

Microbial Translocation and Liver Disease Progression in Women Coinfected With HIV and Hepatitis C Virus

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Background. Microbial translocation has been implicated in the pathogenesis of liver fibrosis and cirrhosis. We sought to determine whether markers of microbial translocation are associated with liver disease progression during coinfection with human immunodeficiency virus (HIV) and hepatitis C virus (HCV).

Methods. We measured serial plasma lipopolysaccharide (LPS), endotoxin core antibody, intestinal fatty acid-binding protein (I-FABP), soluble CD14 (sCD14), interleukin 6 (IL-6), interleukin 10, and tumor necrosis factor α (TNF- α) levels over a 5-year period in 44 HIV/HCV-coinfected women, 21 of whom experienced liver disease progression and 23 were nonprogressors.

Results. While LPS levels did not differ significantly over time between progressors and nonprogressors ($P = .60$), progressors had significantly higher plasma levels of sCD14, a marker of monocyte activation by LPS, at the first time point measured ($P = .03$) and throughout the study period ($P = .001$); progressors also had higher IL-6 and I-FABP levels over the 5-year study period ($P = .02$ and $.03$, respectively). The associations between progression and sCD14, I-FABP, and IL-6 levels were unchanged in models controlling for HIV RNA and CD4⁺ T-cell count.

Conclusions. Although LPS levels did not differ between liver disease progressors and nonprogressors, the association of sCD14, I-FABP, and IL-6 levels with liver disease progression suggests that impairment of gut epithelial integrity and consequent microbial translocation may play a role in the complex interaction of HIV and HCV pathogenesis.

Keywords. HIV; hepatitis C; microbial translocation; fibrosis; liver disease progression; soluble CD14.

Liver disease, most often due to hepatitis C virus (HCV), is an increasingly common cause of morbidity and mortality among persons infected with human immunodeficiency virus (HIV) [1–3]. It is clear that HIV accelerates the course liver disease progression among coinfecting persons, but the cause of this accelerated progression is not fully understood [1, 4].

Microbial translocation has been implicated in the pathogenesis of liver disease associated with alcoholism and graft versus host disease [5–7]. Kupffer cells, which are hepatic macrophages, can be activated by lipopolysaccharide (LPS). Free LPS binds to Kupffer cells via interaction with LPS binding protein and CD14 [8]. The LPS complex, via Toll-like receptor 4 (TLR4) and transcription factor nuclear factor κ -B, sensitizes hepatic stellate cells to transforming growth factor β and the activating effects of Kupffer cells. Activated stellate cells produce a matrix rich in type 1 collagen, leading to hepatic fibrosis [9]. How important these mechanisms are in HIV/HCV-associated liver disease is unclear.

Events early in HIV infection result in translocation of microbes and microbial products across the gut mucosa into the systemic circulation. The destruction of gut-associated lymphoid tissue occurs early in HIV infection through virus-induced apoptosis of CD4⁺ T

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lymphocytes of the intestinal submucosa [10–12] and increases the permeability of the gut. There are limited data on the influence of microbial translocation on HIV/HCV-associated liver disease progression during HIV infection. Balagopal et al, in a cross-sectional study of HIV/HCV-coinfected persons, demonstrated that low CD4⁺ T-cell counts and high LPS levels were independently associated with cirrhosis [13]. In a look-back study of 53 HIV-infected individuals with hepatitis C, elevated LPS levels were seen ≤ 1 year but not > 1 year before cirrhosis diagnosis [13].

We sought to add to the understanding of the influence of microbial translocation on HIV/HCV-associated liver disease by comparing longitudinal plasma markers of microbial translocation, macrophage activation, and inflammation in HIV/HCV-coinfected women who experienced significant liver disease progression to markers in women who experienced minimal or no progression.

METHODS

This study involved women from the Chicago site of the Women's Interagency HIV Study (WIHS). The WIHS is a longitudinal study of HIV-infected and demographically similar uninfected women at 6 sites: Chicago, San Francisco Bay Area, Brooklyn and Bronx/Manhattan, New York, Washington, D. C., and Los Angeles. Women are seen semiannually for an interview, physical examination, and collection of blood and genital specimens. Informed consent was obtained from all participants in accordance with US Department of Health and Human Services guidelines and the institutional review boards of participating institutions. The cohort was designed to reflect the demographic characteristics of US women infected with HIV. Details of cohort recruitment, retention, and demographic characteristics are published elsewhere [14, 15].

Study subjects were HIV/HCV-coinfected women. HCV infection was defined as HCV antibody and RNA positivity at baseline measurement. Soluble markers of microbial translocation, intestinal mucosal integrity, and inflammation were measured from banked specimens, frozen at -80°C since collection. We compared markers from retrospectively defined intervals during which women manifested liver disease progression to intervals during which there was no clinical, pathological, or noninvasive-marker-based evidence of liver disease progression. Liver disease was ascertained by analysis of a liver biopsy specimen, confirmation of liver-associated death, or measurement of the following noninvasive markers: aspartate transaminase (AST) level to platelet count ratio index (APRI), calculated as $([\text{AST level}/\text{upper limit of the normal AST level}]/[\text{platelet count}]) \times 100$, where the AST level is expressed as units/liter and the platelet count is expressed as the number of platelets/liter multiplied by 10^9 ; and the fibrosis 4 (FIB-4) score, calculated as $([\text{age} \times \text{AST level}]/[\text{platelet count} \times \text{ALT level}])^{1/2}$, where

the age is expressed in years, the AST and alanine transaminase (ALT) levels are expressed in units/liter, and the platelet count is expressed as the number of platelets/liter multiplied by 10^9 [16–19]. Liver-associated death was ascertained by review of death certificate. In the case of liver-associated death, the soluble markers were measured on a specimen obtained approximately 1 year before death to ensure that liver disease was present but to avoid measurement of markers that may reflect premortem infectious or inflammatory conditions.

