FOLIA MEDICA CRACOVIENSIA
Vol. LIX, 3, 2019: 53–65
PL ISSN 0015-5616
DOI: 10.24425/fmc.2019.131136

The level of fecal calprotectin significantly correlates with *Clostridium difficile* infection severity

Mirosław Dróżdż[1], Grażyna Biesiada[2], Hanna Pituch[3], Dorota Wultańska[3], Piotr Obuch-Woszczatyński[3], Michał Piotrowski[3], Jolanta Kędzierska[4], Mateusz Michalak[5], Aleksander Garlicki[2], Jacek Czepiel[2]

[1]St. Anne Hospital, Miechów, Poland
[2]Department of Infectious and Tropical Diseases, Jagiellonian University Medical College, Kraków, Poland
[3]Department of Medical Microbiology, Medical University of Warsaw, Warsaw, Poland
[4]Faculty of Microbiology, University Hospital, Kraków, Poland
[5]Students' Scientific Society, Jagiellonian University Medical College, Kraków, Poland

Corresponding author: Jacek Czepiel, MD, PhD
Department of Infectious and Tropical Diseases, Jagiellonian University Medical College
ul. Śniadeckich 5, 31-501 Kraków, Poland
Phone/Fax: +48 12 424 73 49; E-mail: jacek.czepiel@uj.edu.pl

Abstract: Introduction: Fecal calprotectin (FC) rises significantly in intestinal inflammation accompanied by neutrophil activation — such as *Clostridium difficile* infection (CDI). The aim of the study was to evaluate the benefit of FC testing in assessing the severity of CDI.
Materials and Methods: The study group included 76 patients with CDI hospitalized in the Jagiellonian University Hospital in Krakow from July 2017 till January 2018. FC levels were measured using an EIA (Enzyme Immunoassay). Demographic, clinical information and blood tests were recorded using standardized data collection forms. The selection of patients into non-severe and severe groups was carried out in accordance with the ESCMID criteria (European Society of Clinical Microbiology and Infectious Diseases) and some modifications to those criteria were proposed.
Results: The studied population included 76 patients (39 men and 37 women) with CDI aged from 24 to 98 years (mean: 72). Median calprotectin level was 739 (Q_{25}–Q_{75}: 612–799 µg/g), characteristic of patients with colitis. A statistically significant difference in FC concentration in patients with severe vs non-severe CDI was observed (severe — 770 vs non-severe — 659 µg/g, $p = 0.009$). FC directly correlated with platelets level; however, no correlation between FC level and the blood parameters prognostic for CDI (leukocyte, neutrophil count, albumin, creatinine levels) was found.

C o n c l u s i o n: FC level is an indication of ongoing intestinal inflammation in CDI patients. FC level significantly correlated with CDI severity, which demonstrates that FC could serve as a predictive marker for assessing CDI severity.

Key words: *Clostridium difficile*, fecal calprotectin, severity, mortality.

Introduction

Clostridium difficile (*C. difficile*) is a Gram-positive, anaerobic, spore-forming bacillus responsible for one of the most notable healthcare-associated infections of the last two decades, one with a significant morbidity and mortality. The most important risk factors for *C. difficile* infection (CDI) development are antibiotic exposure, older age, and hospitalization. The main protective barrier against CDI is the normal intestinal microflora. Destruction of that barrier as a result of antibiotic use facilitates the colonization of the intestine by *C. difficile* and the progress of CDI symptoms. The pathogen is not invasive, and its virulence is mostly a result of *C. difficile* enzyme production, such as collagenase, hyaluronidase, chondroitin-sulfatase, and toxins (toxin A and B), which damage the epithelial cell cytoskeleton, stimulate neutrophil adhesion and local inflammation [1–3]. The clinical picture of CDI is very heterogeneous, and ranges from the asymptomatic carrier state to life-threatening colitis. Typical symptoms include diarrhea, abdominal pain, fever, nausea and vomiting, weakness, and loss of appetite. The most severe complications are toxic megacolon, colon perforation, intestinal paralysis, kidney failure, systemic inflammatory response syndrome, septicemia and death [4–6]. Currently, several methods of fast diagnosis of CDI are available. The best way to optimize the diagnosis is to combine two tests in an algorithm. The first test should be one with high negative predictive value (either GDH EIA or NAAT). The second test should be a test with a high positive predictive value (toxin A/B EIAs) [7].

