Monitoring myeloablative therapy-induced small bowel toxicity by serum citrulline concentration: a comparison with sugar permeability tests

Monitoring Myeloablative Therapy-Induced Small Bowel Toxicity by Serum Citrulline Concentration

A Comparison with Sugar Permeability Tests

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BACKGROUND. Intestinal mucositis is an important cause of cancer treatment-related morbidity and mortality, carrying a serious economic burden. Currently, objective parameters are lacking that would enable the monitoring of gut damage in routine clinical practice, thus hindering the development of clinical studies designed to investigate potential new strategies aimed at reducing or preventing this side effect. The authors investigated the characteristics of serum citrulline concentration compared with sugar permeability tests with respect to its use as a marker for cancer treatment-induced small bowel injury.

METHODS. In this prospective study, 10 patients with hematologic malignancies who were receiving myeloablative therapy had gut toxicity assessed with sugar permeability tests. Serum citrulline concentrations also were determined using archival serum samples. The association between both parameters and their respective characteristics were analyzed and compared with data from the literature.

RESULTS. Sensitivity and specificity were better for the citrulline assay compared with sugar permeability tests. Maximum gut damage assessed with the citrulline assay was observed 1–2 weeks earlier compared with the sugar permeability test. Similarly, citrulline indicated recovery of gut damage at 3 weeks after transplantation, whereas most sugar permeability tests remained abnormal.

CONCLUSIONS. The simplicity of the method, the low costs, and the lack of drawbacks to the method make the citrulline assay the first choice for measuring and monitoring treatment-related gut damage and provides an objective parameter for cancer treatment-related gut toxicity. ***Cancer* 2005;103:191–9.**

KEYWORDS: citrulline, sugar permeability test, gut damage, assay.

An increased use of multiple treatment modalities is characteristic for current developments in curative cancer treatment. This strategy has yielded superior treatment results in a variety of solid tumors, although treatment-related acute toxicity also is increased.[1–5] In patients with hematologic malignancies, epithelial gut damage has been suggested as a causal factor in the life-threatening graft-versus-host disease that occurs after myeloablative therapy.[6] In general, severe mucositis has a detrimental effect on treatment outcome due to necessary reductions in treatment intensity and/or treatment interruption. In addition to these detrimental acute effects, epithelial gut damage also has been suggested as one of several mechanisms that contribute to late treatment-related sequelae.[7] Taking into account mucositis-associated complications, such as a longer hospital stay, an increased number of infectious events, and nutritional support, Elting et al.[8] recently estimated an incremental cost of $2725 and $5565 per

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TABLE 1
Patient Characteristics and Clinical Parameters

Patient no.	Gender	Age (yrs)	Diagnosis	Max DGS	Bacteremia	LOS (days)	FI[a]
1	Male	32	ALL	2	Yes	33	0.39
2	Female	48	NHL	1	—	33	NA
3	Female	47	AML	1	Yes	30	0.06
4	Male	52	NHL	1	Yes	42	0.24
5	Female	59	MF	1	—	39	0.00
6	Male	32	AML	1	—	42	0.06
7	Male	30	ALL	2	Yes	107	0.29
8	Female	59	CML	2	Yes	44	0.56
9	Male	22	ALL	1	Yes	35	0.06
10	Female	52	CML	2	—	38	0.28

Max DGS: maximum daily gut score; LOS: length of hospital stay; FI: fever index; ALL: acute lymphatic leukemia; NHL: non-Hodgkin lymphoma; MF: myelofibrosis; AML: acute myeloid leukemia; CML: chronic myeloid leukemia; NA: not available.

[a] The ratio between the number of days at which a body temperature ≥ 38 °C was registered and the total number of days during the study period on which the body temperature was registered.

chemotherapy cycle-associated Grade 1–2 mucositis and Grade 3–4 mucositis, respectively. Hence, it is expected that preventing and/or reducing epithelial gut damage will have significant clinical and socioeconomic impact. However, clinical studies addressing treatment-induced gut damage have been hampered by the fact that objective parameters are lacking to enable monitoring of damage in routine clinical practice.