Soluble markers were measured at 3 time points for each woman; time points were approximately 2.5 years apart. Time 1 (T1) for all women was a point when liver biopsy and both APRI (< 0.5) and FIB-4 score (< 1.45) indicated no or minimal fibrosis and self-report indicated no clinical evidence of end-stage liver disease. For liver disease progressors, T3 was a point 5 years later when there was biopsy-confirmed cirrhosis or bridging fibrosis, liver-associated death within approximately 1 year, or the APRI (> 1.5) and FIB-4 score (> 3.25) were consistent with cirrhosis. For nonprogressors, T3 was a point when there was no clinical evidence of end-stage liver disease and when liver biopsy or both APRI (< 0.5) and FIB-4 score (< 1.45) indicated no or minimal fibrosis. T2 was equidistant between T1 and T3, usually 2.5 years from each, with a range of 2 to 3 years.

APRI and FIB-4 score are the most commonly used noninvasive markers in the WIHS because the data necessary to calculate them are available at most WIHS visits. An APRI of > 1.5 has been found to have area under the receiver operating curve of 0.76 for biopsy-proven significant fibrosis (METAVIR stages F2–4) and 0.82 for cirrhosis in a large meta-analysis including HCV-infected patients with and without HIV infection [20] and ranges of 0.71–0.82 for significant fibrosis and 0.81–0.92 and cirrhosis in large series [21]. The negative predictive values of an APRI of < 0.5 for lack of significant fibrosis have been found to be 80%–91% in populations with a prevalence of fibrosis similar to that among WIHS participants [16, 20]. Several reviews have found that the accuracy of the APRI for cirrhosis was the same or better in studies of HIV/HCV-coinfected persons, compared with accuracy in studies of HCV-monoinfected persons [17, 20]. A FIB-4 score of > 3.25 has been found to correlate with severe fibrosis (METAVIR stages F3–4), with an area under the receiver operating curve of 0.72–0.85, and a FIB-4 score of < 1.45 has been found to have a negative predictive value of 95% for significant fibrosis in one large study of HIV/HCV-coinfected persons [20, 22].

Laboratory Methods

Plasma LPS levels were quantified in duplicate by dilution of plasma specimens to 10% with endotoxin-free water, using a Limulus amoebocyte assay (Lonza Group, Switzerland). The background level was subtracted, and LPS levels were calculated by following the manufacturer's recommended protocol.

Immunoglobulin M endotoxin core (EndoCAB) and intestinal fatty acid-binding protein (I-FABP) levels were quantified using an enzyme-linked immunosorbent assay (ELISA; Hycult Biotech, Uden, the Netherlands). Soluble CD14 (sCD14) levels were measured in plasma diluted to 1:200, and data were analyzed by ELISA according to the manufacturer's instructions (R & D Systems, Minneapolis, MN). Commercially available ELISAs were used to measure levels of tumor necrosis factor α (TNF- α ; BD Biosciences, San Jose, CA), interleukin 6 (IL-6; R & D Systems), and interleukin 10 (IL-10; R & D Systems). Laboratory investigators were blinded to the liver disease progression status of participants.

Statistical Methods

Demographic and clinical characteristics were assessed at various periods (T1–3), which correlated with measures of the soluble plasma markers. At the initial study visit, age, mean CD4⁺ T-cell count, mean HIV RNA level, and injection drug or cocaine use in the previous 6 months (by self-report) was assessed. Additional characteristics evaluated included mean CD4⁺ T-cell count and mean HIV RNA level at each visit, use of highly active antiretroviral therapy during the study period, alcohol use (by self-report), and hepatitis C treatment. The crude association between progression and each categorical covariate was assessed using unadjusted odds ratios (ORs), 95% confidence intervals (CIs), and *P* values (calculated by the Fisher exact test); continuous variables were compared by the *t* test. Crude associations between progression and each plasma marker were also assessed at T1, T2, T3, and all visits, using *t* tests. Multiple generalized linear regression models (using Proc GLM) were fit to assess associations and slopes for each serum marker, stratifying by progression status and time and controlling for log HIV RNA level and CD4⁺ T-cell count. *P*-values from regression models are reported for demonstrating differences in slopes between progressors and nonprogressors for each plasma marker. All analyses were performed in SAS software, version 9.2 (SAS Institute, Cary, NC). Graphs were produced using STATA, version 10.0.

RESULTS

Forty-four participants in the Chicago WIHS site were studied, of whom 21 did and 23 did not experience progression of liver disease. Characteristics of the 44 women are in Table 1. The median interval between T1 and T3 was 4.9 years and did not differ between progressors (4.8 years) and nonprogressors (5.0 years). The majority of women in both groups were African American, and the mean age at T1 was 41.6 years. The CD4⁺ T-cell count was relatively preserved, with mean values at T1 of 421 cells/mm³ for progressors and 526 cells/mm³ for nonprogressors (*P* = .27); progressors had a significantly lower mean

CD4⁺ T-cell count than nonprogressors on the basis of values from the entire study period (371 vs 492 cells/mm³; *P* = .02).

