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Systemic Inflammatory Responses in Ulcerative Colitis Patients and *Clostridium difficile* Infection

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Abstract

Background/Aims—Finding differences in systemic inflammatory response in ulcerative colitis (UC), UC with *Clostridium difficile* infection (CDI), and CDI could lead to a better ability to differentiate between UC with symptomatic CDI and UC with *C. difficile* colonization, and could identify specific inflammatory pathways for UC or CDI, which could be therapeutic targets.

Methods—We prospectively collected sera from symptomatic UC patients whose stools were tested for toxigenic *C. difficile*, and from CDI patients who did not have UC (CDI-noUC). The UC patients with positive tests (UC-CDI) were further categorized into responders to CDI treatment (UC-CDI-R) and non-responders (UC-CDI-NR). We compared serum inflammatory mediators among groups using unadjusted and adjusted multivariable statistics.

Results—We included 117 UC [27 UC-CDI, 90 UC without CDI (UC-noCDI)] and 16 CDI-noUC patients. Principal component analysis (PCA) did not reveal significant differences either between UC-CDI and UC-noCDI groups, or between UC-CDI-R and UC-CDI-NR groups. In contrast, the PCA showed significant separation between the UC and CDI-noUC groups ($P=0.002$). In these two groups, hepatocyte growth factor (HGF) and chemokine (C-C motif) ligand 2 (CCL2) levels were significantly lower and IL-23 levels were higher in UC patients in multivariable analyses. The model to distinguish UC from CDI including IL-23, HGF, CCL2, age, gender, and HGB had an AuROC of 0.93.

Conclusion—Inflammatory profiles could not distinguish UC-CDI from UC-noCDI, and UC-CDI-R from UC-CDI-NR. However, the UC and CDI-noUC groups were significantly different.

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Author's contribution PDRH and KR were involved in study concept and design; JL, LJ, AR, and EB acquired data; JL, KR, and PDRH analyzed and interpreted the data; JL and KR drafted the manuscript; RWS, SMG, AKW, AR, and PDRH critically revised the manuscript for important intellectual content; PDRH was involved in study supervision.

Compliance with ethical standards

Conflict of interest All authors report no conflicts.

Future work should examine whether therapeutic agents inhibiting IL-23 or stimulating HGF can treat UC.

Keywords

Ulcerative colitis; *Clostridium difficile*; Cytokine; Chemokine; Growth factor; Inflammatory biomarker

Introduction

There has been lack of published data related to the systemic inflammatory response in *Clostridium difficile* infection (CDI) in patients with ulcerative colitis (UC). Understanding these responses may have clinical benefit. First, in clinically active UC patients with positive testing for toxigenic *C. difficile*, the pattern of inflammatory changes could help guide treatment. It could also help to differentiate *C. difficile* carriers, whose main treatment is escalation of immunosuppressive agents, from the patients with active CDI, whose main treatment is antibiotics. The current diagnostic tests to detect toxigenic *C. difficile* in stool cannot differentiate between true CDI and *C. difficile* colonization [1].

Second, understanding the changes in circulating inflammatory mediators that occur in UC can lead to development of therapeutic agents that target specific inflammatory pathways [2]. Most previous studies of inflammatory markers in UC compared UC patients to healthy controls [3]. Comparing the inflammatory response in UC to CDI, one of the most common causes of infectious colitis, may highlight specific inflammatory pathways for UC as therapeutic targets.

This study aimed to compare inflammatory mediators (cytokines, chemokines, and growth factors) in the sera of the following cohorts: UC patients with positive stool testing for toxigenic *C. difficile*, UC patients with negative stool testing for toxigenic *C. difficile*, and patients with symptomatic CDI, but no UC. In UC patients with CDI, we also compared systemic inflammatory responses of those who responded to CDI therapy, whom we assumed to be the true CDI cases, to those who failed to respond to CDI therapy, whom we assumed to be the *C. difficile* carriers.

