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The analysis of protein deficiency in patients hospitalized due to *Clostridium difficile* infection

Analiza deficytów białkowych u osób hospitalizowanych z powodu biegunki związanej z *Clostridium difficile*

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Key words

diarrhoea, *Clostridium difficile,* protein, albumins

Słowa kluczowe

biegunka, *Clostridium difficile*, białko, albumina

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Summary

Introduction. *Clostridium difficile* infections are one of the major causes of bacterial diarrhoea, particularly in patients aged over 65 years. Hospitalization time depends not only on the severity of the diarrhoea, but mainly on the decompensation of the general status of patients with concurrent chronic diseases. Protein-losing enteropathy, a complication resulting from the exposure of the intestines to toxins released by *C. difficile*, leads to a considerable loss of protein, particularly the albumin fraction, and deterioration of the patient's general health.

Aim. The aim of this study was to evaluate protein deficiency in patients with CDI and to identify the risk factors for such disorders related to demographic and iatrogenic parameters.

Material and methods. The retrospective analysis of *C. difficile*-associated diarrhoea (CDAD) among patients hospitalized at the Department of Infectious Diseases (J. Struś Multispecialist City Hospital, Poznań) in 2013.

An episode of CDI was diagnosed based on the recommendations of the experts.

The aetiology of *Clostridium difficile*-associated diarrhoea was confirmed using TECHLAB immunoenzymatic tests, i.e. a rapid membrane-based assay detecting the glutamate dehydrogenase (GDH) antigen and a rapid assay detecting toxins A and B from *C. difficile*. Protein metabolism was evaluated based on the measured total protein or albumin levels in blood plasma.

Protein disorders were analysed depending on the age and sex of patients, the duration and severity of the diarrhoea, and inflammatory markers, i.e. levels of C-reactive protein and leukocytosis. The correlation between the creatinine levels and risk factors for CDI was also tested.

Test results were analysed using the Shapiro-Wilk and Mann-Whitney tests.

Results. Deficiency in total protein and albumin levels was found in 28 (71,8%) of patients from the studied population. Total protein deficiency correlated with deficiency of the albumin fraction, while the severity of the deficiency depended on the duration of the diarrhoea, and not on the daily number of passed stools. No evidence supporting the assumed correlation between the protein deficiency and the patient's age, renal function or the levels of inflammatory markers (C-reactive protein and leukocytosis) was found.

Conclusions. CDI is a systemic disease correlated with protein loss. Because of the patient's age and numerous concomitant diseases, CDI poses a serious threat to the patient's life, and in each case requires the evaluation of protein metabolism.

Streszczenie

Wstęp. Zakażenia *Clostridium difficile* są jedną z dominujących przyczyn biegunki bakteryjnej, zwłaszcza wśród osób po 65. roku życia. Czas trwania hospitalizacji zależy nie tylko od nasilenia biegunki, ale zwłaszcza od dekompensacji stanu ogólnego osób z towarzyszącymi chorobami przewlekłymi. Z powodu enteropatii wysiękowej, będącej powikłaniem

ekspozycji jelit na toksyny *C. difficile*, dochodzi do znacznego ubytku białka, a zwłaszcza frakcji albumin, co wiąże się z pogorszeniem stanu ogólnego chorujących osób.

Cel pracy. Celem badania była ocena deficytów białkowych u pacjentów leczonych z powodu biegunki związanej z *Clostridium difficile* oraz wyznaczenie czynników ryzyka takich zaburzeń wśród parametrów demograficznych oraz jatrogennych.

Materiał i metody. Retrospektywna analiza zachorowań na biegunkę związaną z *C. difficile* CDI wśród pacjentów Oddziału Zakaźnego (Wielospecjalistyczny Szpital Miejski im. J. Strusia, Poznań) hospitalizowanych w 2013 roku.

