Published in final edited form as:

Eur J Clin Microbiol Infect Dis. 2018 December ; 37(12): 2341–2346. doi:10.1007/s10096-018-3381-9.

Fecal Calprotectin Concentrations in Cancer Patients with *Clostridium difficile* Infection

Taojun He^{1,2}, Samuel E. Kaplan², Luz A. Gomez², Xuedong Lu¹, Lakshmi V. Ramanathan², Mini Kamboj^{2,3}, and Yi-Wei Tang^{2,3}

¹The Eighth Affiliated Hospital of Sun Yat-sen University, Shenzhen, China;

²Memorial Sloan Kettering Cancer Center, New York, NY

³Weill Medical College of Cornell University, New York, NY

Abstract

Fecal calprotectin (fCPT) has been used as a surrogate marker for assessment of intestinal inflammation. We explore the utility of fCPT values as a diagnostic aid in cancer patients with suspected *Clostridium difficile* infection (CDI). A total of 232 stool specimens submitted for GeneXpert *C. difficile* PCR testing were included in the study. All specimens were tested for fCPT and toxin/GDH antigens. Clinical severity of CDI cases was determined by the IDSA/SHEA criteria. Significant differences of median fCPT values ($\mu\text{g/g}$) between CDI ($n=117$, Median 183.6 $\mu\text{g/g}$) and non-CDI ($n=115$, 145.6 $\mu\text{g/g}$, $p=0.006$) patients were seen. In CDI patients, significantly lower fCPT values were found in patients with mild to moderate ($n=95$, 182.1 $\mu\text{g/g}$) than those with severe & severe to complicated ($n=22$, 218.5 $\mu\text{g/g}$, $p=0.014$) scores, and among those that were toxin positive ($n=24$, 200.2 $\mu\text{g/g}$) versus toxin negative ($n=86$, 182.8 $\mu\text{g/g}$, $p=0.044$). Despite this overall trend, wide variations in fCPT values were found in all categories examined. A logistic regression analysis revealed that the fCPT values correlated independently with the severity of clinical manifestations (OR=2.021, 95% CI=1.132–3.608); however, it did not correlate with other clinical outcomes. Our study findings show that high fecal calprotectin levels correlate with toxin positive and clinically severe CDI; however wide variations in individual measurements preclude establishment of reliable cut-offs for routine diagnostic use in cancer patients.

Keywords

Clostridium difficile infection; fecal calprotectin; PCR; colonization

Introduction

Clostridium difficile is an anaerobic, spore-forming, Gram-positive bacillus, which is a leading cause of infection-associated diarrhea in hospitalized patients receiving

Corresponding author: Yi-Wei Tang (tangy@mskcc.org).

Conflict of Interest: The Phical Test was provided free of charge. No other reported conflicts.

Compliance with Ethical Standards

Ethical approval: This study was approved by the Memorial Sloan Kettering Cancer Center Institutional Review Board (WA0477–15).

Informed consent: Waive approved.

antimicrobial therapy [1]. In the human gastrointestinal tract it may exist as a silent colonizer or under certain conditions, cause active infection leading to diarrhea and related complications [2–4]. *C. difficile* infection (CDI) is the leading cause of hospital-acquired gastrointestinal illness and places an enormous burden on the healthcare system, with an estimated cost of 3.2 billion U.S. dollars annually [5]. The reported prevalence of CDI has increased in the past decade across the healthcare continuum [1].

Diagnosis of CDI is a major conundrum at the current time. While several different technologies exist, each has its unique set of limitations [6, 7]. It has been shown that part of the reported increase in CDI incidence is due to the wider implementation of high sensitivity molecular assays over toxin-EIA or culturing methods which are considered reference methods [8, 9]. Whereas toxin-based assays directly assess disease-mediating toxin production in vivo, PCR-based assays detect only the presence of the toxin gene. Planche et al. reviewed PCR positive stool test results from 6,522 inpatient episodes and found that patients positive for toxin were more likely to die compared with those without detectable toxin in stool [8]. Polage et al. reported that patients with positive *C. difficile* PCR (CDPCR) tests but negative CD toxin EIA had similar outcomes when compared to patients without detectable *C. difficile*, and the CD related complications occurred only among patients that were concurrently toxin positive [9].