Methods

Participants

This is a prospective, observational, single-center study at the University of Michigan Health System. We prospectively collected sera from symptomatic adults with UC (age ≥ 18) whose stools were tested for toxigenic *C. difficile* in our center from July 2013 to August 2016. The diagnosis of CDI was based on the testing algorithm in our center as shown in Fig. 1. The patients were classified into two groups: those with positive *C. difficile* testing (UC-CDI) and those with negative *C. difficile* testing (UC-noCDI). The patients in the UC-CDI group were further categorized into those who responded (UC-CDI-R), and those who failed to respond (UC-CDI-NR) to CDI treatment. Patients with no need for escalation of immunosuppressive agents after complete CDI treatment were considered UC-CDI-R. Patients who needed escalation of any immunosuppressive agents (corticosteroids,

immunomodulators, and biological agents) after treatment with antibiotics for at least three days were considered UC-CDI-NR. If patients started both CDI treatment and escalation of any immunosuppressive agents within a three-day interval, the response to CDI treatment was undetermined. Another group of patients who had stool tests positive for CDI, but did not have UC were enrolled as a separate comparator group (CDI-noUC). The consort flow diagram of the study is shown in Fig. 2. We excluded UC patients status-post colectomy and patients who had other concomitant infections.

Demographic information, phenotype of ulcerative colitis, medication use at the time of serum collection, response to CDI treatment, need for escalation of immunosuppressive agents, need for surgery, and conventional laboratory tests including complete blood count, renal function, and liver biochemistries were manually collected from electronic medical records (EPIC Systems, Verona, WI).

Sera and Measurement of Inflammatory Mediators

The sera were obtained within 48 h of collection of the stool samples. All samples were stored at -80°C until used for this study. Measurement of inflammatory mediators was done using a custom, multiplex, polystyrene bead-based assay (R&D Systems, Inc., Minneapolis, MN). A Luminex[®] 200 dual laser detection system was used to analyze samples/ standards. Inflammatory mediators which were potentially related to CDI including cytokines, chemokines, and growth factors, were measured [4]. A list of these mediators and their standard abbreviations are provided in Table 1, selected based on significant associations with CDI seen in prior studies [4–14].

Statistical Methods

All data were analyzed using SAS 9.4 (SAS Institute Inc., North Carolina, US) and R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). A two-tailed P value of < 0.05 was considered significant for all analyses. The continuous data were reported in mean and standard deviation (SD) if normally distributed, and in median and range if not normally distributed. Proportional data were reported in percentage. To compare the continuous data among UC-CDI, UC-noCDI, and CDI-noUC groups, we used one-way ANOVA if the data were symmetrically distributed, and the Kruskal–Wallis test if the data were not symmetrically distributed. With some inflammatory mediators, log transformation was employed if not normally distributed. If there were any significant differences among these three groups, further analyses to determine the differences between each pair were performed; ANOVA contrasts and an independent t test were used for the symmetrically distributed data, and the Wilcoxon Rank Sum test was used for the non-symmetrically distributed data. Comparisons of proportions were performed using the Chi-square test or Fisher's exact test.

Logistic regression analysis was performed to find the independent parameters that helped to differentiate between UC-CDI versus (vs.) UC-noCDI, UC-CDI-R versus UC-CDI-NR, and UC (pooled UC-CDI and UC-noCDI) versus CDI-noUC. The parameters with significant correlations with other parameters (Pearson correlation coefficient, $r \geq 0.5$) were identified by construction of a correlation matrix and were selectively excluded from analyses. The

predictive models including the non-correlated, significant parameters based on logistic regression results were built. The backward selection method was used to select predictors in the final multivariable model. The area under the receiver operator characteristic (AuROC) curve of each model was reported.

For exploratory analysis, principal component analysis (PCA) via the R package *vegan* version 2.4–6 [15] was used to identify the combinations of inflammatory mediators that best explained the variation seen in the dataset. The values of each patient's score for component 1 and component 2 were then placed on a two-dimensional plot, and the centroids for each group were constructed and compared using a permutational multivariate ANOVA (MANOVA).

Since our dataset included a large number of putative predictor variables (i.e. inflammatory mediators), we deployed a technique to address this: elastic net regression. This method uses a penalty for each additional predictor in the model to reduce the chance of overfitting when constructing adjusted logit models. Models are built in a stepwise fashion utilizing cross-validation for the point estimates and errors. Our aim in using elastic net regression was to identify potentially important mediators not revealed through conventional logit models. This was implemented via the R package *glmnet* version 2.0–5 [16], and the optimal model was selected using 10-fold cross-validation with the minimal penalty term (i.e. λ). Where applicable, a λ penalty term one standard error away from the minimum was also used to select variables for inclusion in the model, and the two models were compared.