Relationship of Plasma Markers to Liver Disease Progression

Table 2 summarizes the quantitative plasma marker data and the associations with liver disease progression, and Figures 1–4 depict the plasma markers values over time. Figure 1 shows LPS and EndoCAB levels over time in progressors and nonprogressors. There was no difference between progressors and nonprogressors in the LPS level or slope in unadjusted models or models with adjustment for HIV RNA load or CD4⁺ T-cell count. For EndoCAB, progressors had a nonsignificant trend toward a lower level overall (*P* = .07), which persisted in the model with HIV RNA load (*P* = .07) but was attenuated in the model with CD4⁺ T-cell count (*P* = .22). There was no change over time or difference in slope in crude models or those with adjustment for HIV RNA load and CD4⁺ T-cell count.

Figure 2 depicts levels of sCD14 and IL-6 in liver disease progressors and nonprogressors. The level of sCD14 was significantly higher in progressors at T1 (*P* = .03) and overall (*P* = .001), but the slope was not different (*P* = .75). When the HIV RNA load and CD4⁺ T-cell count data were added to the model testing the association between sCD14 level and liver disease progression, the relationship did not change (*P* = .003 and *P* = .002, respectively). Adding alcohol use to the model attenuated the association, but it remained significant (*P* = .01). Progressors had a higher level of IL-6 overall than nonprogressors (*P* = .02), and the level increased significantly over time for progressors (slope, 1.1; *P* = .03). In the models that adjusted for HIV RNA load and CD4⁺ T-cell count, the association between progression and IL-6 level remained significant (*P* = .04 and *P* = .04, respectively), but when alcohol was added to the model, the association attenuated to nonsignificance (*P* = .10).

Figure 3 shows results for TNF- α and IL-10 levels. TNF- α levels and slopes did not differ between progressors and nonprogressors. For IL-10, progressors had a higher level overall than nonprogressors (*P* = .05), with no change in relationship in adjusted models (HIV RNA load, *P* = .04; CD4⁺ T-cell count, *P* = .03) and no difference in slope. Figure 4 shows results for I-FABP. I-FABP levels were similar at visit 1 but significantly higher at visit 3 (*P* = .01) and overall (*P* = .03) in progressors. The I-FABP level increased significantly with time, with a slope of 388 for progressors (*P* < .001) and a slope of 76.9 for nonprogressors (*P* = .20). These findings were similar in models that included HIV RNA load and CD4⁺ T-cell count.

Relationship of Plasma Markers to HIV RNA Load

Two markers had significant relationships with HIV RNA level: sCD14 and IL-6 levels increased as the HIV RNA load increased (*P* = .0005 and *P* = .0007, respectively). Levels of LPS (*P* = .47), TNF- α (*P* = .86), IL-10 (*P* = .43), and EndoCAB (*P* = .47) showed no significant relationship with the HIV RNA level.

Table 1. Demographic and Clinical Characteristics for 44 Liver Disease Progressor and Nonprogressor Study Subjects Coinfected With Human Immunodeficiency Virus (HIV) and Hepatitis C Virus in Whom Liver Disease Did or Did Not Progress

Characteristic	Progressors (n = 21)	Nonprogressors (n = 23)	Univariate OR (95% CI)	P ^a
Age at T1, y	42.4 ± 7.4	40.8 ± 5.744
Interval between T1 and T3, y	4.8 ± 0.62	5.0 ± 0.3317 ^b
Race/ethnicity				
White, non-Hispanic	8 (38.1)	8 (34.8)	1.04 (.31–3.52)	.62 ^c
White, Hispanic	0 (0)	1 (4.3)	...	
African American, non-Hispanic	13 (61.9)	14 (60.9)	Reference	
CD4 ⁺ T-cell count, cells/mm ³				
T1	421.0 ± 267.8	526.0 ± 343.827 ^b
All study visits (n = 131) ^d	371.0 ± 271.4	491.7 ± 299.802 ^b
CD4 ⁺ T-cell percentage				
T1	20.3 ± 8.5	27.0 ± 10.302 ^b
All study visits (n = 131) ^d	20.5 ± 10.4	27.4 ± 9.40001 ^b
HIV RNA load, copies/mL				
T1	15 420.5 ± 30 190.4	12 156.1 ± 22 007.673 ^b
All study visits (n = 131) ^d	12 789.4 ± 613 973	38 836.8 ± 185 21628 ^b
Visits during which HIV RNA load was undetectable ^e	37/63 (58.7)	36/68 ^e (52.9)	1.27 (.63–2.53)	.60
HIV RNA load at T1 for those with a detectable HIV RNA load (n = 26)	24 667.5 ± 36 585.8	19 079.3 ± 26 179.565 ^b
HIV RNA load for those with a detectable HIV RNA load (n = 73) ^e	213 830 ± 787 349	72 851.7 ± 251 27531 ^b
Visits during which HAART was used ^e	36/63 (57.1)	28/69 (40.6)	1.95 (.98–3.90)	.08
Maximum alcohol use, drinks/wk				
None	7 (33.3)	4 (17.4)16 ^c
<8	5 (23.8)	13 (56.5)	...	
8–14	2 (9.5)	2 (8.7)	...	
≥15	7 (33.3)	4 (17.4)	...	
Liver disease ascertainment				
By liver biopsy	8 (38.1)	8 (34.8)	...	
By confirmation of liver-associated death	6 (28.6)		...	
By serum marker analysis	7 (33.3)	15 (65.2)	...	
Any hepatitis C treatment	1 (4.8)	1 (4.4)	1.10 (.06–18.77)	>.999
IDU since most recent visit before T1	2 (9.5)	5 (21.7)	0.38 (.07–2.21)	.42
Cocaine use since most recent visit before T1	8 (38.1)	6 (26.1)	1.74 (.48–6.28)	.52

Data are mean ± SD or no. (%) of subjects, unless otherwise indicated. See Methods for definitions of T1 and T3.