These data suggest that molecular assays may be overly sensitive, and that a positive *C. difficile* PCR result in stool cannot differentiate between *C. difficile* colonization and infection [10]. This conclusion is especially relevant in a cancer hospital, where the likelihood of encountering toxigenic *C. difficile* and non-infectious diarrhea is higher [2, 11, 12].

A wide variety of biomarkers including fecal lactoferrin and fecal calprotectin (fCPT) have been used for screening for inflammatory bowel disease as well as for monitoring disease progression [13–15]. The recent guidance by UK National Institute for Health and Care Excellent (NICE) recommends using fecal biomarkers to aid in the future diagnosis of CDI [16]. Studies have reported the use of fCPT values as new diagnostic biomarkers in CDI [16–22]. In the current study, we explored the utility of fCPT values to aid in the diagnosis and monitoring of *Clostridium difficile* infection (CDI) in patients with underlying cancer.

Materials and Methods

Clinical specimens

A total of 232 specimens from 156 patients submitted for Cepheid Xpert *C. difficile* PCR assay testing from January 2012 to December 2013 were selected for the study including (i) 80 patients with single specimens (PCR-negative, 39; PCR-positive, 41), (ii) 38 patients with paired specimens from an initial PCR negative and subsequent positive sample, and (iii) 38 patients with paired specimens from an initial PCR positive to a negative second sample. Leftover stool specimens after molecular testing were aliquotted and stored at –80°C until subsequent antigen and calprotectin (fCPT) testing. Solid or formed stool specimens were rejected and the ordering clinician was notified. This study was approved by the Memorial Sloan Kettering Cancer Center (MSKCC) Institutional Review Board (WA0477–15).

C. difficile PCR assay

The GeneXpert C. difficile PCR assay (Cepheid, Sunnyvale, CA) was performed on the GeneXpert system according to the manufacturer's instructions [23].

C. difficile antigen assay

The C. Diff Quik Chek Complete (TechLab, Blacksburg, VA) dual-antigen EIA was performed according to the manufacturer's instructions. In brief, approximately 25µl of stool was added to a tube containing the diluents and conjugate, and the mixture was transferred to the device sample well. After incubation for 15 min at room temperature, the wash buffer and then the substrate were added to the reaction window. Results were read after 10 min. GDH antigen and/or toxins were reported positive if a visible band was seen on the antigen and/or toxin side of the device display window, respectively [24].

Fecal calprotectin assay

Fecal calprotectin (fCPT) was quantitatively detected by the Phical Test (Calpro AS, Oslo, Norway) according to the manufacturer's instructions [25]. In brief, after three freeze-thaw cycles, stool specimens were vortexed thoroughly. The corresponding volume of Diluted Extraction Solution was combined with 80–120 mg of stool to produce a 1:10 dilution. After homogenization in an Eppendorf Thermomixer R (Fisher Scientific) at 1,000 rpm for 30 min and centrifugation at 12,200 rpm for 20 min, 20µl supernatant was transferred to a sterile Eppendorf tube containing 980µl Diluted Extraction Solution. Calprotectin levels from 100 µl of mixture were measured using an enzyme immunoassay on a 96-well microtiter plate in duplicate as previously described [25]. The fCPT value from the duplicate wells were averaged ranging between 11.9 and 278.7 µg/g based on a 6-point standard curve provided with the kit.