Calprotecin is a 36 kDa calcium- and zinc- binding protein, produced mostly by neutrophils and to some extent by monocytes/macrophages. The protein complex belongs to the S100 family and it is composed of two hydrophobic non-covalently linked S100A8/A9 regions [8, 9].

Calprotectin is stored in neutrophil granules, constituting about 5% of proteins, whereas in monocytes and macrophages it can comprise up to 60% of cytosolic proteins [8]. It has antimicrobial properties — bacteriostatic and fungistatic, due to the competitive binding of manganese and zinc [9, 10]. Calprotectin has been shown to exhibit antimicrobial activity against *Escherichia coli*, *Klebsiella* sp., *Staphylococcus aureus* [8]. The fecal calprotectin (FC) level rises in intestinal inflammation, as neutrophils are activated. Crucially, it is thought that unlike other inflammation

markers such as erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP), inflammatory reactions other than intestinal do not cause a significant increase in calprotectin level [9]. Its fecal excretion is highly correlated to that of 111-indium-radiolabeled leukocyte scanning, the gold standard for measurement of severity of intestinal inflammation [11]. Furthermore, the measurement is relatively straightforward, requiring approximately 5 grams of fecal matter. A noteworthy characteristic of calprotectin itself is its stability, reaching up to 7 days in room temperature [12]. However, one study indicates that FC concentration in stool samples stored in room temperature starts to decrease 3 days after collection [13]. Therefore, before assay, the sample can be safely stored in low temperatures, a week minimum in 2–8°C and in −20 C and below a minimum of 4 months without a drop in FC detectability [14]. An exact cut-off has not been defined; however, numerous studies suggest that concentrations of <50 μg/g would be seen as normal; 50–100 μg/g represent a weakly positive test; >100 μg/g indicate a positive result [12]. As mentioned above, an increase in FC is an indication of ongoing intestinal inflammation — in healthy people FC levels are lower and not influenced by lifestyle factors. FC level in healthy people is approximately 50 μg/g for adults and children older than 4 years [12].

Materials and Methods

The study group included 76 patients with CDI hospitalized from 1 July 2017 till 31 January 2018 at the Jagiellonian University Hospital in Krakow, aged from 24 to 98 years (mean: 72 years; 39 men and 37 women). The diagnosis of CDI was based on patient history, epidemiological data, physical examination, and laboratory tests according to the ESCMID (European Society of Clinical Microbiology and Infectious Diseases) guidelines [15]. Outcomes included severe CDI (intensive care unit admission, colectomy, death attributable to CDI within 30 days of diagnosis), and 90 days all-cause mortality. CDI was diagnosed in patients experiencing diarrhea, defined as the passage of 3 or more unformed stools in 24 hours. The infection was then confirmed by the detection of the *C. difficile* antigen and toxins in feces using the C. difficile Quick Check Complete test kit (TechLab Inc., Blacksburg, USA). When the test came back positive for the antigen but negative for the toxin, the test for the *C. difficile* toxin was repeated with the ELISA *C. difficile* toxin A/B II test kit (TechLab Inc., Blacksburg, USA). The study was conducted in accordance with the Declaration of Helsinki (1975), and approved by the Jagiellonian University Ethics Committee [protocol number: 1072.6120.51.2018]. Exclusion criteria included the presence of other acute or chronic inflammatory diseases of the digestive tract.

Patient qualification into the non-severe and severe groups according to the ESCMID criteria

Patient qualification into the non-severe and severe groups was performed based on ESCMID criteria [15]. Severe CDI was defined as an episode of this infection with one or more specific signs and symptoms of severe colitis or a complicated course of disease, with significant systemic toxin effects and shock, resulting in need for ICU (intensive care unit) admission, colectomy or death. One or more of the following unfavorable prognostic factors can be present during the course of the disease: marked leukocytosis (leucocyte count $>15 \times 10^9$/L), decreased blood albumin (<30 g/L), kidney impairment (rise in serum creatinine level ≥133 μM or ≥1.5 times the premorbid level or glomerular infiltration rate (GFR) reduced by 25% from baseline). In adherence to those criteria, 50 people were categorized as severe and 26 as non-severe.