Abbreviations: CI, confidence interval; HAART, highly active antiretroviral therapy; IDU, injection drug use; OR, odds ratio.

^a By the Fisher exact test, unless otherwise indicated.

^b By the t test.

^c By global χ^2 analysis.

^d One subject did not have data for 1 visit.

^e Data are for 3 visits per subject.

Relationship of Plasma Markers to CD4⁺ T-Cell Count

A higher IL-6 level was associated with a lower CD4⁺ T-cell count ($P = .02$), and a higher EndoCAb level were associated

with a higher CD4⁺ T-cell count ($P = .001$). There was no significant relationship between CD4⁺ T-cell count and levels of LPS ($P = .63$), sCD14 ($P = .34$), IL-10 ($P = .82$), or TNF- α ($P = .60$).

Table 2. Associations Between Quantitative Plasma Marker Levels and Liver Disease Progression

Variable, by Time Point(s)	Overall	Progressors	Nonprogressors	<i>P</i> ^a	
				Unadjusted	Adjusted ^b
T1	n = 44	n = 21	n = 23		
LPS level, EU/mL (n = 43) ^c	0.38 ± 0.33	0.35 ± 0.27	0.41 ± 0.38	.53	.59
EndoCAb level, MMU/mL	110.8 ± 82.5	99.5 ± 72.3	121.2 ± 91.3	.39	.42
sCD14 level, pg/mL	2359.9 ± 447.6	2510.7 ± 453.9	2222.2 ± 403.4	.03	.02
IL-6 level, pg/mL	3.1 ± 2.5	3.4 ± 2.0	2.9 ± 2.9	.56	.57
IL-10 level, pg/mL	10.6 ± 9.5	12.7 ± 12.5	8.8 ± 5.1	.20	.11
TNF-α level, pg/mL	2.2 ± 6.8	3.8 ± 8.8	0.6 ± 3.7	.13	.11
I-FABP level, pg/mL	476.1 ± 378.5	475.8 ± 356.6	476.4 ± 405.4	.99	.87
T3	n = 44	n = 21	n = 23		
LPS level, EU/mL	0.32 ± 0.24	0.33 ± 0.18	0.32 ± 0.28	.90	.31
EndoCAb level, MMU/mL	87.7 ± 82.4	68.7 ± 63.6	105.0 ± 94.6	.15	.10
sCD14 level, pg/mL	2473.0 ± 607.6	2615.4 ± 603.7	2342.9 ± 594.3	.14	.33
IL-6 level, pg/mL	4.9 ± 3.8	5.5 ± 3.7	4.4 ± 3.9	.33	.69
IL-10 level, pg/mL	9.6 ± 4.7	9.9 ± 5.6	9.2 ± 3.7	.63	.74
TNF-α level, pg/mL	3.08 ± 8.1	3.2 ± 6.9	3.0 ± 9.2	.94	.97
I-FABP level, pg/mL	926.9 ± 799.3	1252.0 ± 1001.2	630.2 ± 380.2	.01	.001
T1-3	n = 132	n = 63	n = 69		
LPS level, EU/mL (n = 131) ^c	0.37 ± 0.28	0.36 ± 0.25	0.37 ± 0.30	.69	.60
EndoCAb level, MMU/mL	97.9 ± 78.8	85.2 ± 66.7	109.4 ± 87.3	.07	.07
sCD14 level, pg/mL	2416.3 ± 531.0	2571.6 ± 483.6	2274.6 ± 536.0	.001	.003
IL-6 level, pg/mL	3.9 ± 3.3	4.6 ± 3.3	3.2 ± 3.2	.02	.04
IL-10 level, pg/mL	9.8 ± 6.9	11.0 ± 8.7	8.6 ± 4.6	.05	.04
TNF-α level, pg/mL	2.8 ± 7.7	3.8 ± 8.5	1.8 ± 6.9	.13	.14
I-FABP level, pg/mL (n = 131) ^c	673.2 ± 597.9	796.1 ± 734.5	559.4 ± 408.6	.03	.005

Data are mean ± SD. See Methods for definitions of T1-3.

Abbreviations: EndoCAb, antibody to endotoxin; EU, endotoxin units; I-FABP, intestinal fatty acid binding protein; IL-6, interleukin 6; IL-10, interleukin 10; LPS, lipopolysaccharide; MMU, immunoglobulin M median units; sCD14, soluble CD14; TNF-α, tumor necrosis factor α.

^a By the *t* test.

^b Adjusted for log human immunodeficiency virus RNA load.

^c One subject did not have data for 1 visit.