Previously, clinical symptoms were used most frequently as surrogate endpoints for gut damage. In addition to the fact that toxicity grading systems are not used uniformly by investigators,[9] they are undergoing adjustment on a regular basis. More important, however, is that clinical symptoms correlate poorly with objective parameters of gut damage, such as sugar permeability tests (SPT),[10–12] altered histomorphology,[13] or treatment-related parameters,[9] illustrating the complex city of the pathophysiology of clinical symptoms related to cytotoxic treatment-induced small bowel damage.[14–16]

Currently, morphologic parameters and SPTs are the objective endpoints used in preclinical and clinical studies. However, both methods are unsuited for monitoring purposes in routine clinical practice. In a preclinical study, we recently demonstrated that plasma citrulline concentration is a radiation dose-dependent parameter that correlates with radiation-induced small bowel morphologic endpoints, such as mucosal surface or crypt regeneration.[17] In a subsequent clinical study, we demonstrated that plasma citrulline concentration is correlated with small bowel radiation dose and volume parameters.[9] Similarly, we observed decreased citrulline levels in response to myeloablative therapy.[18] In addition to surgery,[19] celiac and nonceliac disease,[20] and acute cellular rejection after small bowel transplantation,[21,22] cytotoxic treatment was identified as another event associated with small bowel epithelial cell loss that can be monitored by plasma citrulline.

The objective of the current study was to compare the citrulline assay with a currently used method for measuring small bowel damage (i.e., the SPT). For this purpose, we analyzed a group of 10 patients who were treated uniformly in a prospective pharmacokinetic study for a hematologic malignancy. We hypothesized that changes in citrullinaemia are correlated with changes in absorption of sugar probes associated with the loss of mucosal surface and with changes in gut permeation, as assessed with multiple sugar probes.

MATERIALS AND METHODS

Patients

Patient characteristics are summarized in Table 1. Between June 1999, and December 2000, 10 patients with a mean age of 43 years (age range, 22–59 years) underwent hematopoietic stem cell transplantation (HSCT) for a hematologic malignancy at the Department of Haematology, University Medical Centre St. Radboud, Nijmegen, The Netherlands. All patients provided informed consent to participate in a prospective study investigating the pharmacokinetics and safety of 14 days of intravenous itraconazole nanocrystals in patients who were undergoing a human leukemic antigen-matched, partial T-cell-depleted sibling bone marrow transplantation. The conditioning regimen consisted of idarubicin given at a dose of 42 mg/m^2 by continuous infusion over 48 hours starting 12 days before transplantation (HSCT Day − 12), followed by 120 mg/kg cyclophosphamide (60 mg/kg

per day on HSCT Days − 6 and − 5) and 9 Gray (Gy) of total body irradiation (TBI) in 2 fractions (4.5 Gy per fraction on HSCT Days − 2 and − 1). The total duration of the study period was 34 days, from HSCT Day − 12 until HSCT Day + 21. For prophylaxis against graft-versus-host disease, patients were administered cyclosporine at 3 mg/kg per day by continuous infusion from HSCT Day − 1 to HSCT Day + 14 followed by 2 mg/kg per day until oral intake was possible. Parenteral nutritional support was started on HSCT Day − 7 and was sustained until the daily oral intake was sufficient. No agents were applied that are believed to ameliorate alimentary tract mucositis (i.e., amifostine, glutamine, keratinocyte growth factor). Antiinfective prophylaxis consisted of ciprofloxacin, acyclovir, and sulphamethoxazole-trimethoprim. Antifungal prophylaxis started on HSCT Day − 6 and consisted of intravenous itraconazole 200 mg twice daily for 2 days followed by 200 mg once daily for 10 days. All patients were treated with meropenem preemptively from the day after HSCT until neutrophil recovery ($> 0.5 \times 10^9$/L). The glomerular filtration rate was determined on admission by measuring renal creatinine clearance (in mL per minute) and was estimated thereafter from the serum creatinine level using the formula from Cockroft and Gault.[23]

Clinical Toxicity Assessment

Gut toxicity was scored daily using six items, i.e., frequency of emesis and diarrhea, occurrence of nausea, abdominal complaints, fecal incontinence, and fecal volume.[12] Each of these items was allocated a score between 0 (normal) and 3 (severe). Summation of the scores yielded a daily gut score (DGS) with 3 grades: mild toxicity (DGS Grade I; 1–6 points), moderate toxicity (DGS Grade II; 7–12 points), and severe toxicity (DGS Grade III; 13–18 points). All gut toxicities after cytotoxic treatment were ascribed to mucositis, unless there was a more plausible alternative cause, such as an adverse drug reaction or proven toxigenic infection.