Modification proposal of the ESCMID criteria of patient classification into non-severe and severe groups

Having analyzed the ESCMID criteria, we have proposed an adjusted version of the distribution criteria. The presence of clinical events such as: ICU admission, colectomy, death indisputably allow to qualify patient to the "severe" CDI group. However, these factors often occur at a later stage of the disease. Significant disturbances in the 3 known factors of poor CDI prognosis (hypoalbuminemia, WBC increase, creatinine and GFR changes) are extremely important, because they often allow to predict a severe course of CDI. On the other hand, deviations in the above parameters appear relatively frequently, hence the qualification to the "severe" group in the presence of two rather than one impaired parameter (hypoalbuminemia, WBC increase, creatinine and GFR changes) in our opinion is much more accurate in predicting the severity of CDI.

Therefore, we have defined the modified criteria of CDI severity as follows:

Severe CDI was defined as an episode of this infection with one or more specific signs and symptoms of severe colitis or a complicated course of disease, with significant systemic toxin effects and shock, resulting in either: need for ICU admission, colectomy or death. Two or more of the following unfavorable prognostic factors can be present during the course of the disease: marked leukocytosis (leucocyte count $>15 \times 10^9$/L), decreased blood albumin (<30 g/L), kidney impairment (rise in serum creatinine level ≥133 μM or ≥1.5 times the premorbid level or glomerular infiltration rate (GFR) reduced by 25% from baseline). In adherence to those criteria, 31 people were categorized as severe and 45 as non-severe. Using this classification, FC was compared in both groups.

Sample collection and data gathering

All samples came from stool left over in the main laboratory. The samples were stored in a frozen (–80°C) state until the calprotectin test was performed. Demographic data (age, sex) and clinical data (outcome of CDI infection, intensive care unit admission, colectomy, death, and blood tests information) were recorded using standardized data collection forms. Blood tests included complete blood count (CBC), creatinine, GFR, albumin and CRP level.

Fecal calprotectin analysis

The stool samples were tested with Ridascreen® Calprotectin immunoassay marketed by R-Biopharm AG in accordance with the supplied instructions. Briefly: 5 ml of extraction buffer was added to every 100 mg sample and homogenized for approximately 30 seconds (depending on consistency), then centrifuged for 10 minutes with the velocity of 3000 G. The obtained supernatant was diluted 50-fold and in 100 µl portions transferred to reaction plates. Several other wells contained 100 µl of negative control in 100 µl (5 standards), 100 µl of positive control and a 100 µl of weak positive control. Every sample was incubated (covered) for 60 minutes in room temperature. After incubation, the samples were rinsed 5 times with a rinse buffer, a conjugate was added and the mixture was again incubated for 60 mins in room temperature. Subsequently, every reaction well was rinsed 5 times with a rinse buffer, the substrate was added and the covered plate was incubated in a darkroom in room temperature for 15 minutes. After the appointed time period the reaction was halted with a STOP reactant. Photometric analysis was performed with the wavelength of 450 nm and reference wavelength of 620 nm in a BIO-RAD PR 3100 spectrophotometer. Calprotectin concentrations were recorded on a standard curve, drawn according to the manufacturer's instructions, for each sample series.

Statistics

All data is presented as medians and lower (Q_{25}), upper (Q_{75}) quartiles. Normal distribution of variables was checked using the Shapiro–Wilk test. Differences between study groups were determined using the Mann–Whitney U-test if normality was not observed. Correlation between selected variables was evaluated using a Pearson correlation coefficient; in the absence of normal distribution, Spearman's rank correlation was used. Calculations were performed using StatSoft, Inc. (2011), STATISTICA, version 13 statistical package software licensed for the Jagiellonian University, and statistical significance was defined as $p \leq 0.05$.