DISCUSSION

In this study, we demonstrated that levels of markers of macrophage activation (sCD14), intestinal mucosal integrity (I-FABP), and inflammation (IL-6) were higher in HIV/HCV-coinfected women during intervals when significant liver disease progression occurred, compared with intervals when minimal or no progression occurred. While LPS levels did not differ between progressors and nonprogressors, there was a nonsignificant trend toward lower EndoCAb levels ($P = .07$), which may suggest increased clearance of LPS from the circulation in liver disease progressors, compared with nonprogressors [23].

Monocyte activation, as evidenced by elevated sCD14 levels, was associated with the most consistent and significant difference between progressors and nonprogressors, and the sCD14 level was significantly higher in progressors even at the first visit studied, 5 years before the liver disease end point. In the models with adjustment for HIV RNA level and CD4⁺ T-cell

count, the relationship of the sCD14 level to liver disease progression remained highly significant ($P = .003$ and 0.002 , respectively), although it was slightly attenuated when excess alcohol use was added to the model ($P = .03$). Although other stimuli may activate monocytes, an elevation in the sCD14 level is usually caused by LPS binding, and sCD14 is considered a marker of LPS-induced inflammation [24, 25]. The relationship between monocyte activation and liver disease progression is supported by what is known about the pathogenesis of liver fibrosis and from previous work involving HCV-monoinfected and HCV/HIV-coinfected persons. Kupffer cells, the hepatic macrophages, when stimulated by LPS, secrete inflammatory cytokines such as interleukin 1, IL-6, and TNF-α, which in turn lead to the activation of stellate cells. When activated, stellate cells produce a matrix rich in collagen, which leads to hepatic fibrosis [9]. In a cross-sectional study comparing patients with hepatitis B virus (HBV) or HCV monoinfection, with or without fibrosis, to uninfected controls, Sandler et al

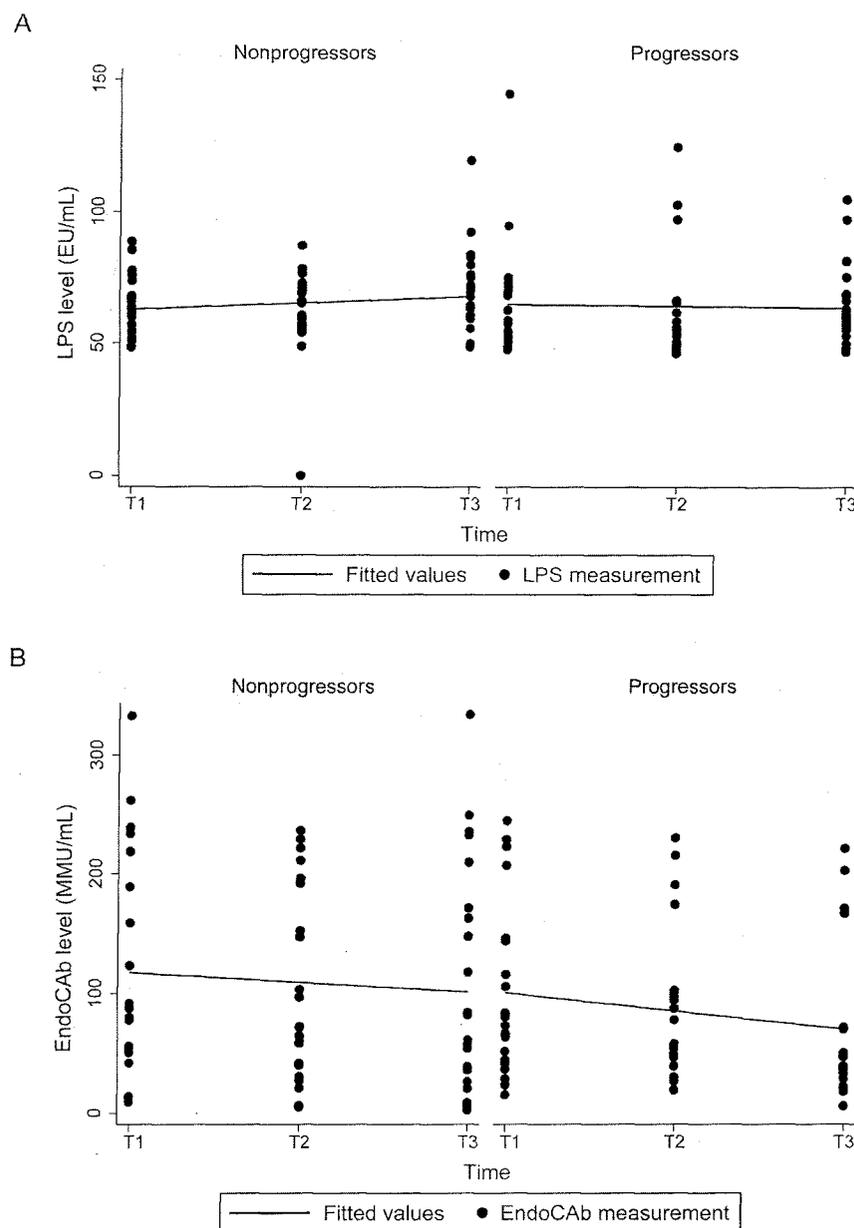


Figure 1. Plasma lipopolysaccharide (LPS) and endotoxin core antibody (EndoCAB) levels among liver disease progressors and nonprogressors over a 5-year period. *A*, There was no significant difference between progressors and nonprogressors in the LPS level in simple models ($P = .70$). There was also no significant difference in the slope, after adjustment for log human immunodeficiency virus (HIV) RNA level ($P = .60$) or $CD4^+$ T-cell count ($P = .63$). *B*, There was a trend toward lower EndoCAB levels at all time points ($P = .07$). The statistical significance of the difference was unchanged in a model with log HIV RNA level ($P = .07$) but was attenuated in a model with $CD4^+$ T-cell count ($P = .22$). There was no significant difference in slope. See Methods for definitions of T1–3. Abbreviations: EU, endotoxin units; MMU, immunoglobulin M median units.

