The Prognostic Value of Inflammatory Markers in *Clostridium difficile*-associated Diarrhea

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ABSTRACT: **Background:** The incidence of *Clostridium difficile*-associated diarrhea (CDAD) is increasing and is associated with significant morbidity and mortality. Therefore, there is a need to find new tools to determine the severity of the disease.

Objectives: To investigate the prognostic values of inflammatory markers such as mean platelet volume (MPV), neutrophil-lymphocyte ratio (NLR), and C-reactive protein (CRP) in patients with CDAD.

Methods: The study comprised of 100 patients diagnosed with CDAD. The study included an additional control group of 69 patients with diarrhea who were negative for *C. difficile* toxin. The control group was age- and sex-matched and hospitalized at the same time period. NLR and MPV were obtained from complete blood count results. Serum CRP levels were measured by the latex particle enhanced immunoturbidimetric assay. Blood samples for all inflammatory markers were collected at time of diagnosis and prior to initiating the antibiotic therapy. Demographic, clinical, laboratory, and prognostic data were collected from medical records for a period of 90 days from the initial diagnosis of CDAD.

Results: The mean age of the CDAD group was 68.6 ± 21.5 years compared to 65.6 ± 24.5 in the control group ($P = 0.29$). Our findings show that patients with CDAD had significantly higher NLR, MPV, and serum CRP levels compared to the control group ($P < 0.001$). Moreover, significantly higher levels were observed when CDAD was fatal ($P < 0.001$).

Conclusions: Elevated NLR, MPV, and serum CRP levels may serve as biomarkers for prediction of recurrence and mortality in patients with CDAD.

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KEY WORDS: *Clostridium difficile* (*C. difficile*), C-reactive protein (CRP), inflammatory markers, mean platelet volume (MPV), neutrophil-lymphocyte ratio (NLR)

Clostridium difficile is a gram positive, toxin-producing, anaerobic rod, and is the main etiological agent of antibiotic-associated diarrhea [1]. In severe cases, *C. difficile* can cause pseudomembranous colitis, a severe inflammation of the colon. The incidence of *C. difficile* colitis has increased during recent years, due to abundant use of broad-spectrum antibiotics. Outbreaks may occur when patients accidentally ingest spores of the bacteria while they are in a hospital, nursing home, or similar facilities [2]. The clinical manifestation of *C. difficile*-associated diarrhea (CDAD) ranges from asymptomatic carrier state to toxic megacolon, colonic perforation, and death [3]. Latent symptoms of *C. difficile* infection (CDI) often mimic flu-like symptoms or inflammatory bowel disease-associated colitis. The most common risk factor for developing CDAD is the use of antimicrobial agents [4]. Other risk factors include advanced age, hospitalization, severe co-morbidities, exposure to cytotoxic chemotherapy, immunosuppressive therapy, and use of acid suppressive therapy, especially proton pump inhibitors [5,6].

During the past decade, the incidence, severity, and recurrence of CDI have considerably increased, concomitant with the increasing prevalence of hypervirulent bacteria strains [7,8]. An early assessment of CDI severity is necessary for prompt and specific treatment initiation. Therefore there is a need to find tools to determine the severity of the disease. The neutrophil–lymphocyte ratio (NLR) is a simple and cheap marker of subclinical inflammation. NLR has recently been used as a systemic inflammation marker in chronic diseases such as chronic kidney disease, as well as a predictor of prognosis in cardiovascular diseases and malignancies [9,10]. NLR provides a rapid indication of the extent of an inflammatory process and thus it is a routinely available marker of the systemic inflammatory response [11].

Mean platelet volume (MPV) is a measurement of the average size of platelets found in blood and is typically included in blood tests as part of the complete blood count. The MPV is higher when there is destruction of platelets which occurs during inflammation [12]. C-reactive protein (CRP) is an acute phase protein synthesized by the liver, primarily in response to interleukin-6 during inflammation. Serum CRP levels have been shown to be useful in assessing the severity of various bacterial infections [13]. In this study, we evaluated the prognostic value of NLR, MPV, and CRP in patients with CDAD.