Results

The study consisted of 76 patients with C. difficile infection (39 women, 37 men) hospitalized from July 2017 till January 2018 in the mean age of 72 (ranged from 24 to 98 years). In the analyzed group there were 64 patients with a first and 12 with a recurring episode of CDI. Patients with a recurring episode of CDI were analyzed only once. The results of biochemical parameter analysis have shown that patients with CDI suffered from very intensive systemic inflammatory reaction, which was manifested by the increase in inflammatory markers. The median level of calprotectin was typical for people with colitis (Table 1). The table does not include the results of a patient with leukemia, after chemotherapy, the results of which were significantly influenced by primary disease and chemotherapy, as well as a patient who died shortly after beginning of diarrhea, hence it was possible to take feces for *C. difficile* diagnosis, but no blood was collected before death. Approximately 50% of patients did not have white blood cell smears, 13% have not had albumin and CRP levels tested. In almost all cases, the levels of albumin and CRP were not performed in people whose diarrhea was characterized by a very mild and short-lasting course of CDI.

Table 1. Results of the assessed parameters in the study groups.

Parameter	Patients with CDI	
	n	median (Q_{25}–Q_{75})
WBC ($\times 10^3$/µl)	74	8.6 (6.8–15.6)
neutrophils ($\times 10^3$/µl)	35	4.9 (3.8–12.3)
RBC ($\times 10^6$/µl)	74	3.8 (3.3–4.2)
Haemoglobin (g/dl)	74	10.6 (9.4–11.7)
Haematocrit (%)	74	31.9 (29.2–35.6)
platelets ($\times 10^3$/µl)	74	246 (181–324)
creatinine (µmol/l)	75	91 (68–141)
GFR (ml/min/1.73m^2)	75	69 (48–91)
Albumin (g/l)	66	28.9 (23.5–33)
CRP (mg/l)	66	61 (17–131)
Fecal calprotectin (µg/g)	76	739 (612–799)

CDI — *Clostridium difficile* infection; CRP — C-reactive protein; GFR — glomerular filtration rate; RBC — red blood cells; Q_{25} — lower quartile; Q_{75} — upper quartile; WBC — white blood cells

Non-severe vs severe CDI — according to ESCMID

Subsequently, a statistical comparison of assessed parameters was performed, dividing the patients into the non-severe and severe subgroups of CDI. A statistically higher FC concentration was observed in the severe vs non-severe CDI (severe — 770 vs non-severe — 659 µg/g, p = 0.009). Furthermore, patients with severe CDI exhibited significantly higher values of WBC, neutrophils, creatinine, CRP and lower GFR, albumin compared to the non-severe group (Table 2).

Table 2. Results of the assessed parameters in the study groups divided into the non-severe and severe subgroups.

Parameter	CDI non-severe		CDI severe		p
	n	median (Q_{25}–Q_{75})	n	median (Q_{25}–Q_{75})	
WBC (×10³/µl)	26	7.3 (6.3–8.0)	48	13.2 (7.5–18.8)	p <0.001
neutrophils (×10³/µl)	13	4.1 (3.4–4.8)	22	10.4 (4.8–18.8)	0.008
RBC (×10⁶/µl)	26	3.8 (3.4–4.2)	48	3.8 (3.2–4.2)	0.42
Haemoglobin (g/dl)	26	10.7 (10.2–11.7)	48	10.3 (8.9–11.8)	0.14
Haematocrit (%)	26	33 (31–35)	48	31.8 (28–36)	0.12
platelets (×10³/µl)	26	245 (180–311)	48	247 (183–330)	0.78
creatinine (µmol/l)	26	75 (60–86)	49	120 (80–169)	0.001
GFR (ml/min/1.73m²)	26	90 (72–93)	49	54 (35–78)	<0.001
Albumin (g/l)	22	34 (32–36)	44	25 (22–28.9)	<0.001
CRP (mg/l)	21	15 (6–58)	45	80 (40–158)	<0.001
Fecal calprotectin (µg/g)	26	659 (369–775)	50	770 (689–802)	0.009

CDI — *Clostridium difficile* infection; CRP — C-reactive protein; GFR — glomerular filtration rate; RBC — red blood cells; Q_{25} — lower quartile; Q_{75} — upper quartile; WBC — white blood cells

Comparison of FC level using proposed modifications of ESCMID criteria

Table 3. Comparison of FC level using proposed modifications of ESCMID criteria.