The SPT

An isotonic solution was prepared by dissolving 5 g of lactulose (LAC), 1 g of L-rhamnose (RHA), 0.5 g of D-xylose (XYL) and 0.2 g of 3-*O*-methyl-D-glucose (OMG) in 100 mL of demineralized water, according to good manufacturing practice by the pharmacy of the University Medicle Centre St. Radboud. After an overnight fast, the patients emptied their bladders and then drank the test solution. A light breakfast with a drink was allowed after 2 hours. Urine was collected for 5 hours. The total volume was recorded, and aliquots of 5 mL were stored in − 80 °C until analysis. The first (baseline) SPT was performed on HSCT Day − 12 before starting conditioning therapy and was repeated on HSCT Days − 7, 0, + 7, + 14, and + 21, yielding 6 time points in 5 weeks per patient.

Urinary Excretion Ratios of Differential SPTs

Urinary excretion was determined using gas chromatography with flame ionization detection and with D-glucoheptose as an internal standard.[24] Data were collected online using Star Chromatography software (Varian, Tempe, AZ), and peak areas were measured and corrected for the internal standard. Urinary recovery of each sugar was expressed as a percentage of the intake, and the lactulose/rhamnose ratio (L/R), the D-xylose/OMG (XYL/OMG) ratio, and the rhamnose/OMG (RHA/OMG) were calculated. The L/R ratio is considered a measure of intestinal permeability, whereas the XYL/OMG and RHA/OMG ratios are used as indices of epithelial absorptive capacity and mediated cellular transport, respectively. L/R ratios, XYL/OMG ratios, and RHA/OMG ratios of ≤ 0.02,[25,26] ≥ 0.28,[27] and ≥ 0.65,[28] respectively, are considered normal.

Determination of Plasma Citrulline Concentration

The protocol required obtaining serum daily from HSCT Day − 6 until HSCT Day + 1, every other day thereafter until HSCT Day + 7, again daily thereafter until HSCT Day + 12, and finally on HSCT Day + 21, yielding 17 time points over 4 weeks per patient. The serum was stored at − 80 °C until analysis. For determination of amino acids, 250 μL serum were deproteinized in 22 mg dry 5-sulfosalicylic acid. The plasma citrulline concentration (μmol/L) was measured by using high-performance liquid chromatography.[29]

Statistical Analysis

SPSS software for Windows (release 11.0; SPSS Inc., Chicago, IL) was used for statistical analyses. All results are expressed as the mean with the 95% confidence interval (95% CI). One-way analyses of variance (ANOVAs) were used to analyze changes in citrulline concentrations and dual sugar test ratios, respectively. A Student *t* test for paired data was used to investigate differences with respect to baseline values. A Pearson correlation procedure was performedusing the raw data on citrulline concentration, urinary recovery, and dual sugar test ratios to determine the correlations between these parameters. *P* values < 0.05 were considered statistically significant.

RESULTS

Clinical Parameters

No treatment-related, lethal sequelae occurred. Patient demographics are summarized in Table 1.

TABLE 2
Time Course of Serum Citrulline Concentration for All Study Time Points (μmol/L)[a]

Day[b]	Mean	95% CI	No.	Student *t* test
− 6	25.7	17.1–34.4	10	—
− 5	21.5	14.4–28.6	10	0.177
− 4	18.1	10.3–26.1	10	0.057
− 3	18.6	9.5–27.8	10	0.075
− 2	15.6	9.9–21.2	10	0.004
− 1	14.5	8.4–20.7	10	0.006
0	12.3	8.0–16.5	10	0.001
+ 1	11.6	6.6–16.6	10	0.000
+ 3	9.7	5.3–14.1	10	0.000
+ 5	8.9	4.4–13.4	10	0.000
+ 7	9.9	5.5–14.2	10	0.000
+ 8	11.3	6.3–16.3	10	0.001
+ 9	11.5	6.1–17.0	10	0.001
+ 10	12.4	10.5–14.2	8	0.016
+ 11	15.7	8.6–22.9	8	0.009
+ 12	13.8	6.1–21.4	9	0.002
+ 21	20.1	11.1–29.1	9	0.109

95% CI: 95% confidence interval; No.: the number of individuals tested.
[a] The two-sided, Student *t* test for paired data test was used. Comparisons with baseline levels were assessed at hematopoietic stem cell transplantation Day −6 (these data correspond to Figure 1).
[b] The number of days to hematopoietic stem cell transplantation.