PATIENTS AND METHODS

The study was approved by the institutional ethics committee at Ziv Medical Center in agreement with the Helsinki Declaration Helsinki Committee. The study comprised 100 patients diagnosed with CDAD during a period of 3 years from January 2014 to January 2017. All patients were older than 18 years of age, with diarrhea and positive stool examination for *C. difficile* toxin (CDT). The patients had available medical records data for a period of at least 90 days from the initial diagnosis of CDAD as well as complete blood count (CBC) and serum measurements of CRP in the initial 2 days of the CDAD. Exclusion criteria were: a negative stool CDT, acute infections, sepsis of causes other than CDI, bacteremia, pregnancy, uncontrolled hypertension, uncontrolled diabetes, hemodialysis, severe hepatic failure, severe chronic heart disease, chronic systemic inflammatory disease, upper or lower gastrointestinal bleeding, medications affecting the number of leukocytes, immunocompromised patients, patients receiving immunosuppressant treatment, hematological disorders that affect platelets. The control group included 69 age- and sex-matched patients with diarrhea who were negative for CDT and had been hospitalized during the same time period. The control group met the exclusion criteria and had documented CBC and serum measurements of CRP in the initial two days of the diarrhea.

MEASUREMENTS AND DEFINITIONS

Diarrhea was defined as the passage of three or more unformed stools per day for at least 2 consecutive days. CDAD was diagnosed as diarrhea attributed solely to *C. difficile* combined with endoscopy revealing pseudomembranous colitis or positive stool enzyme immunoassay for toxin A or B (Techlab®, Inc., Blacksburg, VA, USA). Demographic, clinical indications, laboratory data, treatment, imaging studies, and recurrence and complications of the disease were obtained from medical records. Recurrent *C. difficile* infection is defined by complete abatement of CDI symptoms while on appropriate therapy, followed by subsequent reappearance of symptoms after ending treatment. NLR and MPV were calculated from the CBC, which was obtained by a Beckman-Coulter Gen-S system device (Beckman-Coulter Inc., USA). NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count. Concentrations of CRP were quantified in serum or plasma by latex particle enhanced immunoturbidimetric assay (Beckman-Coulter).

STATISTICAL ANALYSIS

Data were analyzed using SPSS version 19 (IBM SPSS, Chicago, IL, USA). Continuous variables are expressed as the mean standard deviation. The chi-square test was used to test differences in categorical variables between the cases and controls, and analysis of variance (ANOVA) or Student's *t*-test was used for comparisons of continuous variables. $P < 0.05$ was considered statistically significant.

RESULTS

We reviewed the medical records of 210 patients with CDAD. Of these, 100 met the inclusion criteria and an additional 69 patients were in the control group. There were no significant differences between the CDAD and control groups in term of mean age, white blood cell count, temperature, and length of hospitalization (LOH) in days. We found significant differences in the measurements of the NLR, MPV, and CRP, and days of treatment between the two groups ($P < 0.001$, for each) [Table 1].

Of the patients with CDAD, 41 died. The mean age of the patients who lived was 62.0 years compared to 78.2 ($P < 0.001$) in the group who died [Table 2]. All examined parameters including neutrophils, NLR, MPV, MPV, and CRP were significantly higher in the patients who died ($P < 0.001$), while the lymphocyte number were significantly elevated in the patients who lived [Table 2]. There was a significant difference between the groups in LOH which was 10.8 ± 6 days in the patients who lived compared to 15.8 ± 12.9 days ($P = 0.05$) in the group who died. No difference was noted in the type of treatment, duration, and recurrence in patients who lived or died.