Parameter	CDI non-severe		CDI severe		p
	n	median (Q_{25}–Q_{75})	n	median (Q_{25}–Q_{75})	
Faecal calprotectin (µg/g)	45	661 (581–789)	31	780 (714–810)	0.001

CDI — *Clostridium difficile* infection; CRP — C-reactive protein; Q_{25} — lower quartile; Q_{75} — upper quartile

A statistically higher FC concentration was observed in the severe vs non-severe CDI (severe — 780 vs non-severe — 661 µg/g, p = 0.001).

Deaths

The evaluation of patients with CDI showed 20 deaths among the 76 people, 13 of which originated directly from CDI — sequentially or clinically. In the remaining 7 cases, the CDI had been successfully treated with death attributed to different mechanisms. The mortality rate was 17% (n = 13/76) where death was directly attributed to CDI and 26% (n = 20/76) where all deaths were included. Where CDI was the contributing factor, the time from diagnosis to death averaged 10 days (1–22 days). In the remaining 7 cases that timeframe was 36 days (21–45 days). There was no significant difference in FC level between group with no CDI-related deaths and those with CDI-related deaths. However, significantly higher values of WBC, creatinine, and CRP and a lower GFR and albumin were observed in people who had died than in those who had survived CDI (Table 4).

Table 4. Results of the assessed parameters in the study groups divided into patients with no CDI-related deaths (n = 63) and CDI-related deaths (n = 13) subgroups.

Parameter	Patients with no CDI-related deaths		CDI-related deaths		p
	n	median (Q_{25}–Q_{75})	n	median (Q_{25}–Q_{75})	
WBC ($\times 10^3$/μl)	63	8.04 (6.5–14.3)	11	13.7 (9.3–26.9)	0.03
neutrophil ($\times 10^3$/μl)	29	4.8 (3.6–10.6)	6	14.5 (4.9–29)	0.07
RBC ($\times 10^6$/μl)	63	3.8 (3.3–4.1)	11	3.9 (3.1–5.4)	0.52
Haemoglobin (g/dl)	63	10.5 (9.5–11.7)	11	11.1 (8.6–13.7)	0.95
Haematocrit (%)	63	32.3 (30–35)	11	32 (27.4–40.8)	0.92
platelets ($\times 10^3$/μl)	63	247 (180–324)	11	246 (199–404)	0.73
creatinine (μmol/l)	63	85 (60–130)	12	163 (118–350)	0.004
GFR (ml/min/1.73m^2)	63	74 (51–92)	12	37 (14–54)	0.002
Albumin (g/l)	57	29 (25–33)	9	24 (19.7–26.1)	0.009
CRP (mg/l)	55	51 (11.9–104)	11	198 (74–304)	<0.001
Fecal calprotectin (μg/g)	63	727 (607–798)	13	772 (693–800)	0.27

CDI — *Clostridium difficile* infection; CRP — C-reactive protein; GFR — glomerular filtration rate; RBC — red blood cells; Q_{25} — lower quartile; Q_{75} — upper quartile; WBC — white blood cells

The last test was designed to determine whether the calprotectin concentration correlates with the assessed blood parameters and we found only one such correlation — between FC and platelets. Correlations between other parameters are presented in Table 5.

Table 5. Correlations between measured parameters in patients with CDI.