found that, while the LPS level was higher in patients with viral hepatitis, the level did not correlate with liver disease severity. However, the sCD14 level was associated with cirrhosis and markers of hepatic inflammation. As in our study, the downstream effects of microbial translocation, monocyte activation, and IL-6 secretion were associated with liver disease

progression, but the LPS level itself was not. Of interest, Sandler et al found that a higher sCD14 level predicted nonresponse to hepatitis C therapy [25].

Our findings and those of Sandler et al contrast with results of a study by Balagopal et al, who found that a higher LPS level is associated with cirrhosis in HCV-infected persons with and

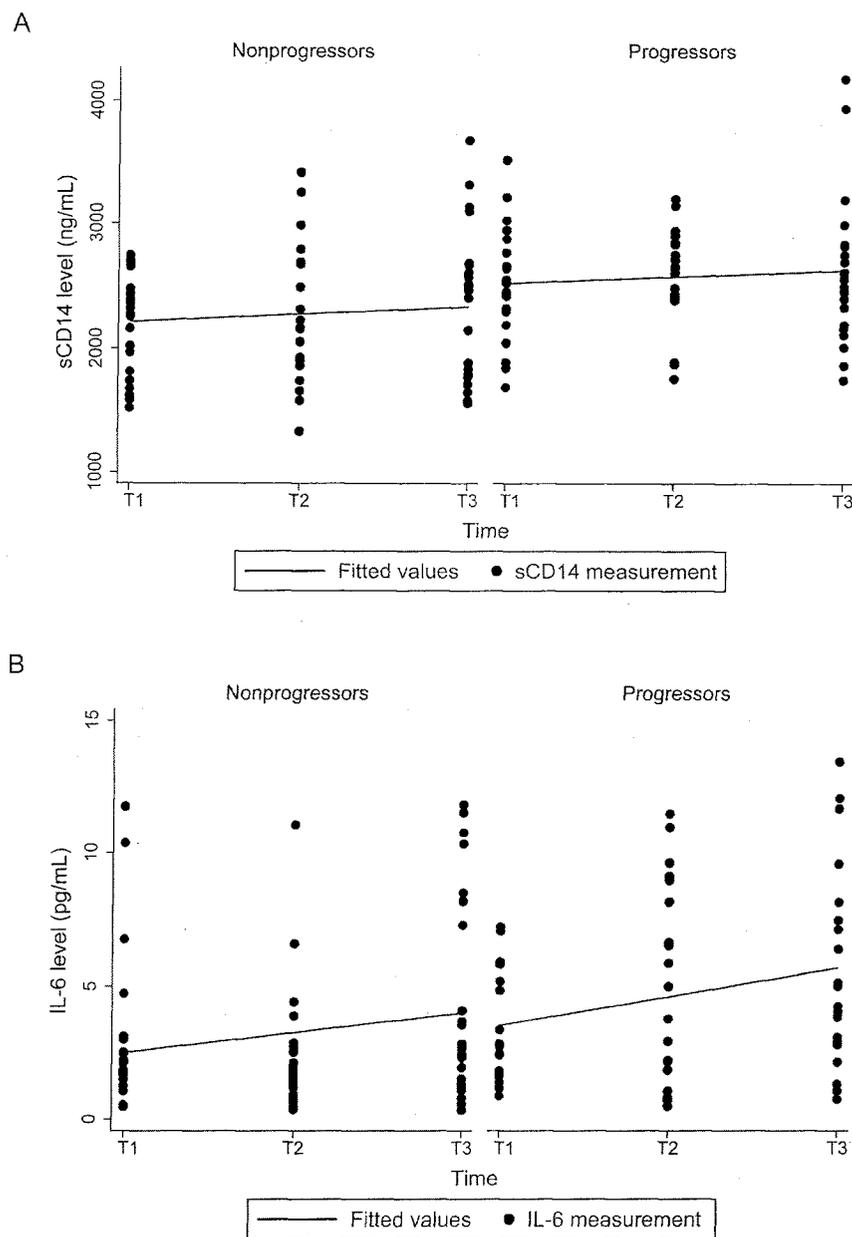


Figure 2. Comparison of soluble CD14 (sCD14) and interleukin 6 (IL-6) levels between liver disease progressors and nonprogressors. *A*, For sCD14, levels were significantly higher in progressors overall ($P = .001$; $P = .003$ after adjustment for log human immunodeficiency virus [HIV] RNA load; $P = .002$ after adjustment for $CD4^+$ T-cell count) and at T1 ($P = .03$). The association remained significant in a model that adjusted for log HIV RNA load and alcohol use ($P = .01$ over all time points). There was no difference in slope between progressors and nonprogressors after adjustment for log HIV RNA level ($P = .75$) or for log HIV RNA level and alcohol use ($P = .94$). *B*, For IL-6, levels were significantly higher in progressors for all visits ($P = .02$; $P = .04$ after adjustment for log HIV RNA load); adding alcohol use to the model attenuated the association to nonsignificance ($P = .10$). IL-6 levels increased significantly with time in progressors, with a slope of 1.1 ($P = .03$); the slope for nonprogressors was 0.74 ($P = .12$). However, when tested in the adjusted model with log HIV RNA load ($P = .80$) or in the adjusted model with log HIV RNA load and alcohol use ($P = .62$), the slopes did not differ. See Methods for definitions of T1–3.