Throughout the study period, mild (DGS Grade I) and moderate (DGS Grade II) intestinal toxicities were observed in 6 patients and 4 patients, respectively. All but one patient (Patient 5) had microbiologically and/or clinically defined infectious events during the study period. Fever (body temperature ≥ 38 °C) was present for ≥ 1 day in all patients who experienced infectious events. A fever index was calculated as the ratio between the number of days with a body temperature ≥ 38 °C and the total number of days on which the body temperature was registered. The mean fever index was 0.21 (range, 0.00–0.56) (Table 1). *Micrococcus sendentarius* and a *Staphylococcus epidermidis* bacteraemia were identified in one patient and five patients, respectively. No association was observed between any of these clinical parameters and bacteraemia (data not shown).

Citrulline Concentration

The mean citrulline concentration in 10 patients at HSCT Day − 6 was 25.7 (95% CI, 17.1–34.4 μmol/L) (Table 2). During the complete study period, a significant decline in citrulline concentration was observed (one-way ANOVA; $P < 0.001$) (Fig. 1), with the lowest concentration measured at HSCT Day + 5 (mean, 8.9 μmol/L; 95% CI, 4.4–13.4 μmol/L). Compared with the first sample at HSCT Day − 6, the citrulline concentration decreased significantly between HSCT Day − 2 and HACT + 12. At HSCT Day + 21, the citrulline concentration no longer differed from the value measured at HSCT Day − 6.

Urinary Sugar Recovery and SPT

The results of urinary sugar excretion (% intake) and SPT ratios determined at HSCT Days − 12 (baseline), − 7, 0, + 7, + 14, and + 21 are summarized in Tables 3 and 4. Comparison with baseline values yielded significantly increased LAC excretion on HSCT Day + 7 ($P = 0.029$) and significantly decreased RHA excretion on HSCT Day + 14. In a one-way ANOVA, only alterations in RHA excretion were significant ($P = 0.025$).

Compared with baseline values, the L/R ratio was

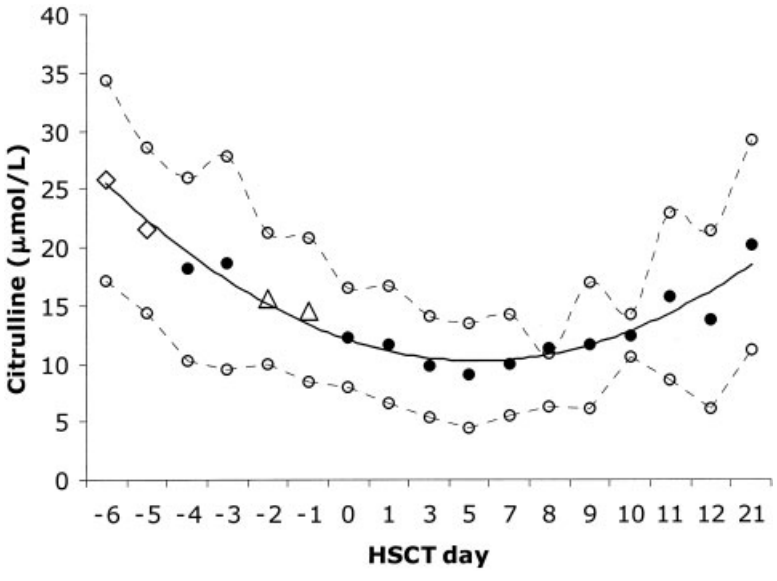


FIGURE 1. Time course of the plasma citrulline concentration (μmol/L) between hematopoietic stem cell transplantation (HSCT) Day − 6 and HSCT Day + 21. Diamonds: 120 mg/kg cyclophosphamide (60 mg/kg); triangles: 4.5 Gray of total body irradiation (trend line: polynomial, second order). Dotted lines represent the upper and lower bounds of 95% confidence intervals, respectively (one-way analysis of variance; $P < 0.001$). The mean citrulline concentrations measured between HSCT Day + 1 and HSCT Day + 9 differed significantly compared with the concentration measured at HSCT Day − 6 (Tukey post-hoc test;: $P = 0.035$, $P = 0.007$, $P = 0.003$, $P = 0.008$, $P = 0.029$, and $P = 0.035$ for HSCT Days + 1, + 3, + 5, + 7, + 8, and + 9, respectively).