Parameters compared	r	p
WBC vs CRP	0.34	0.005
WBC vs albumin	–0.42	<0.001
WBC vs platelets	0.26	0.02
Neutrophil vs albumin	–0.48	0.005
CRP vs albumin	–0.40	0.004
CRP vs haematocrit	–0.25	0.047
albumin vs haemoglobin	0.38	0.001
albumin vs haematocrit	0.37	0.002
Fecal calprotectin vs platelets	0.28	0.02

CRP — C-reactive protein; WBC — white blood cells

Discussion

Numerous studies have shown that the concentration of calprotectin in the stool correlates with the intestinal inflammation. So far, this test has found use primarily in diagnosing and monitoring the course of IBD [16–18]. Van Rheenen *et al.* in their study including 670 adults with suspicion of IBD found that the sensitivity and specificity of calprotectin was 0.93 and 0.96 [19]. Von Roon *et al.* performed a meta-analysis using data from 9 studies comparing FC in IBD subjects in respect to IBS (irritable bowel syndrome) subjects or healthy controls. They showed that the overall sensitivity and specificity of FC equaled 95% and 91% respectively for the identification of patients with IBD. Moreover, the level of FC was higher by 219.2 μg/g in patients with IBD compared with healthy controls. They also demonstrated that as a cut-off, the value of 100 μg/g functions better than 50 μg/g in the diagnosis of IBD [20]. The efficiency of calprotectin in identifying IBD is markedly higher than the widely used CPR, ESR, anti-*Saccharomyces cerevisiae* antibody (ASCA) or perinuclear antineutrophil cytoplasmic antibody (ANCA) [18].

Neutrophil infiltration is one of the basic drivers of the development of symptoms of CDI and it is known that neutrophils play a major role in the pathogenesis of the disease [21]. Toxins produced by *C. difficile* damage the enterocytes, which leads to degradation of the cytoskeleton and an increase in the epithelial barrier permeability, resulting in local inflammation, mast cell degranulation, epithelial cell death, and neutrophil recruitment [22]. The higher inflammation intensity in CDI, the bigger the increase in neutrophil count [21]. Considering the neutrophils' role in CDI pathogenesis, it would seem logical to assume that in this disease a calprotectin test could similarly prove its clinical value. Results of the studies conducted so far have

been inconclusive. Kim *et al.* demonstrated that FC significantly correlated with CDI severity and also was able to discriminate between severe CDI, mild CDI, and healthy controls. FC concentrations were higher ($p < 0.001$) in the 30 severe CDI patients (median, 1.391 μg/g) than in the 50 mild CDI patients (median, 188.2 μg/g), and both were higher than those of the healthy controls (median, 35.6 μg/g) [23]. Rao *et al.* have shown that high FC (>2000 ug/g) was associated with adverse outcomes (complicated/recurrent) CDI in older adults, however, in their study the number of patients with these outcomes was relatively small (5 patients with complicated and 8 with recurrent CDI). Moreover, well known and important for severe CDI parameters like age, comorbidities, WBC did not correspond to CDI severity in their study — the authors seem to think that this could indicate that calprotectin, which was associated with adverse outcomes despite the small sample size, is a stronger predictor than clinical variables. This, however, is a bold theory and only an investigation on a larger scale would do it justice [24]. That argument is furtherly weakened by Swale *et al.* who have observed no statistically significant difference in FC comparing patients with severe CDI vs. non-severe CDI, as well as seen no correlation with all-cause mortality [25]. Peretz *et al.* in a small study (29 patients) assessed FC correlation with virulent ribotype 027 strain infection and clinical and laboratory measures of disease severity. They found higher FC levels in patients with *C. difficile* ribotype 027 ($p < 0.0005$) and a positive correlation between FC and WBC ($p = 0.007$). No correlation was found regarding CDI severity, age, sex, functional status, community versus hospital acquired CDI, antibiotic susceptibility, fever, and creatinine levels [26]. In a Swale *et al.* study with a larger population sample (164 patients) there was no correlation between FC levels and ribotype 027 [25]. Swale *et al.* found significant differences between CDI and controls (684 vs 67 mg/kg), in this study control patients were individuals with evidence of antibiotic-associated diarrhea (AAD), but negative toxin ELISA test and microbiological cultures, with no past history of CDI. No significant differences for FC comparing severe vs non-severe CDI was found. There was also no correlation with 90-day recurrence, prolonged CDI symptoms, positive culture results [25]. In another study Wultańska *et al.* also found that there were no statistically significant differences in the concentration of calprotectin between patients infected with RT027 and different PCR ribotypes than RT027 [27].