Epizod CDI rozpoznawano w oparciu o rekomendacje ekspertów. Etiologię biegunki *Clostridium difficile* potwierdzono testami immunoenzymatycznymi TECHLAB, tj. szybkim testem membranowym na obecność antygenu dehydrogenazy glutaminowej (GDH) oraz szybkim testem do detekcji toksyny A i B *C. difficile*.

Ocenę gospodarki białkowej prowadzono w oparciu o oznaczenie stężenia białka całkowitego lub albumin w surowicy krwi.

Zaburzenia białkowe analizowane były w zależności od wieku i płci pacjentów, czasu trwania i nasilenia biegunki oraz wykładników stanu zapalnego takich jak stężenia białka C-reaktywnego oraz leukocytozy. Sprawdzono również korelację pomiędzy stężeniem kreatyniny oraz uznawanych za czynnik ryzyka wystąpienia CDI.

Zgodność z rozkładem normalnym sprawdzono za pomocą testu Shapir-Wilka. Porównanie miedzy grupami kobiet i mężczyzn wykonano za pomocą testu Manna-Whitney'a.

Wyniki. W badanej populacji potwierdzone niedobory stężenia białka całkowitego oraz albumin wystąpiły u 28 (71,8%) chorych. Niedobór białka całkowitego korelował z niedoborem frakcji albumin, zaś głębokość niedoboru była zależna nie od dobowej liczby stolców, ale od czasu trwania biegunki. Nie potwierdzono również przypuszczenia, że niedobór białka jest zależny od wieku pacjenta, czynności nerek lub wysokości wskaźników stanu zapalnego (białka C-reaktywnego oraz leukocytozy).

Wnioski. Biegunka związana z *C. difficile* jest chorobą ogólnoustrojową, która łączy się z utratą białka. Z uwagi na wiek pacjentów oraz liczne choroby współistniejące, biegunka związana z *C. difficile* jest poważnym zagrożeniem dla życia pacjentów i w każdym przypadku wymaga diagnostyki gospodarki białkowej.

INTRODUCTION

Clostridium difficile is a Gram-positive anaerobic, toxigenic and spore-forming bacterium. It was described for the first time in the 1940s as a nonpathogenic component of the intestinal microbial flora in healthy new-born infants (1). After broad-spectrum antibiotics were introduced in medical practice, attention was drawn to the pathogenic nature of this bacterium due to the growing number of patients suffering from pseudomembranous colitis (2). Currently, it is known that the spectrum of clinical manifestations for *Clostridium difficile* includes asymptomatic colonization, but also cases of colitis of various severity, including watery diarrhoea, pseudomembranous inflammation and toxic megacolon (2). The clinical picture is largely determined by the immune system function in individual patients, but disorders in the composition of intestinal microbial flora are the key factor for the development of *Clostridium difficile* infections (CDI) (3). For this reason, the inducing factors, e.g. the use of antibiotics, proton pump inhibitors, immunosuppressants, as well as hospitalizations or concomitant diseases are the major risk factors for this disease (3).

The worldwide incidence of CDI has been increasing for several decades, particularly in the developed countries (4). This process is attributed to the increased incidence of the above-mentioned risk factors, but also to the emergence of hypervirulent strains, and the ageing of the population (3, 4).

In 2013 in Poland 4716 cases of the disease were reported, which makes *Clostridium difficile* second to *Salmonella* spp. as the most frequent bacterial cause of intestinal infections. The prevalence of CDI is 12.24 per 100 000 people, and is currently lower than in other developed countries (5).

The rate of increase and scale of the problems associated with *Clostridium difficile* stimulate the need for the development and updating of guidelines on the classification, diagnostics, treatment and prevention of CDI. Despite the growing number of community-acquired CDI cases (CA-CDI), the disease is usually contracted through contact with health care and is one of the major iatrogenic types of infection (Health Care-Associated Infection – HCAI) (4). According to reports from the United States, including the analysis of the 5 most frequent HCAIs, these diseases generate annual costs of USD 9.8 bn, of which CDI is responsible for 15.4% (6).