TABLE 3
Time Course of Serum Citrulline Concentration (μmol/L) and Sugar Permeability Test Ratios[a]

	Citrulline			L/R ratio			X/O ratio			R/O ratio		
Day[b]	No.	Mean	95% CI	No.	Mean	95% CI	No.	Mean	95% CI	No.	Mean	95% CI
− 12	—	—	—	10	0.04	0.03–0.06	10	0.68	0.56–0.80	10	0.23	0.17–0.26
− 7	10	25.7	17.1–34.4	10	0.05	0.03–0.07	10	0.65	0.52–0.79	10	0.29	0.22–0.35
0	10	12.3	12.3–16.5[c]	8	0.13	0.00–0.27	8	0.61	0.45–0.77	8	0.21	0.15–0.28
+ 7	10	9.9	5.5–14.2[d]	8	0.12	0.06–0.17[e]	8	0.51	0.47–0.57	8	0.13	0.10–0.16[e]
+ 14	9	13.8	6.1–21.4[c]	7	0.18	0.02–0.34	7	0.51	0.43–0.58	7	0.13	0.07–0.20[e]
+ 21	9	20.1	11.1–29.1	5	0.13	0.06–0.20[e]	5	0.45	0.33–0.58	5	0.09	0.07–0.20[e]

L/R: lactulose/rhamnose; X/O; D-xylose/*O*-methylglucose; R/O: rhamnose/*O*-methylglucose; No.: the number of individuals tested; 95% CI: 95% confidence interval.

[a] Statistics were determined with 2-sided, Student *t* tests for paired data to investigate changes relative to baseline values (i.e., hematopoietic stem cell transplantation [HSCT] Day − 12 for sugar permeability tests and HSCT Day − 7 for citrulline). Citrulline data that were obtained at HSCT Days − 6 and + 12 were used to calculate correlations with sugar permeability test data that were obtained at HSCT Days − 7 and + 14, respectively.

[b] The number of days to hematopoietic stem cell transplantation.

[c] $P < 0.01$.

[d] $P < 0.001$.

[e] $P < 0.05$.

TABLE 4
Time Course of Urinary Sugar Excretion (% Intake)[a]

		Lactulose[c]		Rhamnose[d]		D-xylose[c]		3-*O*-methylglucose[c]	
Day[b]	No.	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
− 12	10	0.2	0.1–0.3	4.4	2.5–6.3	13.2	7.2–19.3	19.5	12.0–27.0
− 7	10	0.4	0.1–0.7	8.1	3.4–12.9	17.5	7.6–27.4	27.4	13.4–41.4
0	8	0.7	− 0.2–0.2	6.2	1.5–10.8	17.8	2.7–33.0	28.3	5.3–51.3
+ 7	8	0.3	0.2–0.4[e]	2.6	1.3–4.0	10.4	5.6–15.2	20.4	11.0–27.9
+ 14	7	0.3	− 0.0–0.7	2.5	0.3–4.8[f]	9.6	4.7–15.5[e]	19.2	7.2–31.2
+ 21	5	0.2	0.1–0.6	1.7	− 0.2–3.5	11.3	− 1.0–23.7	26.0	− 5.1–57.0

No.: the number of individuals tested; 95% CI: 95% confidence interval.

[a] Two-sided, Student *t* tests for paired data were used to investigate changes relative to baseline values at the time of hematopoietic stem cell transplantation.

[b] The number of days to hematopoietic stem cell transplantation.

[c] *P* value not significant (one-way analysis of variance).

[d] $P = 0.025$ (one-way analysis of variance).

[e] $P < 0.05$.

