Can Procalcitonin Contribute to the Diagnosis of *Clostridium difficile* Colitis?

Dvorah S. Shapiro MD[1*], Reuven Friedmann MD[1*], Ashraf Husseini MD[1], Hefziba Ivgi PhD[2], Amos M. Yinnon MD[3,4] and Marc V. Assous MD PhD[2]

[1]Department of Geriatrics, [2]Clinical Microbiology and Immunology Laboratory, [3]Infectious Disease Unit and [4]Division of Internal Medicine, Shaare Zedek Medical Center, affiliated with the Hebrew University Hadassah Medical School, Jerusalem, Israel

ABSTRACT: **Background:** *Clostridium difficile* colitis diagnosis is challenging.

Objectives: To determine, among patients who developed nosocomial diarrhea whether serum procalcitonin (PCT) can distinguish between *C. difficile* toxin (CDT)-positive and CDT-negative patients.

Methods: This prospective study included 50 adults (›18 years) who developed diarrhea during hospitalization, 25 with a positive fecal test for CDT (study group) and 25 CDT negative (control group).

Results: Baseline demographic and underlying illnesses were similar in both groups. Duration of diarrhea was 6 ± 4 days and 3 ± 1 in the study and control groups, respectively (P = 0.001). Mean blood count was 20 ± 15 and 9.9 ± 4, respectively (P = 0.04). CRP level was higher in the study group than in the control (10.9 ± 7.4 and 6.6 ± 4.8, P = 0.028). PCT level was higher in the study group (4.4 ± 4.9) than the control (0.3 ± 0.5, P = 0.102). A PCT level › 2 ng/ml was found in 7/25 patients (28%) and 1/25 (4%), respectively [odds ratio 9.33, 95% confidence interval (0.98 to 220), P = 0.049]. Multivariate analysis showed that only duration of diarrhea and left shift of peripheral leucocytes were significant indicators of CDT (P = 0.014 and P = 0.019, respectively). The mortality rate was 12/25 (48%) vs. 5/25 (20%), respectively (P = 0.04).

Conclusions: We found a non-significant tendency to higher PCT levels in patients with CDT-positive vs. CDT-negative nosocomial diarrhea. However, a PCT level › 2 ng/ml may help distinguish between these patients.

IMAJ 2017; 19: 313–316

KEY WORDS: *Clostridium difficile* colitis, *Clostridium difficile* toxin (CDT), antibiotic associated colitis, nosocomial diarrhea, procalcitonin (PCT)

Procalcitonin (PCT) is a peptide precursor of the hormone calcitonin. The source of production and its mechanism of induction are unknown. It is normally not secreted into serum, although typically during bacterial infections high serum levels can be detected. PCT levels > 2 ng/ml point to an acute bacterial infection, while in severe bacterial infections and sepsis the level can rise to 10–100 ng/ml [1,2]. In the latter situations, PCT rises within 3 to 4 hours, peaks at 6 hours and stays at plateau levels for 24 hours from exposure time [3-5]. PCT decreases to baseline levels within 2 days, its half-life is 25–30 hours and it dissolves through a specific protease. PCT reacts more rapidly to infection than C-reactive protein (CRP) [6], which may contribute to diagnosis of bacterial infection and also to more prudent antimicrobial usage [7-9].

PCT levels have been evaluated in various bacterial infections such as pneumonia, urinary tract infections (UTI), meningitis, and post-operative infections. PCT levels do not increase equally in different infections. Two studies compared PCT levels in infectious diarrhea vs. inflammatory diarrhea, and higher PCT levels were found in infection in the former condition [9,10]. Few studies have assessed PCT levels in nosocomial diarrhea or *Clostridium difficile* associated colitis (CDC), a potentially devastating complication [11-14]. Early diagnosis of CDC is important for initiation of specific treatment, discontinuation of antibiotics and consideration of fecal transplantation [15].

The primary purpose of this study was to evaluate, among patients who develop diarrhea in the hospital, whether serum PCT can distinguish between *Clostridium difficile* toxin (CDT)-positive and CDT-negative patients. The secondary aim was to determine the possible correlation between PCT levels and CDC severity.

PATIENTS AND METHODS

This prospective, comparative and non-interventional study was conducted at Shaare Zedek Medical Center, a 1000-bed general hospital in Jerusalem. Included were adults ≥ 18 years of age who developed nosocomial diarrhea and had a stool specimen sent for CDT testing. The study group consisted of patients with a positive fecal test for CDT, while the control group consisted of patients with diarrhea and a negative result for CDT. We did not attempt to match the two groups

*The first and second authors contributed equally to this study

of patients. Multiple demographic and clinical and laboratory data were collected to allow for comparison of the groups.