In everyday medical practice, protein disorders seem to be frequent among patients hospitalized due to CDI. Proteins are involved in all processes at the cellular level and the entire body. Proteins take part in the coordination of life functions at multiple levels: the generation and transmission of information, buffering changes in the levels of other biologically active substances, the control of growth and differentiation, and cell-cell interactions. Albumins are mainly responsible for the maintenance of the oncotic pressure of plasma and its correct volume. They are also the carrier proteins transporting free fatty acids, bilirubin, certain drugs, metal ions and hormones. Albumins also form the protein reserve in the body and are used as a source of amino acids for the synthesis of proteins in cells of organs other than the liver (7). For this rea-

son protein deficiency frequently results in peripheral oedema, ascites and hypotension. The mechanisms responsible for protein deficiency in patients with CDI and caused by protein-losing enteropathy were described more than twenty years ago (8). However, the results of studies analysing the incidence of protein disorders among patients with CDI were not conclusive and indicated the need for further research (9). Currently available reports contain the analysis of hypoproteinaemia in the context of evaluation of the severity of CDI recurrence (9). We have retrieved only one report evaluating protein deficiency in patients treated for CDI (10). Therefore, our study may set new trends in research and contribute to updating currently available guidelines.

AIM

The aim of this study was to evaluate protein deficiency in patients with CDI and to identify the risk factors for such disorders related to demographic and iatrogenic parameters.

MATERIAL AND METHODS

We carried out a retrospective analysis of CDI among patients hospitalized at the Department of Infectious Diseases in 2013.

An episode of CDI was diagnosed based on the recommendations of the Polish National Programme of Antibiotic Protection, released in 2011, which are in line with the guidelines of the European Society for Clinical Microbiology and Infectious Diseases. According to the guidelines, the diagnosis of CDI was based on bacterial culture to confirm the presence of *C. difficile* in faeces, or detecting bacterial antigens or metabolites (11, 12).

The etiology of CDI was confirmed using TECHLAB immunoenzymatic tests, i.e. a rapid membrane-based assay detecting the glutamate dehydrogenase (GDH) antigen (sensitivity: 92.8%, specificity: 92.6%, positive predictive value: 78.6%, negative predictive value: 98.7%, correlation: 92.6%) and a rapid assay detecting toxins A and B from *C. difficile* (sensitivity: 90.2%, specificity: 99.7%, positive predictive value: 98.6%, negative predictive value: 97.9%, correlation: 98.0%).

Protein metabolism was evaluated based on the measured total protein and/or albumin levels in blood plasma.

Total protein levels were determined using a colorimetric method (burette reaction) by measuring the increase in absorbance at wavelength 540 nm. The sensitivity and range of the test was 1.0-15.0 mg/dl. The range for biological reference values was 6.2-8.5 g/dl.

Albumin levels were determined using a colorimetric method by measuring the increase in colour intensity at the wavelength of 630 nm. The sensitivity and range of the test was 0.5-8.0 g/dl. The range for biological reference values was 3.5-5.3 g/dl.

CRP levels were measured using the latex particle enhanced immunoturbidimetric method. The sensitivity and range of the test was 0-220 mg/l. The range for biological reference values was 0-10 mg/l.

Test results were analysed using the Shapiro-Wilk and Mann-Whitney tests.

Protein disorders were analysed depending on the age and sex of patients, the duration and severity of the diarrhoea, and inflammatory markers, i.e. levels of C-reactive protein and leukocytosis. We also tested the correlation between the creatinine levels and risk factors for CDI.

RESULTS

CDI was diagnosed in 77 hospitalized patients. Protein metabolism was evaluated in 39 patients from the study group. Women were in the majority 22 vs. 17 (56 vs. 44%).

The mean age of patients was 73.21 years (range 18-99 years, standard deviation 17.8 years) (fig. 1).