Results

Initially, blood samples from 151 patients were collected. Eighteen patients were excluded due to presence of other co-infections. In total, 133 patients were included for analysis (27 UC-CDI, 90 UC-noCDI, and 16 CDI-noUC). Baseline characteristics, types of UC, medication use, conventional laboratory tests, and inflammatory mediators of the three groups are shown in Table 2. The patients in UC-CDI and UC-noCDI groups had comparable age, sex, UC types, medication use, and severity of disease assessed by C-reactive protein (CRP), hemoglobin (HGB), and albumin levels. The patients in CDI-noUC group were older and had lower HGB and albumin levels. In the UC-CDI group, 14 patients were UC-CDI-R, eight patients were UC-CDI-NR, and five patients were undetermined.

Comparison of Inflammatory Mediators in UC-CDI, UC-noCDI, and CDI-noUC Groups

As in Table 2, the inflammatory profiles in the UC-CDI group were quite similar to the UC-noCDI group, but significantly different from CDI-noUC group. Significant differences among three groups were observed in platelets, interleukin(IL)-6, IL-15, IL-23, chemokine (C-C motif) ligand 2 (CCL2), CCL4, chemokine (C-X-C motif) ligand 9 (CXCL9), and hepatocyte growth factor (HGF). Further analyses showed that most of the differences were among UC-CDI versus CDI-noUC, and UC-noCDI versus CDI-noUC. When comparing between the UC-CDI group and the UC-noCDI group, CCL4 was the only mediator that was found to be significantly different (medians 292.51 and 412.58; ranges 216.34–527.09 and 200.09–1371 picogram/ mL, respectively).

Comparison of Inflammatory Mediators in UC-CDI and UC-noCDI

As mentioned above, most of the inflammatory mediator levels were not significantly different between these two groups. Only CCL4 levels were significantly different. The CCL4 levels were lower in the UC-CDI group compared to the UC-noCDI group, and it retained its significance in multivariable analysis after adjusting for age, gender, and HGB levels. However, the model to differentiate UC-CDI from UC-noCDI including CCL4, age, gender, and HGB had an AuROC of only 0.68. Furthermore, PCA and MANOVA did not reveal any difference in centroids between groups as shown in Fig. 3a ($P = 0.508$). Elastic net regression with model selection minimizing λ also identified log (CCL4) as potentially inversely associated with UC-CDI (odds ratio [OR] 0.43 and AuROC 0.67); however, the nature of this regression technique currently precludes construction of reliable confidence intervals or significance testing.

Comparison of Inflammatory Mediators in UC-CDI-R and UC-CDI-NR

We excluded the five patients in whom we could not determine the response to CDI therapy. Fourteen UC-CDI-R patients and eight UC-CDI-NR patients were included in this analysis. There were no significant differences in age and gender between these two groups. The patients who responded to CDI treatment had significantly higher levels of TNF- α and IL-4 than those who failed to respond to CDI treatment in bivariate analyses. IL-15 trended higher, but not statistically significantly, in the UC-CDI-R group. Due to a limited number of cases, multivariate analysis could not be performed. The details are in Table 1S in supplementary documents. PCA and MANOVA did not reveal any difference in centroids between groups as shown in Fig. 3b ($P = 0.515$). Elastic net regression with model selection minimizing lambda did not retain any predictors in the model. That is, $\log(\lambda)$ simply decreased and the curve was not parabolic, thus not allowing for selection of a minimum.