CDI severity scoring

A retrospective chart review was done to determine the severity of CDI. A diagnosis of CDI was established when the patient fulfilled the following criteria: (i) the presence of diarrhea, defined as passage of ≥ 3 unformed stools in 24 consecutive hours and (ii) a stool test result positive for GeneXpert C. difficile PCR [26]. CDI severity was divided into the following six categories: (i) no clinical CDI, (ii) mild CDI, (iii) mild-to-moderate CDI, (iv) moderate CDI, (v) moderate-to-severe CDI, and (vi) severe CDI based on the IDSA/SHEA guideline [26] as well as the grouping criteria described previously [27]. CDI severity was correlated with fCPT concentration.

Data statistical analysis

All samples were categorized into five groups (Table 2) based on their PCR, toxin and GDH antigen results and fCPT median, mean, and standard deviation (SD) were calculated for each group. Chi-square and Fisher exact tests were used to compare categorical variables and the Student's t-test was used to compare continuous variables. Quantitative variables were compared by the non-parametric Mann-Whitney U-test (for 2 groups) and Kruskal-Wallis test (for >3 groups), respectively, in uni-variable analysis. Multi-variate logistic regression was performed to identify risk factors for death, as well as for composite severe

outcomes that were associated with fCPT levels. Odds ratios (OR), 95% confidence intervals (CI), and *p* values were calculated, and all *p* value less than 0.05 were considered statistically significant. On univariate analysis, variables with *p*<0.3 were included in a logistic regression model to further identify independent risk factors associated with fCPT levels. All data analysis was carried out in SPSS24 and *p* value equal or less than 0.05 was considered to be of statistical significance.

Results

We first determined reproducibility of the fCPT levels of the Phical Test. Two specimens with adequate volumes were selected and tested in triplicate on three different calendar days. The average fCPT values of the two specimens were 88.7 and 248.0 µg/g. Intra- and the inter-assay coefficients of variation (CV) were 1.32–4.31% and 9.63–14.48%, respectively (Table 1).

A total of 232 specimens from 156 patients were included and tested for fCPT levels by the Phical Test. These specimens were divided into five groups based on their *C. difficile* GDH, toxin antigen and PCR results (Table 2). The fCPT levels were the highest in the GDH+/TOX+/PCR+ group and the lowest in the GDH-/TOX-/PCR- group (200.2 ± 398.6 µg/g vs. 139.8 ± 595.7 µg/g; *p*=0.019). Large variations in fCPT values as indicated by high standard deviations were noticed. Significant differences in fCPT medians between the five groups were also detected (*p*=0.024). Notably, differences in fCPT medians between GDH+/TOX+/PCR+ and GDH+/TOX-/PCR+ groups values (200.2 µg/g vs. 182.8 µg/g) were statistically significant (*p*=0.044). Median values were used for subsequent further analysis.

Among the 232 specimens, 117 (50.4%) were positive by *C. difficile* PCR: the median fCPT values for these specimens (183.6 µg/g) was significantly higher than those that were PCR-negative (145.6 µg/g, *p*=0.006) (Table 3). Among the 117 PCR-positive specimens, fCPT median values were significantly higher in patients with severe and severe to complicated CDI (218.5 µg/g) compared to those with mild to moderate disease (182.1 µg/g, *p*=0.014).

We further assessed characteristics, risk factors, and outcomes in relation to fCPT levels for 156 patients with the first stool samples only. On univariate analysis, statistically significant factors associated with higher fCPT levels were found for those without history of chemotherapy within 30 days (*p*=0.025), fever $\geq 38^{\circ}\text{C}$ (*p*=0.048) at the time of diarrhea, lower blood white blood cell (WBC $<1 \times 10^9/\text{L}$) count (*p*=0.039), and those with severe-complicated CDI (*p*=0.036) (Table 4). A logistic regression analysis of all the characteristics with *p*<0.3 on univariate analysis was performed, and the only independent risk factor associated with fCPT values was severe, complicated manifestations of CDI (OR=2.021, 95% CI=1.132–3.608, *p*=0.017). Recurrent CDI and death within 30 days were not associated with fCPT values at the time of clinical evaluation.

