s test showed that the difference in plasma citrulline concentrations before and after therapy was not significant for PN-dependent patients and was nearly statistically significant (P = 0.008) for PN-weaned patients.

enterocyte damage per se, except in the case of significant intestinal resection. It was reported that systemic inflammation, defined as a CRP concentration >10 mg/L and a Crohn disease activity index (CDAI) of >150, did not significantly influence citrulline concentrations in 31 patients with Crohn disease (15). In this study of HIV patients, citrulline seems to be negatively influenced by the level of CRP-identified systemic and/or intestinal inflammation. This suggests a cytokine effect on enterocyte citrulline production, for example, due to active HIV replication in intestinal lamina propria with depletion of the resident CD4 T cells leading to a loss of the mucosal barrier. Therefore, the actual dependency of citrulline concentrations on inflammation is open to question, requiring further study. This study did not measure glutamine, which is the major in vivo precursor for citrulline production, but glutamine can decrease in severe inflammation and so might also influence enterocyte citrulline production. However, this hypothesis remains unlikely, because the rate of glutamine appearance increases in HIV patients with (31) or without (29) wasting, even though plasma glutamine concentrations can decrease slightly (29).

In most (17 of 26, 65%) patients in group 1b with adverse toxic intestinal effects of antiretroviral drugs, the HAART combination contained lopinavir in its previous capsule formulation. This drug, as with ritonavir, is known for its digestive intolerance, which is estimated to be at least grade 2 (4–5 stools/d or a duration >1 wk) in 10% of treated patients in clinical trials. Lopinavir also has the most frequent rate of adverse events leading to treatment discontinuation (8). In this last case, diarrhea has been linked to the presence of small amounts of sorbitol and of ricinoleic acid as excipient in the capsule, and ricinoleic acid is well-known for its structural and functional toxicity on small-bowel and colic (32) mucosa. The patients in group 2b, who were treated with the same antiretroviral combinations, had citrulline concentrations similar to those of group 1b (ie, 22–30 μmol/L). Hence, citrullinemia is moderately decreased in both of these groups, which confirms that citrulline is indeed a good indicator of enterocyte loss, which increases and normalizes in response to the protease inhibitor switch or discontinuation. There was no significant difference in citrulline concentrations between groups 1a and 2a compared with 3a, although CD4 was low in group 3a. This finding suggests that the citrulline concentration is in fact linked to intestinal disease and not to HIV per se.

Acute and chronic mucosal enteropathy can cause significant enterocyte loss (20) and lead to acute intestinal failure. HIV enteropathy immunology clearly shows enterocyte loss with a decrease in surface area-to-volume ratio with crypt hyperplasia in the jejunal mucosa (7, 21). Citrulline is lowered in situations involving enterocyte loss due to acute infectious processes in HIV-negative patients, for example, in infectious intestinal diseases with high cytopathic effects (18). We recorded the same patterns in 4 of our patients, with acute common (presumably viral) gastroenteritis. Citrulline normalizes rapidly in these situations, ie, within <1 mo. This is consistent with data observed during chemotherapy after bone marrow allograft, which induces a decrease in citrulline concentrations (33). The effect is also concordant with the known cytokinetic renewal of intestinal mucosa with a nadir in citrulline concentrations (40% of pretreatment concentration) 5–8 d after the initiation of hematopoietic stem cell transplantation (34) and is equally concordant with previous observations in acute gastroenteritis in children with short-bowel syndrome (35). Citrulline variations are more sensitive, occurring earlier than permeability test modifications (34). Furthermore, the decrease in citrulline concentrations after bone marrow transplantation appeared to be a risk factor for bacterial infection (36), possibly because of intestinal translocation.

An important finding in this study was the very good correspondence shown between citrullinemia normalization and clinical improvement, especially weaning off PN assistance. The citrulline threshold can be used as an indicator to guide nutritional support strategy: a citrulline value <10 μmol/L is associated with a high probability of PN dependency (intestinal failure by enterocyte reduction), whereas a citrulline concentration >10 μmol/L is compatible with nutritional supplementation through the enteral route (eg, with tube feeding). Prospective studies are required to establish whether this is just an association without causality or a predictive factor. It appears that a low citrulline concentration (<10 μmol/L) is clearly a risk factor for PN, but at the same time we have no data on the possible decline in citrulline concentrations before symptoms of enteropathy appear. This result makes a strong case for citrulline as a good reliable biomarker of overall intestinal enterocyte absorptive function. The major practical interest in citrulline dosage concerns follow-up on the course of disease in cases of immunoinfectious or toxic processes. The normality of citrulline in cases of localized stenoses, exclusive of diffuse small-bowel involvement (13), highlights that citrulline is only significantly modified in diffuse small-bowel disease (10). Hypocitrullinemia

![FIGURE 2. Evolution of plasma citrulline concentrations in patients with HIV-associated chronic enteropathy requiring parenteral nutrition (PN; n = 8).](image-url)
should not be present in cases in which there is a lack of significant reduction in enterocyte mass—a finding of (sub)normal citrulline concentrations can be used to exclude extensive small-bowel disease in patients with malabsorptive diarrhea.

As previously shown in other situations, it is equally important to specify that the citrulline concentration does not provide etiologic diagnosis. Plasma or serum citrulline is a biomarker of the expected course of intestinal disease and prognosis in severe enteropathy or intestinal failure. In addition, plasma citrulline is easy to measure through ion-exchange or reversed-phase liquid chromatography, which can generally be performed in any specialized hospital biochemistry laboratory, including university centers.

The authors’ responsibilities were as follows—PC and PDT: study design; PC, PDT, TG, and JCM: clinical monitoring; NN and LC: citrulline analysis; PC: writing of the manuscript; PDT, LC, and JCM: critical review of the manuscript; and PC, PDT, NN, TG, LC, and JCM: approval of the final edition. None of the authors had any potential conflicts of interest.

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