Comparison of Inflammatory Mediators in Patients with UC and Patients with CDI, but Not UC

Due to similar inflammatory profiles between UC-CDI and UC-noCDI, we pooled these two groups together (UC) and compared their profiles to the patients in the CDI-noUC group. In bivariable analyses, albumin levels, HGB, platelets, IL-6, IL-15, IL-23, CCL2, CXCL9, and HGF were significantly different between the UC and CDI-noUC groups as shown in Table 3. A correlation matrix showed significant associations with $r +0.5$ among IL-6 and IL-15, and HGB and albumin levels. IL-6 and HGB were selectively included in multivariable analysis, because the model performance when including these variables was superior to the model comprised by the others. In multivariable analyses, HGF ($P = 0.003$), IL-23 ($P = 0.03$), and CCL2 ($P = 0.04$) remained significantly different between UC patients and CDI-noUC patients after adjusting for age, gender, and HGB levels. The model to differentiate UC patients from CDI-noUC patients including HGF, IL-23, CCL2, age, gender, and HGB had an AuROC curve of 0.93. Furthermore, the model including only HGF, IL-23, and CCL2 provided an AuROC of 0.815 (Figure 1S in Supplementary documents). PCA and MANOVA revealed a significant difference in the centroids between groups ($P = 0.001$) (Fig. 4a). Along the primary axis of separation (Component 1), the following mediators were revealed by the PCA biplot to be driving the separation between UC and CDI-noUC: HGF,

IL-6, IL-15, IL-23, and CXCL5 (Fig. 4b). Finally, elastic net regression with the minimum λ penalty term resulted in a model that included the log-transformed variables IL-8 (OR for CDI-noUC 0.58), CCL2 (OR 1.19), CCL4 (OR 45.3), CXCL9 (OR 0.87), HGF (OR 2.98), and IL-15 (OR 1.003), with an AuROC of 0.95. An elastic net model restricted to just CCL4 (OR 7.34) and HGF (OR 1.55), which corresponded to a λ penalty term one standard error from the minimum, retained most of this performance with an AuROC of 0.93.

Discussion

Our study characterizes the systemic inflammatory response to CDI in patients with UC and without UC. We found that the inflammatory profiles were not obviously different between the patients with UC who had CDI and who did not have CDI. Only serum CCL4 was an independently significant predictor of UC with CDI. There are some significant differences when we compared UC patients with CDI who responded versus those who failed to respond to antibiotics for CDI, but due to the limited number of patients, the significance could not be evaluated in an adjusted, multivariable model. However, the inflammatory responses were clearly different when comparing UC patients and CDI patients who did not have UC, with higher IL-23 and lower HGF and CCL2 levels in UC patients. These specific responses in UC, but not in CDI, may provide a better understanding of the inflammatory processes underlying UC, and could be evidence to support the use of specific therapeutic agents in UC patients, such as monoclonal antibodies, or agents to increase HGF-driven mucosal healing.

Diagnosis of bacterial infections in patients with idiopathic inflammatory disorders is usually difficult because these two conditions share many clinical features. This includes the diagnosis of *C. difficile* infection in ulcerative colitis patients. The current standard tests for diagnosis of CDI can only detect toxigenic *C. difficile* in stool, but cannot distinguish between a true infection, in which the detected *C. difficile* contributes to symptoms and the inflammatory process, and colonization with *C. difficile* during an IBD flare. In addition, understanding how the host reacts to *C. difficile* infection and UC may guide future treatments or predictive models for adverse outcomes.

Our results, however, did not reveal clear differences in inflammatory profiles between UC patients who had positive testing for toxigenic *C. difficile* and those with negative tests. When adjusting for age, gender, and hemoglobin levels, only CCL4 was significant in the multivariable analysis. However, the AuROC was only 0.68. Therefore, CCL4 is not a sufficiently accurate parameter to discriminate between these two groups in clinical settings. This suggests that the inflammatory response in UC flares and in UC with CDI is quite similar, and appears to be more similar to a UC flare than a typical CDI in a patient without ulcerative colitis. This suggests that treatment for UC with CDI should not be very dissimilar from treatment for a flare of UC.

However, the negative results in differentiating between UC and UC with CDI could be due to some cases where the detected toxigenic *C. difficile* was only a bystander. Therefore, we did further analyses focusing on the differences between UC-CDI-R and UC-CDI-NR, hypothesizing that the UC-CDI-R patients represented cases of true, symptomatic infection.