Discussion

Our study demonstrates that in patients with underlying cancer who experience diarrhea, fCPT values are higher in those with CDI and significantly higher among toxin positive and those with higher level of disease severity. The fCPT levels were independently associated

with CDI severity in multivariate analysis. Despite these findings, due to the wide variation in individual measurements, we were unable to establish a clear cut off that would reliably distinguish between infectious and carrier states, an important clinical and epidemiological distinction [3, 12], or predict disease outcomes.

The role of fCPT is not established in the diagnosis of CDI mainly due to observed intra-individual variability in previous studies, which has mostly been attributed to sample handling and processing [28]. It has previously been reported that fCPT analysis on the first bowel movement is most appropriate [14, 15]. In our study, we processed fecal specimens that had undergone three freeze-thaw cycles followed by thorough vortexing. This allowed us to reach intra- and inter-assay CV values less than 5% and 15%, respectively. No significant fCPT value differences (4,585.3 and 4,490.3 µg/g) were revealed between the two specimens collected from the same patient with PCR result conversions, suggesting that fCPT concentration in stool was relatively stable independently of *C. difficile* PCR results.

Current standard-of-care molecular assays targeting *C. difficile*-specific toxin A or B genes do not distinguish between colonization and infection [29, 30], and thereby may result in over-diagnosis, over-treatment, and increased health care costs [8, 9]. Furthermore, only 15 to 25% of nosocomial antibiotic-associated diarrhea cases are attributed to CDI [3, 12, 31, 32]. Previous publications have demonstrated that fCPT is a useful marker in differentiating inflammatory bowel disease from functional gastrointestinal disorders [33–36]. A series of recent studies have described that the median values of fCPT is highly sensitive in differentiating CDI from non-CDI patients with hospital-acquired diarrhea [16, 22, 37]. Fecal calprotectin levels may be used as an adjunctive measure to PCR to support the diagnosis of CDI, especially in older adults [20, 21].

Our study has several limitations. We noted a great deal of variability in individual fCPT values within the same diagnostic and disease severity tier. In addition, two extremely high fCPT levels (4585.3 and 4490.3 µg/g) were detected from one patient with gastrointestinal cancer. While our study demonstrated that fCPT values independently correlated with severity at the time of evaluation, it is emphasized that fCPT levels did not correlate with other clinical outcomes including 30-day mortality and 90-day recurrence [16]. Finally, total sample numbers recruited in the study were relatively small, causing difficulty in achieving reliable conclusions. When fCPT is used to manage patients, it should be interpreted under the specific clinical context.

In summary, while the fCPT values correlate with clinically significant CDI in persons with cancer, the large variability in values precludes widespread application as a diagnostic aid. Future large studies in this population are needed to generate potential cut-off values that may be used to distinguish between carrier and disease states.

Acknowledgments

We thank laboratory staff in the Clinical Microbiology Service at MSKCC for their excellent assistance.

Funding: NIH/NCI Cancer Center Support Grant P30 (CA008748).