PATIENT POPULATION

This was a pilot study, and due to an absence of prior data it was impossible to calculate a study size that could have led to statistically significant results. It was decided arbitrarily to include 25 CDT-positive patients and 25 CDT-negative patients. Excluded were patients receiving chemotherapy in order to avoid inclusion of chemotherapy-induced colitis.

STUDY DESIGN

The clinical microbiology laboratory informed the investigators of each CDT-positive patient. Control patients consisted of patients who developed nosocomial diarrhea and had a stool sample sent for CDT, which turned out to be negative. No attempts were made to match study and control patients, except that the stool specimens had been tested on the same day. On the day of inclusion 2 ml of blood with EDTA was obtained for determination of PCT and C-reactive protein (CRP), simultaneously with blood samples drawn for clinical purposes.

LABORATORY METHODS

Stool specimen assessment for CDT was performed by the ELISA method for toxin detection (C. DIFFICILE TOX A/B II™ T5015 TechLab® (Techlab, Inc., USA) until the middle of 2013, and then the procedure was changed according to current guidelines [16] to combined glutamate dehydrogenase (GDH) and toxin A/B screening method (C DIFF Quick Check Complete®, Alere™ (Techlab, Inc., USA). Study patients were both GDH and toxin A/B positive, while control patients were GDH negative. PCT was determined by chemiluminescence immunoassay (CLIA) according to the manufacturer's instructions (Liaison Brahms PCT, Brahms Diagnostica, Berlin, Germany). CRP was determined with the immune nephelometric method according to the manufacturer's instructions (Behring Diagnostics Inc, Westwood, MA, USA). This project was approved by the ethics committee of Shaare Zedek Medical Center.

Table 1. Baseline demographic and clinical characteristics of patients and controls who developed diarrhea in the hospital

Clinical characteristics	*Clostridium difficile* toxin		Odds ratio univariate	95%CI	*P* value
	Positive n=25 (%)	Negative n=25 (%)			
Age (year), mean ± SD	81 ± 10	75 ± 17	1.04	0.99–1.09	0.134
Gender			0.599	0.19–1.90	0.384
Male	8 (32)	11 (44)			
Female	17 (68)	14 (56)			
Admission diagnosis					
LRTI*	8 (32)	7 (28)	1.21	0.31–4.81	1.00
Sepsis	6 (24)	2 (8)	3.63	0.55–29.77	0.247
Soft tissue infection§	4 (16)	3 (12)	1.40	0.22–9.17	1.00
UTI	1 (4)	4 (16)	0.22	0.01–2.21	0.349
Gastrointestinal#	1 (4)	5 (20)	0.17	0.01–1.17	0.189
Other	5 (20)	4 (16)			
Duration of hospitalization (Mean ± SE)	22 ± 3	21 ± 4	1.00	0.97–1.04	0.896
Abdominal pain	5 (20)	10 (40)	0.38	0.09–1.55	0.217
Protein pump inhibitors	15 (60)	19 (76)	0.47	0.12–1.87	0.364

*Includes pneumonia, infected bronchiectases

§Soft tissue infection includes cellulitis, infected decubitus ulcers and ischemic legs

#Includes Crohn's disease and acute gastroenteritis

LRTI = lower respiratory tract infection, UTI = urinary tract infection, CI = confidence interval, SD = standard deviation, SE = standard error

DATA PROCESSING

Demographic, clinical and microbiological data were entered into an Excel spreadsheet (version 2010, Microsoft, Redmond, WA, USA). Statistical analysis was performed using SPSS software (SPSS Inc., version 20, Chicago, IL, USA). For categorical variables we used chi-square analysis or Fisher's exact test, as appropriate. For continuous variables we used *t*-tests or non-parametric tests as needed. When necessary, the Mann-Whitney test was used. To rule out a possible association between various background conditions and the presence of CDC, the odds ratio, confidence interval and statistical significance were calculated. Multivariate regression was performed, first forced background variables were introduced and then clinical variables approaching a significance threshold of $P < 0.05$ were implemented.

RESULTS

The study comprised 50 consecutive patients who developed diarrhea during hospitalization, including 25 patients whose stool specimen was CDT positive (study group) and 25 patients whose samples were CDT negative (controls). Table 1 shows the baseline demographic and clinical data of both groups. There were no significant differences between the two groups.