Our results showed that TNF- α and IL-4 were significantly higher in the UC-CDI-R group in unadjusted analyses. In UC patients, it is well known that TNF- α has pro-inflammatory effects; the serum and tissue levels of TNF- α were reported to be elevated in UC patients [17, 18]. In contrast, IL-4 reportedly had an anti-inflammatory effects; its production by isolated lamina propria cells from UC patients is less than the production in controls [19]. In CDI, plasma TNF- α is also increased, and the level seems to be associated with poor prognosis [20]. Therefore, it seems reasonable that the levels in the CDI-R group of these two inflammatory mediators with previously established pathologic mechanisms associated with true *C. difficile* infection were higher than the levels in the CDI-NR group. IL-4 potentially plays a role in CDI susceptibility in IBD patients. A recent study by Connelly et al. reported the strong association between the IL-4-associated single-nucleotide polymorphisms rs2243250 and the development of CDI in IBD patients [8]. They hypothesized that abnormal IL-4 expression in gut epithelium could increase risk of CDI due to impaired tight junctions which allows gut luminal *C. difficile* to cross the epithelial barrier. However, how this SNP affects serum IL-4 levels has not been studied.

Clear differences in inflammatory profiles were observed between the patients with UC and the patients with CDI, but not UC. Hepatocyte growth factor, CCL2, and IL-23 were the significant mediators in multivariable analyses, with HGF and CCL2 higher in CDI and IL-23 higher in UC. Hepatocyte growth factor is an angiogenesis-promoting cytokine that may promote inflammation by increasing vascular permeability and facilitate intestinal mucosal healing [21, 22]. The previous data relating to the association of serum HGF levels in UC and CDI are limited and inconclusive. The serum HGF level has been reported to be significantly higher in UC patients compared to controls who had functional abdominal pain, and its level was correlated directly with disease activity [22]. However, another study did not reveal statistically significant differences of plasma HGF levels between UC patients and healthy controls [23]. HGF was reportedly markedly elevated in patients with CDI [4]. In our study, the level in patients with CDI was significantly higher than in those with UC. These results may be explained by the role that HGF has in acute inflammation, acting as an acute phase protein. Sorour et al. reported using a stool test to evaluate HGF as a local acute phase response marker in stool that could distinguish infectious gastroenteritis from chronic, non-infectious inflammatory causes of diarrhea (positive test in 87.9 vs. 10%, respectively) [24]. One could speculate that HGF is a response to intestinal inflammation UC, but that this response may be inadequate to produce mucosal healing. It is not clear whether additional HGF might improve mucosal healing in UC. The level of CCL2, a potent chemoattractant of monocytes, has been reported to be elevated in both colonic mucosal tissues and serum in patients with ulcerative colitis [25]. This chemokine has also been reported to be up-regulated in human intestinal epithelial cancer cell line in response to *C. difficile* toxin [26]. Serum CCL2 level significantly elevated in inpatients with CDI compared to outpatient controls [4]. However, no study has been done to compare the levels between ulcerative colitis and CDI.

The IL-23 levels in UC patients were significantly higher than in CDI patients in our study. IL-23 and Th17 cells constitute one of the important inflammatory pathways in IBD. When IL-23 interacts with its receptor on the Th17 cell surface, the JAK-STAT signaling pathway is activated, which induces gene expression patterns mediating intestinal inflammation [2].

Previous studies showed that both tissue and serum levels of IL-23 were elevated in UC patients compared to healthy controls, and there was a positive correlation between the serum level of IL-23 and the severity and duration of disease [27–29]. Furthermore, an IL-23 receptor mutation is associated with ulcerative colitis in two recent meta-analyses [30, 31]. Our study demonstrated that despite UC appearing clinically similar to *C. difficile* colitis, the serum IL-23 levels in UC patients were significantly higher. These results emphasized the potentially important role of IL-23 in UC pathogenesis. Therefore, ustekinumab, an agent targeting the IL-23/Th17 pathway, which has been studied and approved to treat patients with Crohn's disease, could be effective in UC patients as well. Future studies to establish its benefit and those of other anti-IL23 therapies are warranted.