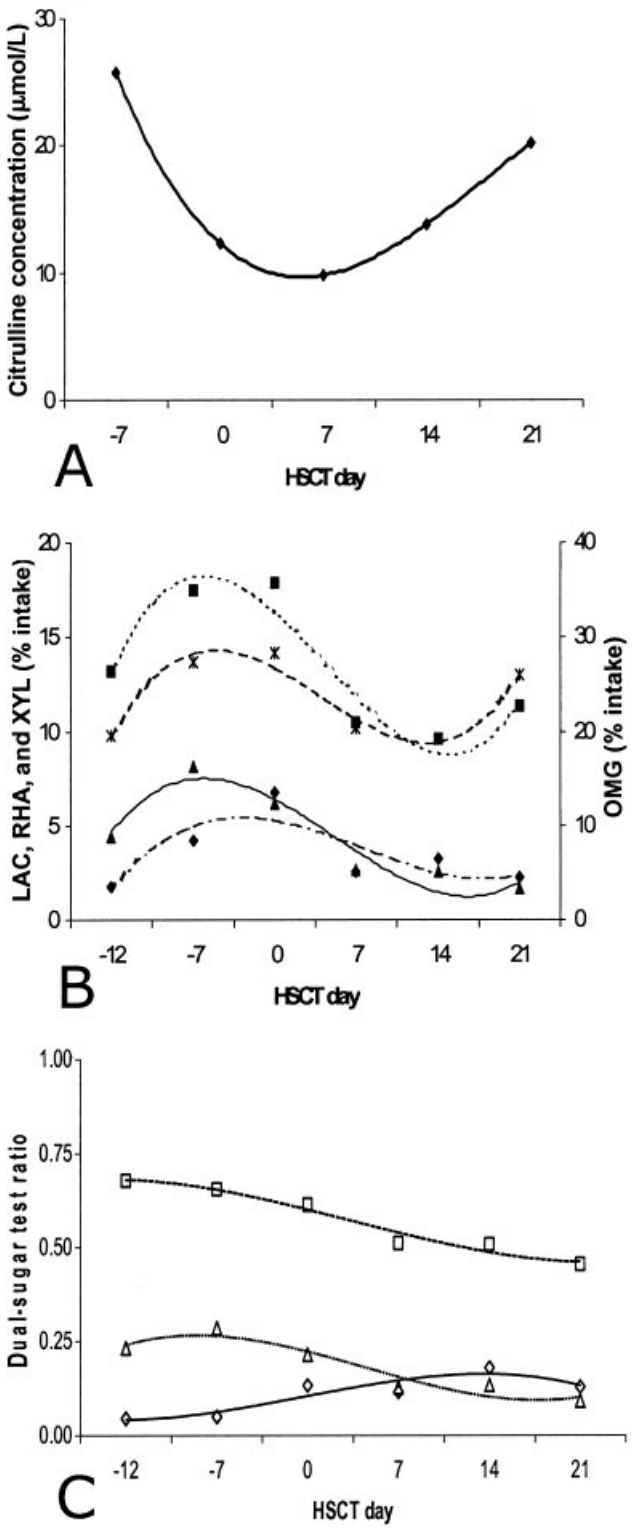
[f] $P < 0.01$.

increased significantly on HSCT Days + 7 and + 21 ($P = 0.020$ and $P = 0.012$, respectively). The RHA/OMG ratio was decreased significantly on HSCT Days + 7, + 14, and + 21 ($P = 0.027$, $P = 0.033$, and $P = 0.036$, respectively). In a one-way ANOVA, only alterations in the XYL/OMG ratio and the RHA/OMG ratio were significant ($P = 0.032$ and $P < 0.001$, respectively).

Correlation between Citrulline Concentration, Clinical Parameters, Urinary Sugar Excretion, and Dual-Sugar Test Ratios

Because no serum was available before the start of treatment, the value assessed at HSCT Day − 6 is referred to as the baseline in this analysis. The citrulline nadir, calculated as a percentage of the baseline but not of the absolute value (μmol/L), was correlated significantly with the maximum DGS ($P = 0.033$). The maximum DGS was correlated significantly with urinary RHA and XYL recovery ($P = 0.047$ and $P = 0.050$, respectively). No correlation was observed between these parameters and dual-sugar test ratios (data not shown).

The citrulline data obtained at HSCT Day − 6 and HSCT Day + 12 were used for the calculation of correlations with SPT data obtained at HSCT Day − 7 and HSCT Day + 14, respectively. Thus, citrulline and SPT data were available for comparison at 5 time points during 4 weeks, i.e., on HSCT Days − 7, 0, + 7, + 14,



and + 21. The time course of the respective parameters is shown in Figure 2. The time course of citrulline indicates maximum damage at HSCT Day + 7, compared with HSCT Day + 14 for urinary recovery of the individual sugar probes and, to some extent, for the L/R ratio and compared with HSCT Day + 21 for the XYL/OMG and RHA/OMG ratios. A recovery of intestinal damage during the study period is indicated by the citrulline, RHA, XYL, and OMG urinary recovery data and by the L/R ratio in contrast to the LAC recovery data and the XYL/OMG and RHA/OMG ratios. It is interesting to note that urinary recovery of all sugar probes was increased 1 week after initiation of the conditioning regimen.