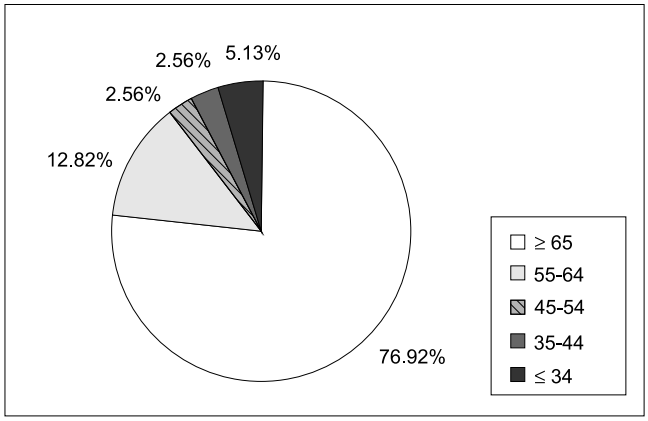

Fig. 1. Distribution of the study group (in per cent) by age.

Mean duration of diarrhoea was 9.24 days. The mean number of stools reported per day was 6.61 (max. 19). For most of the patients this was the first episode of CDI, but the maximum number of recurrences was 3 (fig. 2).

Diarrhoea was associated with increased levels of inflammatory markers. The mean level of C-reactive protein was 102 mg/dl, and the maximum CRP level was above 240 mg/dl. Importantly, the increase in C-reactive protein level was not associated with the increased leukocyte count (mean 14.57 G/L), and only in one case was it 36.20 G/L (data not shown).

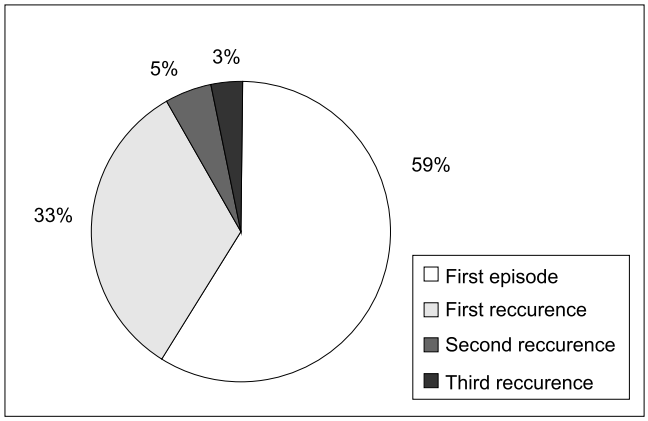

Fig. 2. Distribution of the study group (in per cent) by the number of CDI episodes.

Decreased levels of total protein or the albumin fraction were found in 28 (71.8%) patients. Mean total protein level was 5.32 g/dl, and 2.85 g/dl for albumins. In nine (28,125%) patients the albumin level was below 2.5 g/dl (fig. 3).

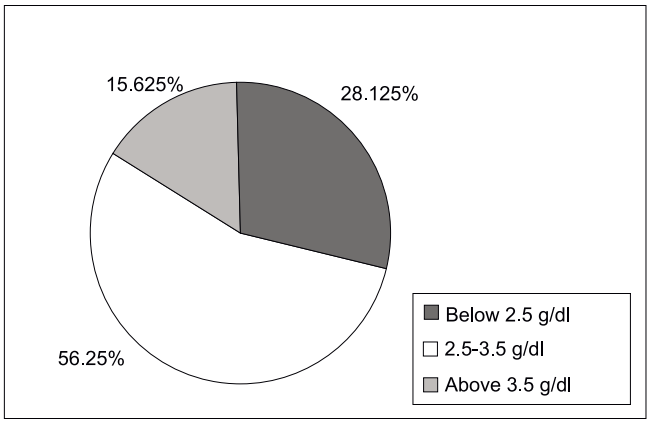

Fig. 3. Distribution of the study group (in per cent) depending on the albumin plasma level.