The present study has limitations. First, we did not try to match the patients by age or gender or severity across groups. However, age, gender, type of UC, medications use, hemoglobin and albumin levels were not different between the patients in the UC-CDI and UC-noCDI groups and we did adjust for these covariates when possible. Nonetheless, the patients in the CDI-noUC group were older and had lower hemoglobin and albumin levels, which indirectly suggests that they may have been sicker. This could have affected their inflammatory profiles. However, we do not believe this would reduce the significance of the IL-23 analysis in our study, as more severe inflammation should have produced a higher pro-inflammatory cytokine level, but the IL-23 levels in the patients with CDI, who appeared to have more severe inflammation, were lower than in the patients with UC. Second, some of the immunosuppressive agents used as therapies may have altered some of the inflammatory mediator serum levels. For example, the serum TNF- α level may be decreased by anti-TNFs, and about 70% of UC patients in our cohort were receiving anti-TNFs when their blood samples were collected. However, this may accentuate the importance of IL-23, since the UC patients in our cohort were symptomatic despite taking their UC medications. High levels of IL-23 in these patients may suggest that their inflammation may be driven more by this IL-23/Th17 pathway, and the agents targeting this pathway may have clinical benefit. Third, there have been no standard criteria in categorizing UC with a true CDI and UC with colonized *C. difficile*. We categorized our UC patients with positive *C. difficile* toxin to UC-CDI-NR and UC-CDI-R, respectively, based on clinical practice [32]. UC-CDI-NR may reflect patients with UC flare and coincidental *C. difficile* colonization who did not respond to antibiotics, but with current diagnostic methods it is impossible to confirm.

Conclusion

Although our study failed to show significant differences in inflammatory mediator patterns between patients with only UC and those with both UC and CDI, we did observe significant differences in inflammatory profiles between UC patients and CDI patients. The significantly higher levels of IL-23 observed in our UC cohort reinforce the important of the IL-23/Th17 pathway in UC and suggest that future studies that characterize the benefit of agents targeting this pathway in UC patients should be pursued.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Testing algorithm for *Clostridium difficile* infection. *CDI* *clostridium difficile* infection, *EIA* enzyme immunoassay, *GDH* glutamate dehydrogenase, *PCR* polymerase chain reaction. Adapted from Rao et al. [4]

Fig. 2. Consort flow diagram of the study. *UC* ulcerative colitis, *CDI* *clostridium difficile* infection, *UC-CDI-R* UC patients with CDI and responded to CDI therapy, *UC-CDI-NR* UC patients with CDI but failed to responded to CDI therapy

Fig. 3.

Global systemic inflammatory responses in the ulcerative colitis (UC) patients who had *Clostridium difficile* infection (CDI) and who did not have CDI. **a** Principal component analysis (PCA) results are shown for UC patients with CDI and UC patients without CDI. **b** PCA results are shown for UC patients with CDI who responded to CDI treatment and who did not respond to CDI treatment. The PCA centroids were not significantly different by permutational MANOVA testing in both graphs (**a** $P = 0.508$, **b** $P = 0.515$)

Fig. 4. Global systemic inflammatory responses in the all ulcerative colitis (UC) patients and patients with *Clostridium difficile* infection (CDI) who did not have UC. **a** Principal component analysis (PCA) results are shown for UC patients (both with CDI and without CDI) and CDI patients without UC. The PCA centroids were significantly different by permutational MANOVA ($P=0.001$). **b** A PCA biplot reveals that specific mediators were responsible for separation of the two groups along the primary axis

Table 1

Sixteen inflammatory mediators (cytokines, chemokines, and growth factors) measured in the sera of study subjects and prior studies supporting their evaluation in the present study