Table 2 presents the follow-up clinical and laboratory data of these patients. CDT-positive patients experienced a longer period of diarrhea ($P = 0.001$), higher leukocyte count ($P = 0.004$), and a more significant left shift ($P = 0.001$). CRP level was higher in the study group than the control group ($P = 0.028$). PCT level was higher in the study group than the control group ($P = 0.102$); however, a level > 2 ng/ml was found in 7/25 (28%) CDT-positive patients, compared to 1/25 (4%) of the CDT-negative ones (odds ratio 9.33, 95% confidence interval 0.98–220, $P = 0.049$). The in-hospital mortality rate was 12/25 (48%) vs. 5/25 (20%) in the study and control groups, respectively ($P = 0.04$). The PCT level was higher in CDT-positive compared to CDT-negative patients for short (≤ 7 days), medium (8–14 days) or longer (≥ 15 days) duration of hospitalization (P = NS, Table 2).

Table 3 presents the multivariate analysis. Except for the duration of diarrhea and left shift of peripheral leucocytes, there were no independent predicting factors for CDT.

DISCUSSION

PCT has been a focus of intense research for more than a decade and has demonstrated diagnostic value for various clinical situations such as exacerbation of chronic obstructive pulmonary disease, pneumonia and other bacterial infections [7,8].

Our study comprised 50 patients who developed diarrhea during hospitalization, including 25 study patients whose stool specimen was CDT positive and 25 control patients who tested negative for CDT; baseline demographic and clinical characteristics were similar. Our main findings showed that the study patients had a considerably longer duration of diarrhea, a higher white blood count, and a more significant shift to the left of peripheral leucocytes ($P < 0.05$). In addition, the CRP level was higher in the CDT-positive than CDT-negative group as was the absolute PCT value, but the difference did not reach statistical difference. PCT levels were higher in CDT patients than controls, not reaching statistical significance. However, 7 patients (28%) in the CDT-positive group reached a PCT level > 2 ng/ml (a value considered to predict the presence of a bacterial infection) compared to only one patient (4%) in the control group ($P = 0.049$). This result indicates that some of the patients with CDT-positive nosocomial diarrhea had high levels of PCT. It should be noted that in this small study, high PCT levels did not predict mortality.

Recently it has been shown that PCT levels may reflect severity of CDC [11]. One study included 69 patients with diarrhea and a diagnosis of CDC and showed that high levels of PCT correlated with disease severity [11]. In patients with a low-PCT level (< 0.2 ng/ml), the risk of severe disease was exceedingly low. In the second study, which evaluated the correlation between PCT level and severity of CDT infection, the authors used a PCT level of > 0.5 ng/ml as a marker of severity. They demonstrated a specificity of 86%, a sensitivity of 88%, a positive predictive value of 94% and a negative predictive value of 75% [12]. In our study, we used a similar cutoff (< 0.5 ng/ml) and found that a low-PCT level occurred in 11 (44%) of the CDT-positive group compared with 18 (72%) in the control group ($P = 0.04$). Our study has the additional strength of having a control group. A third study assessed the efficacy of PCT in a variety of infections, including seven patients with CD infection [13]. These patients surprisingly had a mean PCT level of 47.2 ng/ml, which is much higher than encountered in our study. This difference could indicate a higher disease severity or presence of a concomitant infection. A recent study of 64 patients, including 44 with a PCR-positive test for CDT and 20 PCR-negative patients found that the median PCT levels in CDT-positive patients did not significantly differ from that in CDT-negative patients ($P = 0.08$), similar to our findings [14]. However, we stratified the PCT levels and demonstrated that a high level (> 2 ng/ml) significantly correlates with presence of CDT infection [Table 2].

Table 2. Clinical and laboratory characteristics of patients and controls with nosocomial diarrhea pertaining to the diarrheal episode

Variables	*Clostridium difficile* toxin		Odds ratio univariate	95%CI	*P* value
	Positive n=25 (%)	Negative n=25 (%)			
No. of diarrhea/day	3 ± 1	3 ± 2	1.08	0.73–1.60	0.697
Duration (days) of diarrhea	6 ± 4	3 ± 1	1.97	1.30–2.98	0.001
Temperature, max (°C)	37.5 ± 1.0	37.5 ± 0.9	1.04	0.60–1.81	0.877
WBC, max, x10³/µl	20.3 ± 15	9.9 ± 4	1.29	1.99–1.53	0.004
PMN, %	83 ± 8	73 ± 9	1.15	1.05–1.25	0.001
CRP, range 0–0.5 mg/dl	10.9 ± 7.4	6.6 ± 4.8	1.13	1.01–1.25	0.028
PCT, mean ± SD ≤ 0.5 0.6–1.9 ≥ 2	4.4 ± 10.9 13 (52) 5 (20) 7 (28)	0.3 ± 0.5 20 (80) 4 (16) 1 (4)	2.11 9.33	0.86–5.16 0.98–220.62	0.102 0.049
PCT, mean ± SD and duration of hospitalization: ≤ 7 days (0.89 ± 1.98) 8–14 days (5.50 ± 13.77) ≥ 15 days (1.04 ± 2.43)	 3.79 ± 3.44 7.95 ± 16.58 1.75 ± 3.29	 0.16 ± 0.14 0.42 ± 0.56 0.32 ± 0.65	 1.65 1.41 1.61	 0.39–7.01 0.61–3.26 0.70–3.69	 0.416 0.500 0.265