In our study, we have demonstrated a clear-cut ($p = 0.009$), statistically significant difference between patients with severe and non-severe CDI using the ESCMID criteria. One plausible explanation, partial at least, for the divergence in study results could be the lack of uniform criteria stratifying the non-severe and severe. Therefore we have proposed more accurate, modified criteria and statistically significant difference of FC level between patients with severe and non-severe CDI using modified criteria was also confirmed ($p = 0.001$).

Presently, the antibiotics in CDI management are vancomycin, fidaxomicin and metronidazol. The drug and dose of choice depend on CDI severity — in the most severe fulminant form vancomycin 500 mg orally 4 times a day and metronidazole 500 mg IV 3 times a day is used [4]. It is paramount to correctly categorize the patients into risk strata for CDI severity early on, as timely and suitable treatment increases the chance of recovery and survival rate. Therefore, the search for new markers of severe CDI could prove extremely beneficial. That, coupled with the FC test being inexpensive, easy and FC itself stable in room temperature makes it a promising candidate [14]. Despite having demonstrated a significant increase in FC in patients with severe CDI, a similar correlation with deaths in our study has not been observed. This could be attributed to the relatively small group of patients who died — only 13. Therefore, it is vital to continue with a larger population sample, performing a detailed clinical analysis — patients with CDI usually suffer from a number of comorbidities, often fatal — e.g. terminal cancer. The overall-mortality in our group was high, reaching 26%. It is worth noting that after a clinical analysis, 35% of overall deaths (7 out of 20) were deemed unrelated to CDI.

We have found that FC correlated with platelets, but no other correlation was found between FC and the assessed blood parameters. However, a statistically significant and directly proportional relation was found between the WBC count and CRP and also platelets what reflects the nature of CDI as a disease with a strong inflammatory component. Some interesting results have been obtained pertaining to CRP and albumin relation to RBC parameters — as CDI is not known to directly cause anemia, which can be explained by numerous comorbidities of varying severity. The comorbidity analysis was not the aim of our study; however, the large percentage of all-mortality (26%) and particularly the percentage of CDI-unrelated deaths (35%, 7 out of 20 deaths) may confirm this hypothesis.

Conclusions

FC is an indication of ongoing intestinal inflammation in CDI patients. The FC levels significantly correlated with CDI severity, which shows it could prospectively serve as a predictive marker for assessing CDI severity. The ongoing search for CDI severity markers is particularly vital, enabling fast and optimal treatment approach, which is crucial in CDI therapy.

Conflict of interest

None declared.