References

- [1]. Lessa FC, Mu Y, Bamberg WM, Beldavs ZG, Dumyati GK, Dunn JR, Farley MM, Holzbauer SM, Meek JI, Phipps EC, Wilson LE, Winston LG, Cohen JA, Limbago BM, Fridkin SK, Gerding DN, McDonald LC (2015) Burden of *Clostridium difficile* infection in the United States. The New England journal of medicine 372 (9):825–834 [PubMed: 25714160]
- [2]. Dominguez SR, Dolan SA, West K, Dantes RB, Epson E, Friedman D, Littlehorn CA, Arms LE, Walton K, Servetar E, Frank DN, Kotter CV, Dowell E, Gould CV, Hilden JM, Todd JK (2014) High colonization rate and prolonged shedding of *Clostridium difficile* in pediatric oncology patients. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 59 (3):401–403 [PubMed: 24785235]
- [3]. Furuya-Kanamori L, Marquess J, Yakob L, Riley TV, Paterson DL, Foster NF, Huber CA, Clements AC (2015) Asymptomatic *Clostridium difficile* colonization: epidemiology and clinical implications. BMC infectious diseases 15:516 [PubMed: 26573915]
- [4]. Loo VG, Bourgault AM, Poirier L, Lamothe F, Michaud S, Turgeon N, Toye B, Beaudoin A, Frost EH, Gilca R, Brassard P, Dendukuri N, Beliveau C, Oughton M, Brukner I, Dascal A (2011) Host and pathogen factors for *Clostridium difficile* infection and colonization. The New England journal of medicine 365 (18):1693–1703 [PubMed: 22047560]
- [5]. McFarland LV, Ozen M, Dinleyici EC, Goh S (2016) Comparison of pediatric and adult antibiotic-associated diarrhea and *Clostridium difficile* infections. World J Gastroenterol 22 (11):3078–3104 [PubMed: 27003987]
- [6]. Bagdasarian N, Rao K, Malani PN (2015) Diagnosis and treatment of *Clostridium difficile* in adults: a systematic review. Jama 313 (4):398–408 [PubMed: 25626036]
- [7]. Sammons JS, Toltzis P (2015) Pitfalls in Diagnosis of Pediatric *Clostridium difficile* Infection. Infectious disease clinics of North America 29 (3):465–476 [PubMed: 26188603]
- [8]. Planche TD, Davies KA, Coen PG, Finney JM, Monahan IM, Morris KA, O'Connor L, Oakley SJ, Pope CF, Wren MW, Shetty NP, Crook DW, Wilcox MH (2013) Differences in outcome according to *Clostridium difficile* testing method: a prospective multicentre diagnostic validation study of *C difficile* infection. Lancet Infect Dis 13 (11):936–945 [PubMed: 24007915]
- [9]. Polage CR, Gyorke CE, Kennedy MA, Leslie JL, Chin DL, Wang S, Nguyen HH, Huang B, Tang YW, Lee LW, Kim K, Taylor S, Romano PS, Panacek EA, Goodell PB, Solnick JV, Cohen SH (2015) Overdiagnosis of *Clostridium difficile* Infection in the Molecular Test Era. JAMA Intern Med 175 (11):1792–1801 [PubMed: 26348734]
- [10]. Peng Z, Ling L, Stratton CW, Li C, Polage CR, Wu B, Tang YW (2018) Advances in the diagnosis and treatment of *Clostridium difficile* infections. Emerg Microb Infect:in press
- [11]. Otto CC, Shuptar SL, Milord P, Essick CJ, Nevrekar R, Granovsky SL, Seo SK, Babady NE, Martin SC, Tang YW, Pessin MS (2015) Reducing Unnecessary and Duplicate Ordering for Ovum and Parasite Examinations and Clostridium difficile PCR in Immunocompromised Patients by Using an Alert at the Time of Request in the Order Management System. Journal of clinical microbiology 53 (8):2745–2748 [PubMed: 26063860]
- [12]. Kamboj M, Babady NE, Marsh JW, Schlackman JL, Son C, Sun J, Eagan J, Tang YW, Sepkowitz K (2014) Estimating risk of *C. difficile* transmission from PCR positive but cytotoxin negative cases. PLoS One 9 (2):e88262 [PubMed: 24523882]
- [13]. Lundberg JO, Hellstrom PM, Fagerhol MK, Weitzberg E, Roseth AG (2005) Technology insight: calprotectin, lactoferrin and nitric oxide as novel markers of inflammatory bowel disease. Nature clinical practice Gastroenterology & hepatology 2 (2):96–102 [PubMed: 16265127]
- [14]. Calafat M, Cabre E, Manosa M, Lobaton T, Marin L, Domenech E (2015) High within-day variability of fecal calprotectin levels in patients with active ulcerative colitis: what is the best timing for stool sampling? Inflammatory bowel diseases 21 (5):1072–1076 [PubMed: 25793326]
- [15]. Lasson A, Stotzer PO, Ohman L, Isaksson S, Sapnara M, Strid H (2015) The intra-individual variability of faecal calprotectin: a prospective study in patients with active ulcerative colitis. Journal of Crohn's & colitis 9 (1):26–32
- [16]. Swale A, Miyajima F, Roberts P, Hall A, Little M, Beadsworth MB, Beeching NJ, Kolamunnage-Dona R, Parry CM, Pirmohamed M (2014) Calprotectin and lactoferrin faecal levels in patients