CRP = C-reactive protein, PCT = procalcitonin, normal ‹ 0.5 ng/ml, PMN = polymorphonuclear white blood cells, WBC = white blood cells, CI = confidence interval, SD = standard deviation

Table 3. Forward conditional logistic regression predicted *Clostridium difficile* toxin

	*B**	Standard error	Degrees of freedom	Odds ratio#	*P* value
Age	0.060	0.040	2.26	1.06	0.133
Gender	-1.43	1.09	1.74	0.239	0.188
Duration of admission	-0.004	0.031	0.017	0.996	0.895
Duration of diarrhea	0.767	0.327	5.51	2.15	0.019
No. diarrhea/day	-0.050	0.359	0.019	0.951	0.890
PMN	0.180	0.073	6.05	1.20	0.014
Constant	-21.3	8.38	6.47	NS	0.011

**B* = gross regression co-efficient
#Odds ratio, univariate CDT
PMN = polymorphonuclear white blood cells
NS = not significant

As expected, CRP was higher in CDT-positive than CDT-negative patients. This reflects the fact that patients with nosocomial diarrhea and positive CDT results have a condition with high inflammation markers, as compared to patients with nosocomial diarrhea secondary to other conditions such as osmotic diarrhea (due to tube feeding) or drug induced diarrhea. These causes are not expected to significantly increase CRP levels.

STUDY LIMITATIONS

Our study has several limitations. First, the sample size was small. The study was defined as a pilot study because in the absence of information about PCT in the context of CDT it

was impossible to determine an adequate sample size that could have led to statistical significance. Based on the results of this study, a larger study including 40 to 50 patients in each arm may lead to a statistically significant differences between CDT-positive and CDT-negative groups. The second limitation refers to a more fundamental issue. Some or even most of the included patients with nosocomial diarrhea may harbor active concomitant diseases that can potentially increase the level of PCT. However, rates of evaluated co-morbidities were similar in both groups. Third, at the time the study was initiated, CDT was tested with the toxin enzyme immunoassay. The enzyme immunoassay alone has a high specificity rate (up to 99%), but a low sensitivity rate (73%) [17,18]. During our study, the routine methodology for testing CDT followed existing guidelines [19]. The current method includes the addition of the GDH enzyme immunoassay and in case of discrepancy (i.e., positive GDH and negative toxin test) a PCR for CDT toxin. This combination substantially improves sensitivity of CDT detection. Therefore, the use of the enzyme immunoassay (in the first half of the study) for detection of CDT-positive patients could have inadvertently led to inclusion of false-negative patients in the control group. Of the 25 patients in the control group, 10/13 patients tested by the previous method for CDT detection had a PCT level ≤ 0.5 ng/ml and 3/13 had a level of 0.6–1.9; while 10/12 tested by the current methodology for CDT detection had a PCT level ≤ 0.5, 1/12 had a level 0.6–1.9, and 1/12 had a level > 2. Therefore, it seems highly unlikely that inclusion of a CDT false-negative patient in the control group would have led to different PCT results in the two study arms.

PCT is being introduced in many clinical laboratories because of its usefulness in diagnosing the presence or absence of a bacterial infection and, consequentially, for the initiation of antimicrobial therapy. At a cost of about 10 NIS/test it is one of the least expensive laboratory tests. Our data support testing for PCT in patients with nosocomial diarrhea as additional evidence for severity of CDT.

CONCLUSIONS

Patients with *C. difficile* colitis more often have a have a higher (> 2 ng/ml) PCT level than do patients with CDT-negative nosocomial diarrhea. More research is necessary before PCT can be considered a reliable test for differentiation between these patients.