DISCUSSION

In this retrospective study, we found that NLR, MPV, and CRP were significantly associated with severity and mortality in patients with CDAD. To the best of our knowledge, this is the first study evaluating the connection between inflammatory markers and CDAD.

CDAD is one of the most common hospital-acquired (nosocomial) infections and is an increasingly frequent cause of morbidity and mortality among elderly patients [14]. *C. dif-*

Table 1. Baseline characteristics of the *Clostridium difficile*-associated diarrhea and control groups

	Control (n=69)		CDAD (n=100)		
Variables	**Mean**	**SD**	**Mean**	**SD**	***P* value**
Age (years)	65.6	24.5	68.6	21.5	0.29
WBC (cells/µl)	9.0	3.1	27.3	112.1	0.107
NLR	2.8	1.0	7.7	7.1	‹ 0.001
MPV	6.8	0.9	9.3	1.3	‹ 0.001
CRP (mg/l)	12.9	10.7	121.7	9.2	‹ 0.001
Temperature (°C)	36.8	0.8	37.2	0.9	0.21
Days of treatment	4.2	3.7	11.6	13.6	‹ 0.001
LOH (days)	11.7	10.7	12.6	9.7	0.676

CDAD = *Clostridium difficile*-associated diarrhea, CRP = C-reactive protein, LOH = length of hospitalization, MPV = mean platelet volume, NLR = neutrophil-lymphocyte ratio, SD = standard deviation, WBC = white blood cells

Table 2. Characteristics of patients with CDAD according to endpoint

	Clostridium difficile-associated diarrhea				
	Alive (n=59)		Dead (n=41)		
Variables	**Mean**	**SD**	**Mean**	**SD**	***P* value**
Age, years	62.0	24.1	78.2	11.8	‹ 0.001
WBC, cells/µl	38.3	145.9	11.8	5.8	0.173
Neutrophils	68.4	12.6	83.5	7.2	‹ 0.001
Lymphocytes	20.2	9.0	9.2	3.9	‹ 0.001
NLR	4.7	3.8	12.0	8.3	‹ 0.001
MPV	8.5	1.0	10.4	0.9	‹ 0.001
CRP	70.6	68.7	145.1	72.9	‹ 0.001
Temperature, °C	37.2	0.9	37.1	0.9	0.853
Days of treatment	8.4	10.6	16.2	16.1	0.008
LOH, day	10.8	6.0	15.8	12.9	0.05

CRP = C-reactive protein, LOH = length of hospitalization, MPV = mean platelet volume, NLR = neutrophil-lymphocyte ratio, SD = standard deviation, WBC = white blood cells

ficile colonizes the human intestinal tract after the normal gut flora has been altered by antibiotic therapy and is the cause of antibiotic-associated pseudomembranous colitis. Once patients have been infected, they are at significantly increased risk for further recurrences [15]. Currently, there is no consensus how to define the severity of CDI [16,17].

Recently, several studies have found an association between serum CRP levels and the severity of adverse bacterial infections [18]. Higher procalcitonin levels were found in patients with CDT-positive vs. CDT-negative nosocomial diarrhea [19]. However, a procalcitonin level > 2 ng/ml may help distinguish between these patients. Our study showed a significant correlation between serum CRP levels and CDAD severity. We believe that there is a need for larger study to determine the role of CRP as a prognostic factor in CDI.

NLR is an accessible and cheap assay with results readily determined. This inflammatory marker is associated with the pathophysiological mechanism of SIRS, which is characterized by an increased number of neutrophils. However, lymphocytopenia appears as a consequence of lymphocyte margination and redistribution in the lymphatic system, with accelerated apoptosis [20]. The predictive role of NLR has been evaluated not only in patients with infections but also in patients with cancer, cardiovascular, or intestinal inflammatory diseases. We think that NLR will be a useful prognostic marker in patients with CDAD.