those without HIV coinfection [13]. That the sCD14 level alone, rather than the LPS and sCD14 levels [25], is associated with liver disease progression in HIV/HCV-coinfected persons

may reflect the rapid clearance of circulating LPS in vivo, the lack of bioactive LPS production by some translocated bacteria (eg, gram-positive organisms), or the limitations of the Limulus

Current State of *Clostridium difficile* Treatment Options

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Recent reports of reduced response to standard therapies for *Clostridium difficile* infection (CDI) and the risk for recurrent CDI that is common with all currently available treatment agents have posed a significant challenge to clinicians. Current recommendations include metronidazole for treatment of mild to moderate CDI and vancomycin for severe CDI. Results from small clinical trials suggest that nitazoxanide and teicoplanin may be alternative options to standard therapies, whereas rifaximin has demonstrated success in uncontrolled trials for the management of multiple recurrences. Anecdotal reports have also suggested that tigecycline might be useful as an adjunctive agent for the treatment of severe complicated CDI. Reports of resistance will likely limit the clinical use of fusidic acid and bacitracin and, possibly, rifaximin if resistance to this agent becomes widespread. Treatment of patients with multiple CDI recurrences and those with severe complicated CDI is based on limited clinical evidence, and new treatments or strategies are needed.

Ten to 20 years ago, there was little interest in developing new treatment agents for *Clostridium difficile* infection (CDI) because CDI in most patients responded to metronidazole or vancomycin therapy, recurrent disease was common but more easily managed, and severe complicated cases of CDI were infrequent [1]. During the past decade, the epidemiology and clinical picture of CDI have changed dramatically and the limitations of current treatment options have become more apparent. Before the approval of fidaxomicin earlier this year, oral vancomycin was the only agent approved for the treatment of CDI in the United States by the Food and Drug Administration. Multiple dosing requirements, cost, relative efficacy, risk for recurrence, development of resistance, and adverse reactions limit other treatment options (Table 1). Here, we review the currently available CDI

treatment agents with emphasis on their limitations and the general approach to managing recurrent CDI and severe complicated CDI.

CURRENTLY AVAILABLE TREATMENT OPTIONS

Vancomycin

Oral vancomycin has remained a highly effective treatment agent for CDI and is the preferred comparison antibiotic for treatment trials of new therapeutic agents [2]. The agent is not absorbed, and the concentrations of vancomycin in milligrams per gram of feces that are achieved vastly exceed the minimum inhibitory concentration (MIC) for *C. difficile* by multiple folds [3]. Although vancomycin is highly effective for initial cure, a recurrence rate of 20% has been demonstrated repeatedly [2]. Perhaps for this reason, clinicians are often tempted to increase the dose of vancomycin or extend the length of treatment for the subsequent episode. Neither of these strategies has been tested, and with data showing high fecal concentrations of vancomycin achieved by the 125 mg dose [3], there is little justification for this approach. Vancomycin treatment delays recovery of the indigenous fecal microbiota [4],

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to this manifestation, a potential intervention might be the administration of monoclonal antibodies against toxins A and B [41] or the use of hyperimmune intravenous immunoglobulin if they become available.

In summary, treatment of CDI has relied primarily on metronidazole and vancomycin for the past 30 years. Although these and other agents will still have a role in treatment of patients with CDI, limitations of these agents have stimulated the development of newer therapies. It is hoped that the recently approved agent fidaxomicin and other agents that are still in development will improve the treatment of patients with CDI.