The correlation between the duration of diarrhoea and albumin deficiency ($p < 0.05$) was confirmed in the study group (fig. 4) (tab. 1).

The analysis of total protein and the albumin fraction levels confirmed that the protein loss mainly concerns the albumin fraction, and there is a strong correlation between the loss of total protein and albumins ($p = 0.0281$).

No correlation was found between the number of stools, inflammatory markers (leukocytosis, CRP levels), GFR, hematocrit and age, sex or loss of total protein or albumin (tab. 1).

The correlation between the use of individual antibiotics and the incidence of CDI is presented in table 2. According to the table, the most frequently used antibiotics were ceftriaxone, amoxicillin, ciprofloxacin and cefuroxime. Interestingly, one hospitalized patient had an episode of CDI following the use of metronidazole.

Table 1. Correlation between protein loss and/or albumin loss and the age of the patient, duration of the diarrhoea, number of stools, eGFR, leukocyte count, C-reactive protein level, hematocrit level and creatinine level.

Analysed parameters	N	Correlation coefficient r	p
% protein loss & age	28	-0.1470	0.4553
% protein loss & duration of diarrhoea	23	0.0055	0.9801
% protein loss & creatinine	28	0.1425	0.4695
% protein loss & eGFR	28	-0.1574	0.4238
% protein loss & number of stools	27	0.1029	0.6096
% protein loss & leukocytes	28	0.0115	0.9537
% protein loss & CRP	28	-0.0761	0.7004
% protein loss & HCT	28	0.0454	0.8184
% albumin loss & age	29	0.1476	0.4447
% albumin loss & duration of diarrhoea	24	0.6567	0.0281
% albumin loss & creatinine	29	0.0489	0.8010
% albumin loss & eGFR	29	-0.1097	0.5712
% albumin loss & number of stools	29	0.0612	0.7526
% albumin loss & leukocytes	29	0.2529	0.1856
% albumin loss & CRP	28	0.0151	0.9393
% albumin loss & HCT	29	0.0628	0.7463

Table 2. Correlation between the use of individual antibiotics and incidence of CDI.

Antibiotic taken	Number of patients	% of patients
Ceftriaxone	4	18.18%
Ciprofloxacinum	3	13.63%
Amoxicillinum, Acidum clavulanicum	3	13.63%
Sulfamethoxazolum, Trimethoprimum	2	9.09%
Metronidazolum	1	4.55%
Piperacillin, Tazobactam	1	4.55%
Cefuroximum	1	4.55%
Not specified	7	31.82%

DISCUSSION

CDI is a growing epidemiological problem in Poland and other countries (5, 13). In 2013 the prevalence of CDI in Poland was estimated at 12.24 cases per 100 000 people. Because the obligation to report intestinal infections caused by *Clostridium difficile* in Poland was introduced in early 2013, it is impossible to describe the epidemiological trend in an objective fashion (5). However, epidemiological data from hospital departments indicate a clear and significant increase in the number of CDI cases. For this reason *Clostridium difficile* is regarded as an alert pathogen. For example,