Correspondence
Dr. A.M. Yinnon
Division of Internal Medicine, Shaare Zedek Medical Center, P.O. Box 3235, Jerusalem 91031, Israel

Phone: (972-2) 655-5076
Fax: (972-2) 666-6840
email: yinnon@szmc.org.il

References

1. De Jong E, van Oers JA, Beishuizen A, et al. Efficacy and safety of procalcitonin guidance in reducing the duration of antibiotic treatment in critically ill patients: a randomised, controlled, open-label trial. *Lancet Infect Dis* 2016; pii: S1473-3099 (16) 00053-0.
2. Trásy D, Tánczos K, Németh M, et al. Early procalcitonin kinetics and appropriateness of empirical antimicrobial therapy in critically ill patients: a prospective observational study. *J Crit Care* 2016; 34: 50-5.
3. Adamik B, Smiechowicz J, Jakubczyk D, Kübler A. Elevated serum PCT in septic shock with endotoxemia is associated with a higher mortality rate. *Medicine* (Baltimore) 2015 94 (27): e1085.
4. Lipińska-Gediga M, Mierzchała-Pasierb M, Durek G. Procalcitonin kinetics – prognostic and diagnostic significance in septic patients. *Arch Med Sci* 2016; 12: 112-9.
5. Von Landenberg P, Shoenfeld Y. New approaches in the diagnosis of sepsis. *IMAJ* 2001; 3: 439-42.
6. Hoeboer SH, Groeneveld AB. Changes in circulating procalcitonin versus C-reactive protein in predicting evolution of infectious disease in febrile, critically ill patients. *PLoS One* 2013; 8 (6): e65564.
7. Stolz D, Christ-Crain M, Bingisser R, et al. Antibiotic treatment of exacerbations of COPD: a randomized, controlled trial comparing procalcitonin-guidance with standard therapy. *Chest* 2007; 131: 9-19.
8. Christ-Crain M, Jaccard-Stolz D, Bingisser R, et al. Effect of procalcitonin-guided treatment on antibiotic use and outcome in lower respiratory tract infections: cluster-randomised, single-blinded intervention trial. *Lancet* 2004; 363: 600-7.
9. Thia KT, Chan ES, Ling KL, et al. Role of procalcitonin in infectious gastroenteritis and inflammatory bowel disease. *Dig Dis Sci* 2008; 53: 2960-8.
10. Herrlinger KR, Dittmann R, Weitz G, et al. Serum procalcitonin differentiates inflammatory bowel disease and self-limited colitis. *Inflamm Bowel Dis* 2004; 10: 229-33.
11. Rao K, Walk ST, Micic, D et al. Procalcitonin levels associate with severity of Clostridium difficile infection. *PLoS One* 2013; 8: e58265.
12. Dazley J, Shaaban H, Afridi S, et al. The role of procalcitonin levels in assessing the severity of Clostridium difficile infection. *J Glob Infect Dis* 2015; 7: 120-1.
13. Friend KE, Burgess JN, Britt RC, et al. Procalcitonin elevation suggests a septic source. *Am Surg* 2014; 80: 906-9.
14. Popiel KY, Gheorghe R , Eastmond J, et al. Usefulness of adjunctive fecal calprotectin and serum procalcitonin in individuals positive for Clostridium difficile toxin gene by PCR assay. *J Clin Microbiol* 2015; 53: 3667-9.
15. Cohen NA, Ben Ami R, Guzner-Gur H, Santo ME, Halpern Z, Maharshak N. Fecal microbiota transplantation for Clostridium difficile-associated diarrhea. *IMAJ*; 2015; 17: 510-4.
16. Grotto I. Israel Ministry of Health Standard for the laboratory diagnosis of Clostridium difficile. Guideline from the Director of Public Health Services, Israel Ministry of Health Publication 12/12, June 2012.
17. Swindells J, Brenwald N, Reading N, et al. Evaluation of diagnostic tests for Clostridium difficile infection. *J Clin Microbiol* 2010; 48: 606-8.
18. Cheng JW, Xiao M, Kudinha T, et al. The role of glutamate dehydrogenase (GDH) testing assay in the diagnosis of Clostridium difficile infections: a high sensitive screening test and an essential step in the proposed laboratory diagnosis workflow for developing countries like China. *PLoS One* 2015;10 (12): e0144604.
19. Samra Z, Madar-Shapiro L, Aziz M, Bishara J. Evaluation of a new immuno-chromatography test for rapid and simultaneous detection of Clostridium difficile antigen and toxins. *IMAJ* 2013; 15: 373-6.

"Education is the best provision for old age"

Aristotle (384-322 BC), Greek philosopher and scientist whose writings cover physics, biology, zoology, metaphysics, logic, ethics, aesthetics, poetry, theater, music, rhetoric, linguistics, politics and government, and constitute the first comprehensive system of Western philosophy