MPV is another inflammatory marker, which has gained importance in the management of septic patients. Sepsis causes changes in hemostasis, platelets activation including excessive aggregation and destruction, and platelet number and size. Platelet activation is caused by endothelial injuries triggered by the infectious agent. In this context, an increased MPV and a reduced platelet count are associated with an unfavorable prognosis and with an elevated mortality risk [21]. In our study, we found that MPV could be used as a prognostic marker in patients with CDAD.

LIMITATIONS

Our retrospective study included a small number of participants. Moreover, we did not measure other inflammatory biomarkers, such as procalcitonin, or other factors that could affect the prognosis such as albumin or creatinine.

CONCLUSIONS

Our findings indicate that NLR, MPV, and CRP levels may predict disease severity of CDAD. Measurements of these markers are easy, quick, and cheap. We suggest conducting larger studies to determine the usefulness of these markers in infectious diseases.

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Capsule

Prediction system for risk of allograft loss in patients receiving kidney transplants: international derivation and validation study

Loupy et al. developed and validated an integrative system to predict long-term kidney allograft failure. The authors prospectively recruited 4000 consecutive kidney recipients in four French centers between 2005 and 2014. Validation cohorts: 2129 kidney recipients from three centers in Europe and 1428 from three centers in North America. Participants were recruited between 2002 and 2014. Among the 7557 kidney transplant recipients, 1067 allografts (14.1%) failed after a median post-transplant follow-up time of 7.12 (interquartile range 3.51–8.77) years. In the derivation cohort, eight functional, histological, and immunological prognostic factors were independently associated with allograft failure and were then combined into a risk prediction score (iBox). This score showed accurate calibration and discrimination (C index 0.81, 95% confidence interval [95%CI] 0.79–0.83). The performance of the iBox was also confirmed in the validation cohorts from Europe (C index 0.81, 95%CI 0.78–0.84) and the North America (C index 0.80, 95%CI 0.76–0.84). The iBox system showed accuracy when assessed at different times of evaluation post-transplant. It was validated in different clinical scenarios including type of immunosuppressive regimen used and responsive to rejection therapy, and outperformed previous risk prediction scores as well as a risk score based solely on functional parameters, including estimated glomerular filtration rate and proteinuria. The accuracy of the iBox risk score in predicting long-term allograft loss was confirmed in the three randomised controlled trials.

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Capsule

Intestinal glucocorticoid synthesis enzymes in pediatric inflammatory bowel disease patients

Inflammatory bowel disease (IBD), such as Crohn's disease and ulcerative colitis, are devastating chronic immunopathologies of the intestinal mucosa, which are frequently treated by immunosuppressive glucocorticoids. Endogenous glucocorticoids are not only produced by the adrenal glands, but also by the intestinal epithelium. Local glucocorticoid synthesis critically contributes to the immune homeostasis of the intestinal mucosa. As defective intestinal glucocorticoid synthesis has been associated with the development of IBD, **Ahmed** et al. investigated the expression of steroidogenic enzymes and the key transcriptional regulator Liver Receptor Homolog-1 (LRH-1/NR5A2) in ileal and colonic biopsies from human pediatric IBD and control patients. Furthermore, the induction of steroidogenic enzymes and their transcriptional regulation by LRH-1 was investigated in a mouse model of experimental colitis. These analyses revealed that colitis-induced expression of steroidogenic enzymes in the murine colon is dependent on the presence of LRH-1, as intestinal deletion of LRH-1 strongly reduced their colitis-induced expression. Similarly, a strong correlation between the expression of LRH-1 and different steroidogenic enzymes was seen in intestinal biopsies of human pediatric patients. Importantly, reduced expression of hydroxysteroid dehydrogenase 11B1 was observed in IBD patients compared to control patients, suggesting that defective local reactivation of glucocorticoids could contribute to the pathogenesis of IBD.

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"Man is certainly stark mad: he cannot make a flea, yet he makes gods by the dozens"

Michel de Montaigne (1533–1592), essayist