with *Clostridium difficile* infection (CDI): a prospective cohort study. PLoS One 9 (8):e106118 [PubMed: 25170963]

- [17]. Usacheva EA, Jin J-P, Peterson LR (2016) Host response to *Clostridium difficile* infection: Diagnostics and detection. Journal of Global Antimicrobial Resistance 7:93–101 [PubMed: 27693863]
- [18]. Kim J, Kim H, Oh HJ, Kim HS, Hwang YJ, Yong D, Jeong SH, Lee K (2017) Fecal Calprotectin Level Reflects the Severity of *Clostridium difficile* Infection. Annals of laboratory medicine 37 (1):53–57 [PubMed: 27834066]
- [19]. Peretz A, Tkhawkho L, Pastukh N, Brodsky D, Halevi CN, Nitzan O (2016) Correlation between fecal calprotectin levels, disease severity and the hypervirulent ribotype 027 strain in patients with *Clostridium difficile* infection. BMC infectious diseases 16:309 [PubMed: 27334992]
- [20]. Popiel KY, Gheorghe R, Eastmond J, Miller MA (2015) Usefulness of Adjunctive Fecal Calprotectin and Serum Procalcitonin in Individuals Positive for *Clostridium difficile* Toxin Gene by PCR Assay. Journal of clinical microbiology 53 (11):3667–3669 [PubMed: 26354814]
- [21]. Rao K, Santhosh K, Mogle JA, Higgins PD, Young VB (2016) Elevated fecal calprotectin associates with adverse outcomes from *Clostridium difficile* infection in older adults. Infect Dis (Lond):1–7
- [22]. Whitehead SJ, Shipman KE, Cooper M, Ford C, Gama R (2014) Is there any value in measuring faecal calprotectin in *Clostridium difficile* positive faecal samples? J Med Microbiol 63 (Pt 4): 590–593 [PubMed: 24464697]
- [23]. Babady NE, Stiles J, Ruggiero P, Khosa P, Huang D, Shuptar S, Kamboj M, Kiehn TE (2010) Evaluation of the Cepheid Xpert *Clostridium difficile* Epi assay for diagnosis of *Clostridium difficile* infection and typing of the NAP1 strain at a cancer hospital. Journal of clinical microbiology 48 (12):4519–4524 [PubMed: 20943860]
- [24]. Quinn CD, Sefers SE, Babiker W, He Y, Alcabasa R, Stratton CW, Carroll KC, Tang YW (2010) C. Diff Quik Chek complete enzyme immunoassay provides a reliable first-line method for detection of *Clostridium difficile* in stool specimens. Journal of clinical microbiology 48 (2):603–605 [PubMed: 19955275]
- [25]. Kronborg O, Ugstad M, Fuglerud P, Johne B, Hardcastle J, Scholefield JH, Vellacott K, Moshakis V, Reynolds JR (2000) Faecal calprotectin levels in a high risk population for colorectal neoplasia. Gut 46 (6):795–800 [PubMed: 10807890]
- [26]. Cohen SH, Gerding DN, Johnson S, Kelly CP, Loo VG, McDonald LC, Pepin J, Wilcox MH (2010) 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 31 (5):431–455 [PubMed: 20307191]
- [27]. Huang B, Jin D, Zhang J, Sun JY, Wang X, Stiles J, Xu X, Kamboj M, Babady NE, Tang YW (2014) Real-time cellular analysis coupled with a specimen enrichment accurately detects and quantifies *Clostridium difficile* toxins in stool. J Clin Microbiol 52 (4):1105–1111. doi: 1110.1128/JCM.02601-02613 Epub 2014 Jan 02622. [PubMed: 24452160]
- [28]. Kristensen V, Malmstrom GH, Skar V, Roseth A, Moum B (2016) Clinical importance of faecal calprotectin variability in inflammatory bowel disease: intra-individual variability and standardisation of sampling procedure. Scandinavian journal of gastroenterology 51 (5):548–555 [PubMed: 26634305]
- [29]. Rodriguez C, Van Broeck J, Taminiau B, Delmee M, Daube G (2016) *Clostridium difficile* infection: Early history, diagnosis and molecular strain typing methods. Microb Pathog 97:59–78 [PubMed: 27238460]
- [30]. Sewell B, Rees E, Thomas I, Ch'ng CL, Isaac M, Berry N (2014) Cost and Impact on Patient Length of Stay of Rapid Molecular Testing for *Clostridium difficile*. Infect Dis Ther 3 (2):281–293 [PubMed: 25183400]
- [31]. Dubberke ER (2010) Prevention of healthcare-associated *Clostridium difficile* infection: what works? Infect Control Hosp Epidemiol 31 Suppl 1:S38–41 [PubMed: 20929366]
- [32]. Dubberke ER, Haslam DB, Lanzas C, Bobo LD, Burnham CA, Grohn YT, Tarr PI (2011) The ecology and pathobiology of *Clostridium difficile* infections: an interdisciplinary challenge. Zoonoses Public Health 58 (1):4–20 [PubMed: 21223531]