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References

1. Rolfe R, Finegold SM, eds. *Clostridium difficile*: its role in intestinal disease. San Diego, CA: Academic Press, 1988.
2. Louie TJ, Miller MA, Mullane KM, et al. OPT-80-003 Clinical Study Group. Fidaxomicin versus vancomycin for *Clostridium difficile* infection. *N Engl J Med* 2011; 364:422–31.
3. Johnson S, Homann SR, Bettin KM, et al. Treatment of asymptomatic *Clostridium difficile* carriers (fecal excretors) with vancomycin or metronidazole. A randomized, placebo-controlled trial. *Ann Intern Med* 1992; 117:297–302.
4. Tannock GW, Munro K, Taylor C, et al. A new macrocyclic antibiotic, fidaxomicin (OPT-80), causes less alteration to the bowel microbiota of *Clostridium difficile*-infected patients than does vancomycin. *Microbiology* 2010; 156:3354–59.
5. Johnson S. Recurrent *Clostridium difficile* infection: a review of risk factors, treatments, and outcomes. *J Infect* 2009; 58:403–10.
6. Recommendations for preventing the spread of vancomycin resistance. Recommendations of the Hospital Infection Control Practices Advisory Committee (HICPAC). *MMWR Recomm Rep* 1995; 44:1–13.
7. Wilcox MH, Howe R. Diarrhoea caused by *Clostridium difficile*: response time for treatment with metronidazole and vancomycin. *J Antimicrob Chemother* 1995; 36:673–9.
8. Zar FA, Bakkanagari SR, Moorthi KM, Davis MB. A comparison of vancomycin and metronidazole for the treatment of *Clostridium difficile*-associated diarrhea, stratified by disease severity. *Clin Infect Dis* 2007; 45:302–7.
9. Louie T, Gerson M, Grimmard D, et al. Results of a phase III trial comparing tolevamer, vancomycin, and metronidazole in patients with *Clostridium difficile*-associated diarrhea (CDAD). 47th Interscience Conference on Antimicrobial Agents and Chemotherapy, 17 September 2007. [Abstract L-3826]. In: Program and Abstracts of the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy (Chicago, IL). Washington, DC: American Society for Microbiology, 2007.
10. Bouza E, Dryden M, Mohammed R, et al. Results of a phase III trial comparing tolevamer, vancomycin and metronidazole in patients with *Clostridium difficile*-associated diarrhea [Session O-464]. In: 18th European Congress of Clinical Microbiology and Infectious Diseases; Barcelona, Spain; 19–22 April 2008.
11. Cohen SH, Gerding DN, Johnson S, et al. Society for Healthcare Epidemiology of America; Infectious Diseases Society of America. Clinical practice guidelines for *Clostridium difficile* infection in adults: 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). *Infect Control Hosp Epidemiol* 2010; 31:431–55.
12. Fujitani S, George WL, Murthy AR. Comparison of clinical severity score indices for *Clostridium difficile* infection. *Infect Control Hosp Epidemiol* 2011; 32:220–8.
13. Baines SD, O'Connor R, Freeman J, et al. Emergence of reduced susceptibility to metronidazole in *Clostridium difficile*. *J Antimicrob Chemother* 2008; 62:1046–52.
14. Fox LM, Saravolatz LD. Nitazoxanide: a new thiazolidine antiparasitic agent. *Clin Infect Dis* 2005; 40:1173–80.
15. Musher DM, Logan N, Hamill RJ, et al. Nitazoxanide for the treatment of *Clostridium difficile* colitis. *Clin Infect Dis* 2006; 43:421–7.
16. Musher DM, Logan N, Bressler AM, Johnson DP, Rossignol JF. Nitazoxanide versus vancomycin in *Clostridium difficile* infection: a randomized, double-blind study. *Clin Infect Dis* 2009; 48:e41–6.
17. Musher DM, Logan N, Mehendiratta V, Melgarejo NA, Garud S, Hamill RJ. *Clostridium difficile* colitis that fails conventional metronidazole therapy: response to nitazoxanide. *J Antimicrob Chemother* 2007; 59:705–10.
18. Curry SR, Marsh JW, Shutt KA, et al. High frequency of rifampin resistance identified in an epidemic *Clostridium difficile* clone from a large teaching hospital. *Clin Infect Dis* 2009; 48:425–9.
19. O'Connor JR, Galang MA, Sambol SP, et al. Rifampin and rifaximin resistance in clinical isolates of *Clostridium difficile*. *Antimicrob Agents Chemother* 2008; 52:2813–7.
20. Garey KW, Jiang ZD, Bellard A, Dupont HL. Rifaximin in treatment of recurrent *Clostridium difficile*-associated diarrhea: an uncontrolled pilot study. *J Clin Gastroenterol* 2009; 43:91–3.
21. Johnson S, Schriever C, Galang M, Kelly CP, Gerding DN. Interruption of recurrent *Clostridium difficile*-associated diarrhea episodes by serial therapy with vancomycin and rifaximin. *Clin Infect Dis* 2007; 44:846–8.
22. Johnson S, Schriever C, Patel U, Patel T, Hecht DW, Gerding DN. Rifaximin redux: treatment of recurrent *Clostridium difficile* infections with rifaximin immediately post-vancomycin treatment. *Anaerobe* 2009; 15:290–1.
23. Patrick Basu P, Dinani A, Rayapudi K, et al. Rifaximin therapy for metronidazole-unresponsive *Clostridium difficile* infection: a prospective pilot trial. *Ther Adv Gastroenterol* 2010; 3:221–5.
24. Stein GE, Craig WA. Tigecycline: a critical analysis. *Clin Infect Dis* 2006; 43:518–24.
25. Baines SD, Saxton K, Freeman J, Wilcox MH. Tigecycline does not induce proliferation or cytotoxin production by epidemic *Clostridium difficile* strains in a human gut model. *J Antimicrob Chemother* 2006; 58:1062–5.
26. Jump RL, Li Y, Pultz MJ, Kypriotakis G, Donskey CJ. Tigecycline exhibits inhibitory activity against *Clostridium difficile* in the colon of mice and does not promote growth or toxin production. *Antimicrob Agents Chemother* 2011; 55:546–9.
27. Cheong EY, Gottlieb T. Intravenous tigecycline in the treatment of severe recurrent *Clostridium difficile* colitis. *Med J Aust* 2011; 194:374–5.
28. Herpers BL, Vlamincx B, Burkhardt O, et al. Intravenous tigecycline as adjunctive or alternative therapy for severe refractory *Clostridium difficile* infection. *Clin Infect Dis* 2009; 48:1732–5.