Inflammatory mediator	Alternative name(s)/abbreviation(s)	Prior studies in CDI/UC
Tumor necrosis factor-alpha (TNF- α)		Olsen et al. [5], Brito et al. [6] Klapproth et al. [7]
Interleukin-2 receptor α (IL-2R α)	CD25	Rao et al. [4]
Interleukin-4 (IL-4)		Connelly et al. [8]
Interleukin-6 (IL-6)		Rao et al. [4]
Interleukin-8 (IL-8)	Neutrophil chemotactic factor	Rao et al. [4], Steiner et al. [9], Jiang et al. [10]
Interleukin-15 (IL-15)		Rao et al. [4]
Interleukin-22 (IL-22)		Sadighi et al. [11]
Interleukin-23 (IL-23)		Cowardin et al. [12], Buonomo et al. [13]
Chemokine (C-C motif) ligand 2 (CCL2)	Monocyte chemotactic protein-1 (MCP-1) or small inducible cytokine A2 (SCYA2)	Rao et al. [4]
CCL5	RANTES	Rao et al. [4]
Chemokine (C-C motif) ligand 4 (CCL4)	Macrophage inflammatory protein-1beta (MIP-1b)	Rao et al. [4]
Chemokine (C-X-C motif) ligand 5 (CXCL5)		El Feghaly et al. [14]
Chemokine (C-X-C motif) ligand 9 (CXCL9)	Monokine induced by gamma interferon (MIG)	Rao et al. [4]
Hepatocyte growth factor (HGF)		Rao et al. [4]
Epidermal growth factor (EGF)		Rao et al. [4]
Chemokine (C-X-C motif) ligand 10 (CXCL10)	Interferon gamma-induced protein 10 (IP-10) or small inducible cytokine B10 (SCYB10)	Rao et al. [4]

Baseline characteristics, types of UC, medication use, conventional laboratory values, and inflammatory mediators of patients in UC-CDI, UC-noCDI, and CDI-noUC groups

	Group 1, UC with CDI (n = 27)	Group 2, UC, no CDI (n = 90)	Group 3, CDI (n = 16)	P (across groups)	P (Group 1 vs. 2)	P (Group 1 vs. 3)	P (Group 2 vs. 3)
Age (years)	46.34 ± 21.30	42.10 ± 17.25	60.76 ± 18.78	< 0.01	0.29	0.01	< 0.01
Male (%)	13 (48.15%)	44 (48.89%)	7 (43.75%)	0.93			
UC type (E1:E2:E3)	0%:33.3%:66.7%	2.3%:26.4%:71.3%		0.60			
Immunomodulators	8 (29.63%)	24 (26.97%)		0.79			
Biologics	17 (62.96%)	63 (70.79%)		0.44			
Albumin (g/dL)	3.85 ± 0.52	3.97 ± 0.61	3.46 ± 0.49	0.02	0.35	0.07	< 0.01
CRP (mg/dL)	0.9 (0.05–15.4)	1.2 (0.1–22.6)		0.92			
Hemoglobin (g/dL)	12.5 ± 1.67	12.5 ± 2.14	9.86 ± 2.34	< 0.01	0.44	< 0.01	< 0.01
WBC (10 ³ cells/μL)	9.03 ± 5.51	9.49 ± 3.13	10.98 ± 7.37	0.36			
Neutrophil count (10 ³ cells/μL)	6.06 ± 4.52	6.59 ± 3.45	6.41 ± 4.56	0.84			
Platelet (10 ³ cells/μL)	302.63 ± 93.63	352.3 ± 135.4	252.3 ± 147.9	0.01	0.08	0.22	< 0.01
TNF-α	31.09 ± 5.81	33.2 ± 9.5	32.0 ± 10.1	0.54			
IL-2Ra	2136.04 ± 1131.22	2027.01 ± 1016.80	2192.93 ± 952.27	0.78			
IL-4	127.28 ± 27.68	142.09 ± 31.66	145.31 ± 37.72	0.08			
IL-6	7.77 (4.94–233.20)	9.55 (3.42–62.93)	22.30 (6.61–106.22)	< 0.01	0.34	< 0.01	< 0.01
IL-8	41.13 (16.25–167.38)	38.59 (10.02–681.44)	28.71 (3.71–724.96)	0.31			
IL-15	2.78 (0.8–15.75)	3.56 (0.80–25.26)	7.41 (1.02–20.29)	< 0.01	0.20	< 0.01	< 0.01
IL-22	568.08 ± 119.36	558.84 ± 120.66	538.78 ± 204.13	0.78			
IL-23	49.19 (20.52–329.82)	43.63 (12.43–295.40)	32.71 (12.43–93.38)	0.03	0.18	0.03	0.03
CCL2	307.38 (118.28–588.34)	256.19 (75.95–1028)	382.21 (137.5–838.15)	0.02	0.18	0.07	< 0.01
CCL5	13,131.97 ± 4270.78	12,711.74 ± 2876.19	12,801.69 ± 4325.08	0.86			
CCL4	292.51 (216.34–527.09)	412.58 (200.09–1371)	456.06 (150.36–548.06)	0.01	< 0.01	0.01	0.48
CXCL5	1338 (240.52–21,335)	1748 (236.28–18,948)	1282 (161.34–6018)	0.20			
CXCL9	322.34 (101.89–659.32)	326.29 (82.66–1130)	249.48 (82.66–854.08)	< 0.01	0.99	< 0.01	< 0.01
HGF	320.79 (120.10–1350)	347.55 (78.68–2105)	771.85 (92.94–4363)	0.01	0.64	0.01	< 0.01
EGF	110.49 (5.22–327.68)	130.08 (11.63–615.4)	99.64 (5.22–260.17)	0.28			
CXCL10	11.75 (3.83–42.51)	10.85 (3.89–168.74)	12.62 (3.92–173.64)	0.86			