- [33]. Dabritz J, Musci J, Foell D (2014) Diagnostic utility of faecal biomarkers in patients with irritable bowel syndrome. *World J Gastroenterol* 20 (2):363–375 [PubMed: 24574706]
- [34]. Dhaliwal A, Zeino Z, Tomkins C, Cheung M, Nwokolo C, Smith S, Harmston C, Arasaradnam RP (2015) Utility of faecal calprotectin in inflammatory bowel disease (IBD): what cut-offs should we apply? *Frontline Gastroenterol* 6 (1):14–19 [PubMed: 25580205]
- [35]. Kawashima K, Ishihara S, Yuki T, Fukuba N, Oshima N, Kazumori H, Sonoyama H, Yamashita N, Tada Y, Kusunoki R, Oka A, Mishima Y, Moriyama I, Kinoshita Y (2016) Fecal calprotectin level correlated with both endoscopic severity and disease extent in ulcerative colitis. *BMC Gastroenterol* 16:47 [PubMed: 27071448]
- [36]. Mosli MH, Zou G, Garg SK, Feagan SG, MacDonald JK, Chande N, Sandborn WJ, Feagan BG (2015) C-Reactive Protein, Fecal Calprotectin, and Stool Lactoferrin for Detection of Endoscopic Activity in Symptomatic Inflammatory Bowel Disease Patients: A Systematic Review and Meta-Analysis. *Am J Gastroenterol* 110 (6):802–819; quiz 820 [PubMed: 25964225]
- [37]. Darkoh C, Turnwald BP, Koo HL, Garey KW, Jiang ZD, Aitken SL, DuPont HL (2014) Colonic immunopathogenesis of *Clostridium difficile* infections. *Clin Vaccine Immunol* 21 (4):509–517 [PubMed: 24477852]

Table 1.Reproducibility of fCPT concentrations ($\mu\text{g/g}$) in cancer patients with diarrhea.

Sample	Day	Day 1	Intra-CV (%)	Day 2	Intra-CV (%)	Day 3	Intra-CV (%)	Inter-CV (%)
1	1	94.9	3.45	108.7	3.09	81.8	1.32	14.48
	2	89.3		103.8		80.8		
	3	89.7		110.2		79.7		
2	1	238.9	1.49	266.9	3.67	224.7	4.31	9.63
	2	232.2		273.0		244.4		
	3	234.1		286.7		231.0		

CV, coefficients of variation.