References

1. *Leffler D.A., Lamont J.T.*: *Clostridium difficile* infection. N Engl J Med. 2015; 372: 1539–1548.
2. *Vaishnavi C.*: Clinical spectrum & pathogenesis of *Clostridium difficile* associated diseases. Indian J Med Res. 2010; 131: 487–499.
3. *Moore S.C.*: *Clostridium difficile*: more challenging than ever. Crit Care Nurs Clin North Am. 2018; 30: 41–53.
4. *McDonald L.C., Gerding D.N., Johnson S., et al.*: Clinical practice guidelines for *Clostridium difficile* infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis. 2018; 66: e1–e48.
5. *Surawicz C.M., Brandt L.J., Binion D.G., et al.*: Guidelines for diagnosis, treatment, and prevention of *Clostridium difficile* infections. Am J Gastroenterol. 2013; 108: 478–498.
6. *Bartlett J.G.*: *Clostridium difficile* infection. Infect Dis Clin N Am. 2017; 31: 489–495.
7. *Crobach M.J.T., Planche T., Eckert C., et al.*: European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for *Clostridium difficile* infection. Clin Microbiol Infect. 2016; 22: S63–S81.
8. *Summerton C.B., Longlands M.G., Wiener K., Shreeve D.R.*: Faecal calprotectin: a marker of inflammation throughout the intestinal tract. Eur J Gastroenterol Hepatol. 2002; 14: 841–845.
9. *Montalto M., Gallo A., Santoro L., D'Onofrio F, Landolfi R., Gasbarrini A.*: Role of fecal calprotectin in gastrointestinal disorders. Eur Rev Med Pharmacol Sci. 2013; 17: 1569–1582.
10. *Manceau H., Chicha-Cattoir V, Puy H., Peoc'h K.*: Fecal calprotectin in inflammatory bowel diseases: update and perspectives. Clin Chem Lab Med. 2017; 55: 474–483.
11. *Roseth A.G., Schmidt P.N., Fagerhol M.K.*: Correlation between faecal excretion of indium-111-labelled granulocytes and calprotectin, a granulocyte marker protein, in patients with inflammatory bowel disease. Scand J Gastroenterol. 1999; 34: 50–54.
12. *Gisbert J.P, McNicholl A.G.*: Questions and answers on the role of faecal calprotectin as a biological marker in inflammatory bowel disease. Dig Liver Dis. 2009; 41: 56–66.
13. *Lasson A., Stotzer P.O., Ohman L., Isaksson S., Sapnara M., Strid H.*: The intra-individual variability of faecal calprotectin: a prospective study in patients with active ulcerative colitis. J Crohns Colitis. 2015; 9: 26–32.
14. *Hessels J., Douw G., Yildirim D.D., Meerman G., van Herwaarden M.A., van den Bergh F.A.*: Evaluation of prevent ID and quantum blue rapid tests for fecal calprotectin. Clin Chem Lab Med. 2012; 50: 1079–1082.
15. *Debast S.B., Bauer M.P., Kuijper E.J.*: ESCMID European Society of Clinical Microbiology and Infectious Diseases update of the treatment guidance document for *Clostridium difficile* infection. Clin Microbiol Infect. 2014; 20: 1–26.
16. *Angriman I., Scarpa M., D'Inca R.*: Enzymes in feces: useful markers of chronic inflammatory bowel disease. Clin Chim Acta. 2007; 381: 63–68.
17. *Vermeire S., van Assche G., Rutheerts P.*: Laboratory markers in IBD: useful, magic, or unnecessary toys? Gut 2006; 55: 426–431.
18. *Sutherland A.D., Gearry R.B., Frizelle F.A.*: Review of fecal biomarkers in inflammatory bowel disease. Dis Colon Rectum. 2008; 51: 1283–1291.
19. *van Rheenen P.F., van de Vijver E., Fidler V*: Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. Br Med J. 2010; 341: c3369.
20. *von Roon A.C., Karamountzos L., Purkayastha S.*: Diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy. Am J Gastroenterol. 2007; 102: 803–813.
21. *Czepiel J., Biesiada G., Brzozowski T., et al.*: The role of local and systemic cytokines in patients infected with *Clostridium difficile*. J Physiol Pharmacol. 2014; 65: 695–703.

22. *Penichea A.G., Savidge T.C., Dann S.M.*: Recent insights into *Clostridium difficile* pathogenesis. Curr Opin Infect Dis. 2013; 26: 447–453.
23. *Kim J., Kim H., Oh H.J.*: Fecal calprotectin level reflects the severity of *Clostridium difficile* Infection. Ann Lab Med. 2017; 37: 53–57.
24. *Rao K., Santhosh K., Mogle J.A., Higgins P.D., Young V.B.*: Elevated fecal calprotectin associates with adverse outcomes from *Clostridium difficile* infection in older adults. Infect Dis (Lond). 2016; 48: 663–669.
25. *Swale A., Miyajima F., Roberts P, et al.*: Calprotectin and lactoferrin faecal levels in patients with *Clostridium difficile* infection (CDI): a prospective cohort study. PLoS One. 2014; 9: e106118.
26. *Peretz A., Tkhawkho L., Pastukh N., Brodsky D., Halevi C.N., Nitzan O.*: Correlation between fecal calprotectin levels, disease severity and the hypervirulent ribotype 027 strain in patients with Clostridium difficile infection. Infect Dis. 2016; 16: 309.
27. *Wultańska D., Obuch-Woszczatyński P, Piotrowski M., Pituch H.*: Assessment of fecal calprotectin concentration in samples from patients with *Clostridium difficile* infection. Med Dośw Mikrobiol. 2018; 70: 159–165.