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All inflammatory mediator units are picogram/mL if not otherwise specified. Bold letters indicate significant values ($P < 0.05$)

UC ulcerative colitis, *CDI* *Clostridium difficile* infection, *CRPC*-reactive protein, *WBC* white blood cell count, *TNF* tumor necrosis factor, *IL* interleukin, *CCL* chemokine (C-C motif) ligand, *CXCL* chemokine (C-X-C motif) ligand, *HGF* hepatocyte growth factor, *EGF* epidermal growth factor

Table 3

Baseline characteristics, conventional laboratory values, and inflammatory mediators of the patients with UC and CDI without UC

	UC (<i>n</i> = 117)	CDI (<i>n</i> = 16)	<i>P</i> value
Age (years)	43.08 ± 18.25	60.76 ± 18.78	< 0.01
Male, <i>n</i> (%)	57 (48.72%)	7 (43.75%)	0.71
Albumin (g/dL)	3.94 ± 0.59	3.46 ± 0.49	0.01
Hemoglobin (g/dL)	12.41 ± 2.04	9.86 ± 2.34	< 0.01
WBC (10 ³ cells/μL)	9.38 ± 3.81	10.98 ± 7.37	0.41
Neutrophil count (10 ³ cells/μL)	6.48 ± 3.69	6.41 ± 4.56	0.95
Platelet (10 ³ cells/μL)	340.43 ± 128.07	252.31 ± 147.94	0.01
TNF-α	32.71 ± 8.82	31.99 ± 10.09	0.76
IL-2Rα	2052.17 ± 1040.29	2192.93 ± 952.27	0.61
IL-4	138.67 ± 31.30	145.31 ± 37.72	0.44
IL-6	8.68 (3.42–233.20)	22.30 (6.61–106.22)	< 0.01
IL-8	39.75 (10.02–681.44)	28.71 (3.71–724.96)	0.16
IL-15	3.12 (0.80–25.26)	7.41 (1.02–20.29)	< 0.01
IL-22	560.98 ± 119.91	538.78 ± 204.13	0.68
IL-23	43.63 (12.43–329.82)	32.7 (12.43–93.38)	0.02
CCL2	265.88 (75.95–1028)	382.21 (137.50–838.15)	0.01
CCL5	12,806.8 ± 3227.5	12,801.7 ± 4325.1	0.99
CCL4	361.99 (200.09–1371)	456.06 (150.36–548.06)	0.21
CXCL5	1639 (236.28–21,335)	1282 (161.34–6018)	0.20
CXCL9	325.72 (82.66–1130)	249.48 (82.66–854.08)	< 0.01
HGF	336.84 (78.68–2105)	771.85 (92.94–4363)	< 0.01
EGF	127.73 (5.22–615.40)	99.64 (5.22–260.17)	0.24
CXCL10	10.91 (3.83–168.74)	12.62 (3.92–173.64)	0.60

All inflammatory mediator units are picogram/mL if not otherwise specified. Bold letters indicate significant value (*P* < 0.05)

UC ulcerative colitis, CDI *Clostridium difficile* infection, CRPC-reactive protein, WBC white blood cell count, TNF tumor necrosis factor, IL interleukin, CCL chemokine (C-C motif) ligand, CXCL chemokine (C-X-C motif) ligand, HGF hepatocyte growth factor, EGF epidermal growth factor