The L/R ratio and the urinary recovery of all four sugars were correlated significantly with each other, although they were not correlated with the citrulline concentration (Fig. 2B). The citrulline concentration (μmol/L) was correlated with the RHA/OMG ratio ($P = 0.025$). No correlation was observed with the L/R or XYL/OMG ratios.

DISCUSSION

In the current study, further support was provided for the use of serum citrulline concentration as a marker for cytotoxic treatment-induced gut damage.[9,17,18] We observed a discrepancy between the time course of two objective endpoints for epithelial gut damage (i.e., the serum citrulline concentration and the SPT). Compared with the sugar probe results, the time course for citrulline was in agreement with the known kinetics of epithelial cell loss after radiotherapy[30] and chemotherapy.[13]

Gut-permeability testing is a noninvasive method for the assessment of altered gut-barrier function as an endpoint for intestinal damage.[25,26] Currently, the SPT is the objective endpoint used in clinical studies to quantify gut damage[12,31] and to assess treatment

FIGURE 2. Time course of (A) plasma citrulline concentration (μmol/L), (B) urinary sugar recovery, and (C) dual-sugar test ratios determined on hematopoietic stem cell transplantation (HSCT) Days − 12, − 7, 0, + 7, + 14, and + 21. Solid diamonds and heavy solid line: citrulline; solid diamonds and dotted-and-dashed line: lactulose (LAC); solid triangles and light solid line: L-rhamnose (RHA); solid squares and dotted line: D-xylose (XYL); and asterisks and dashed line: 3-*O*-methyl-D-glucose (OMG). Dual-sugar test ratios: open diamonds: LAC/RHA ratio; open squares: XYL/OMG ratio; open triangles: RHA/OMG ratio (trend line, third-order polynomial). For presentation purposes, the urinary sugar recovery data presented in A were multiplied by a factor of 10^3 (for LAC) or by a factor of 10^2 (for RHA, XYL, and OMG). Data points in A and B represent mean values. Note that sugar baseline values are not shown (i.e., HSCT Day − 12).

effects aimed at mucoprotection.[32] By simultaneously using multiple sugar probes, which are chosen on behalf of different permeation pathways, information about intestinal absorptive capacity is obtained in addition to information regarding intestinal permeability. Currently, it is accepted generally that alterations in the L/R ratio provide an index for intestinal permeability, whereas the recovery of RHA, XYL, and OMG and their ratios provide an index the intestinal absorptive capacity.[26,33] After myeloablative therapy, increased gut permeability (the L/R ratio) and decreased absorptive capacity (the RHA/OMG ratio) was observed (Table 3), in agreement with previous results[12,18] and with reports from the literature.[10,13] Remarkably, the decrease in the XYL/OMG ratio was not statistically significant compared with previous findings from a group of 56 similarly treated patients in our department.[12] Similarly, urinary recovery data did not display major changes (Table 4). The small numbers of patients in the current series and the relatively large standard variations may explain this finding.

We performed a series of preclinical and clinical experiments to develop an objective parameter that would enable us to monitor gut damage in routine clinical practice.[9,17] Cytotoxic treatment induces intestinal epithelial cell loss.[13,30] Plasma citrulline concentration is a surrogate endpoint for the small intestinal epithelial cell mass, irrespective of the underlying cause of cell loss, such as surgical resection,[19,34,35] celiac and nonceliac disease,[20] or cellular rejection after small bowel transplantation.[21,22] We identified plasma citrulline concentration as a simple and sensitive marker enabling quantitation of cytotoxic treatment-induced small bowel epithelial cell loss.[9,17] This was confirmed in patients who received to myeloablative therapy.[18] In the current study, the citrulline concentration was determined in archival serum samples that were obtained during an observation period of 5 weeks in a series of 10 patients who were treated uniformly with intensive myeloablative therapy. The results were in complete agreement with previous data obtained in similarly treated patients[18] and clearly demonstrated the reproducibility and simplicity of the assay and its ability to enable close monitoring of this type of treatment-induced gut damage, as illustrated in Figure 1.