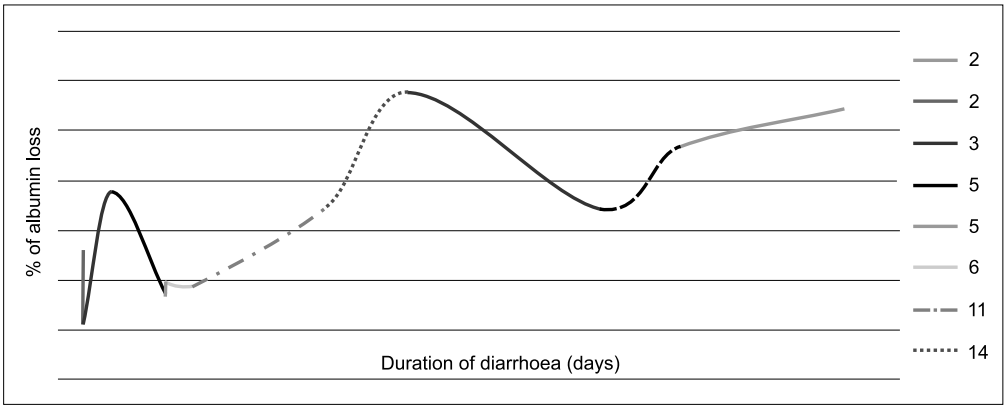

Fig. 4. Correlation between albumin loss and duration of diarrhoea.

among patients discharged from hospitals in the United States of America, the number of diagnosed cases of CDI increased from 3.82/1000 in 2000 to 8.75/1000 in 2008 (13). A significant increase was reported among patients aged over 65 years (13).

Results from our study also indicated a significantly higher prevalence in this age group. Mean patient age was 72.21 years, and patients aged over 65 years accounted for more than 75% of the studied population. This result correlates with findings by other researchers, who estimate that the risk of infection in people older than 65 years is 20-times greater than in people under 20 years of age (14). For this reason being over 70 years of age is one of the risk factors for a severe course of CDI (9).

In 1996 Dansinger et al. studied the differences between asymptomatic colonization and diarrhoea associated with the presence of toxins A and B from *C. difficile*, and found a significant protein loss leading to malnutrition in patients with diarrhoea, but no protein loss in asymptomatic patients (8). The severity of malnutrition was not correlated with age, but resulting complications were correlated with concomitant diseases (8).

Data suggest that when hospitalizing elderly patients with CDI particular attention should be paid to concomitant diseases and disorders resulting from them, as well as to the need for the use of multiple medications. According to our findings, the hospitalization time depends not so much on the severity of the diarrhoea, but mainly on the decompensation of the general status of the patient. Hospitalization time was frequently prolonged due to cardiopulmonary insufficiency caused by the primary protein loss. The presented results point out the fact that protein deficiency greatly depends on the duration of the diarrhoea, and not on the number of daily stools. This should particularly draw the attention of general practitioners, who should consider CDI, confirmed by rapid and inexpensive tests for the detection of toxins A and B, in differential diagnosis of moderately-severe cases of diarrhoea (2-3 stools daily) lasting for longer than 14 days. In addition, the diagnostics of protein deficiencies has to be implemented, as they may cause a considerable deterioration in the patient's general status.

Results from our study indicated that total protein and albumin levels are an important predictive factor in patients hospitalized due to CDI. It should be pointed out that an albumin level below 2.5 g/dl is one of the risk factors for severe cases of CDI (9, 11). Mean total protein level in the study group was 5.32 g/dl, and 2.85 g/dl for albumins. In our material, covering a small study group, 28.125% of patients had albumin levels below 2.5 g/dl. Similar findings (27.7%) were made by researchers who evaluated a group of 336 patients with CDI (15).

It is worth noting that the priorities of the Polish National Health Plan for 2007-2015 for the prevention of infectious diseases and infections include, e.g. a reduction in the number of food poisoning cases and gastrointestinal infections caused by biological factors, and an improvement in the control of nosocomial infections on a national scale (16). Therefore, the problem of *Clostridium difficile* infections requires further investigation, and protein deficiencies in patients with CDI have to be analysed in detail.

CONCLUSIONS

1. CDI is correlated with protein loss, especially in the albumin fraction, while the protein deficiency depends on the duration of diarrhoea, not on the number of stools.
2. CDI is a systemic disease, as suggested by the increased levels of C-reactive protein.
3. Because of the patient's age and numerous concomitant diseases, CDI poses a serious threat to the patient's life, and in each case requires the evaluation of protein metabolism.

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received/otrzymano: 10.09.2014
accepted/zaakceptowano: 14.10.2014