Table 2.

Median, Mean & Standard deviation of fCPT values in cancer patients with diarrhea

Group	Number	Median	p-value	Mean	SD	p-value
GDH+/TOX+/PCR+	24	200.2	0.024	390.7	398.6	0.210
GDH+/TOX-/PCR+	86	182.8		344.8	381.6	
GDH-/TOX-/PCR+	7 ^a	162.0		819.4	1664.2	
GDH+/TOX-/PCR-	9	178.4		198.3	78.0	
GDH-/TOX-/PCR-	106	139.8		332.7	595.7	

-, negative; +, positive.

^a Included two specimens with extremely high fCPT values of 4585.3 and 4490.3 µg/g from one patient with gastrointestinal cancer.

Table 3.

Fecal calprotectin level and clinical severity in cancer patients with diarrhea

Clinical severity	Single group			N to P group			P to N group			Total	
	No.	Median	p-value	No.	Median	p-value	No.	Median	p-value	No.	Median
<i>C. difficile</i> PCR–	39	138.7	0.014 ^a	38	138.0	0.194 ^a	38	164.3	0.155 ^a	115	145.6
Mild to moderate	39	138.7	N.D.	38	138.0	N.D.	38	164.3	N.D.	115	145.6
Severe & severe to complicated	0			0			0			0	
<i>C. difficile</i> PCR+	41	207.6		38	177.3		38	186.0		117	183.6
Mild to moderate	31	202.5	0.023 ^b	30	175.5	0.590 ^b	34	186.0	0.640 ^b	95	182.1
Severe & severe to complicated	10	272.5		8	181.5		4	205.4		22	218.5

N.D., not done.

^aBetween *C. difficile* PCR- and + groups;^bBetween Mild to moderate and Severe & severe to complicated groups.

Table 4.Risk factors and outcomes associated with fecal calprotectin levels^a

Variable	Stratum	Uni-variant analysis			Multi-variant Logistic		
		No.	Median	p-value	OR	95% CI	p-value
Age	60y	82	165.7	0.618			
	>60y	74	154.7				
Gender	female	69	149.7	0.259	2.009	0.905–4.461	0.087
	male	87	167.6				
Cancer type	Liquid	82	156.1	0.521			
	Solid, non GI	32	166.7				
	Solid, GI	42	182.3				
Surgery	No	87	163.2	0.629			
	Yes	69	149.7				
Chemotherapy	No	76	192.0	0.025	0.750	0.353–1.595	0.455
	Yes	80	139.8				
Antibody therapy	No	40	150.3	0.463			
	Yes	116	164.3				
Laxative-use	No	112	154.7	0.286	0.700	0.290–1.687	0.426
	Yes	44	170.5				
Abdominal pain	No	124	151.6	0.113	0.756	0.284–2.015	0.576
	Yes	32	169.1				
Fever	<38°C	151	157.0	0.048	0.860	0.093–7.955	0.895
	38°C	5	209.5				
Albumin	30mg/dl	128	156.1	0.167	1.858	0.754–4.582	0.178
	<30mg/dl	28	181.9				
WBC count	<1×10 ⁹ /L	18	218.1	0.039	2.240	0.486–10.330	0.301
	(1–20)×10 ⁹ /L	128	166.7				
	>20×10 ⁹ /L	10	128.2				
Clinical recurrence	No	153	156.5	0.560			
	Yes	3	178.5				
Manifestation	Mild to moderate	115	145.6	0.036	2.021	1.132–3.608	0.017
	Severe	31	202.5				
	Severe to compl.	10	272.5				
Death in 30 days	No	138	164.3	0.316	0.986	0.303–3.212	0.982
	Yes	18	148.6				

^aBased on single first specimen from each patient.

OR, odds ratio; CI, confidence level.