In contrast to Crenn et al.,[20] we were not able to correlate the citrulline level with the degree of mucosal atrophy. Instead, clinical symptoms[12] associated with mucosal atrophy were used as a surrogate endpoint in addition to SPTs. The absolute citrulline concentration (μmol/L) did not correlate with these endpoints, whereas the normalized citrulline level (% baseline) correlated with the maximal gut toxicity observed during the observation period. A similar correlation was observed between the urinary recovery of RHA and XYL (% intake) and maximal gut toxicity. However, there were several limitations to the use of these surrogate endpoints in the current study. First, compared with the serum citrulline concentration, which is considered highly dependent on the small intestinal cell mass,[36,37] the gut toxicity score we used does not specifically address small intestinal toxicity but addresses only gastrointestinal toxicity in general. Second, mucosal denudation is merely one of several events underlying clinical symptoms related to cytotoxic treatment.[13–16]

Comparison of the citrulline assay results with the SPT results yielded some striking differences between both endpoints. The citrulline level (μmol/L or % baseline) did not correlate with gut permeability (the L/R ratio). Except for a correlation with the RHA/OMG ratio, no other correlation was observed with parameters indicative for the absorptive capacity used in this study (i.e., the XYL/OMG ratio or the urinary recovery of RHA, XYL and OMG). Furthermore, the time course of both endpoints essentially was different (Fig. 2). The maximum damage and recovery were recorded between 1 week and 2 weeks earlier using the citrulline assay compared with the sugar probe test results. In contrast to the time course of urinary sugar probe recovery, the time course observed for citrulline was in complete agreement with the time course observed for other function tests related to a reduced number of epithelial cells[17] and with known kinetics of injured gut epithelium after radiotherapy[30] or chemotherapy.[13] Remarkably, the RHA recovery, which was considered the most appropriate estimate of the mucosal surface available for absorption[33] and citrulline concentration during the initial study period, yielded contradictory results concerning the relations with mucosal atrophy and known kinetics of this mucosal event.[13,30] The initial increase in the sugar probe recovery (and in RHA recovery in particular) may be explained by alterations in the permeation pathway or by altered preabsorptive factors, such as gastric emptying or small bowel transit time, and both factors frequently are affected by this type of treatment. Unfortunately, no serum was available for citrulline analysis at HSCT Day − 12. However, the citrulline concentrations measured at HSCT Day − 6 (i.e., 1 week after initiation of the conditioning regimen) were comparable to baseline citrulline concentrations in a previous study[9] (mean, 30.9 μmol/L; 95% CI, 26.6–35.2 μmol/L). Therefore, it is reasonable to assume that a change, if any, occurring in the citrulline level dur-

ing the first week of the conditioning regimen would be a minor decline. Finally, the average 95% CI was significantly greater for LAC, RHA, XYL, and OMG compared with citrulline ($P = 0.001$) (Tables 2–4). Although the sugar tests showed remarkable changes during the study period (Fig. 2), statistical significance was moderate (Tables 2–4) compared with citrulline.

Gastrointestinal mucositis is a common side effect of cancer treatment that, not infrequently, is persistent[38] and that may cause a major economic burden.[8] In addition to tumor control, data on normal tissue damage are required to assess the therapeutic value of treatment regimens. In patients with small bowel damage, clinical signs and symptoms expressed in toxicity scores are the current standard. For the reasons described above, there is an urgent need for an objective parameter. Ideally, such a marker should be accessed easily and should be independent of medication and metabolic events, such as diet and nutritional status. Previous attempts have been made to develop simple blood tests for measuring gut damage[39,40]; however, to date, none are used in clinical practice. We suggest that serum citrulline fulfils most, if not all, of these requirements. In addition to yielding highly reproducible results and functioning as a sensitive and specific marker for epithelial cell loss, the citrulline assay can be applied as a simple blood test and is relatively inexpensive.

The results of the current study, in which serum citrulline concentration was used as a marker for cytotoxic treatment-induced intestinal damage are highly reproducible. Overall, the citrulline concentration appears to be a quantitative parameter that is independent of the underlying cause for epithelial cell loss.[9,17–21] Compared with SPTs, the citrulline assay is more sensitive and more specific for measuring small bowel epithelial cell loss. In addition, this assay enables more accurate assessment of induction and repair kinetics of damage. The simplicity of the method, the low costs, and the lack of methodological drawbacks, as discussed earlier, make this assay the first choice for measuring and monitoring treatment-related gut damage. In addition, the citrulline assay provides an objective parameter to the clinician that enables standardized assessment of treatment-related morbidity.

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