Faecal lactoferrin and calprotectin in patients with Clostridium difficile infection: a case–control study

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Abstract Calprotectin and lactoferrin are released by the gastrointestinal tract in response to infection and mucosal inflammation. Our objective was to assess the usefulness of quantifying faecal lactoferrin and calprotectin concentrations in *Clostridium difficile* infection (CDI) patients with or without free toxins in the stools. We conducted a single-centre 22-month case–control study. Patients with a positive CDI diagnosis were compared to two control groups: group 1 = diarrhoeic patients negative for *C. difficile* and matched (1:1) to CDI cases on the ward location and age, and group 2 = diarrhoeic patients colonised with a non-toxigenic strain of *C. difficile*. Faecal lactoferrin and calprotectin concentrations in faeces were determined for patients with CDI and controls. Of 135 patients with CDI, 87 (64.4%) had a positive stool cytotoxicity assay (free toxin) and 48 (35.6%) had a positive toxigenic culture without detectable toxins in the stools. The median lactoferrin values were 26.8 μg/g, 8.0 μg/g and 15.8 μg/g in CDI patients and groups 1 and 2, respectively. The median calprotectin values were 218.0 μg/g, 111.5 μg/g and 111.3 μg/g, respectively. Among patients with CDI, faecal lactoferrin and calprotectin levels were higher in those with free toxins in their stools (39.2 vs. 10.2 μg/g, $p = 0.003$ and 274.0 vs. 166.0 μg/g, $p = 0.051$, respectively). Both faecal calprotectin and lactoferrin were higher in patients with CDI, especially in those with detectable toxin in faeces, suggesting a correlation between intestinal inflammation and toxins in stools.

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Introduction

Clostridium difficile is recognised as the main causative agent responsible for nosocomial diarrhoea in adults [1]. The symptoms of *C. difficile* infections (CDI) range from self-limited diarrhoea to fulminant and sometimes fatal pseudomembranous colitis [2]. A study from the Netherlands found that patients with CDI had a higher 30-day mortality rate than controls who had diarrhoea but tested negative for *C. difficile* toxins (hazard ratio 1.6; 95% confidence interval [CI] 0.9–2.8). This finding suggests that CDI, rather than diarrhoea per se, increased the mortality risk [3]. Major virulence factors include toxin A (tcdA) and toxin B (tcdB) that trigger intestinal inflammation and cell death [4]. Accurate and rapid diagnosis of CDI is essential for optimal treatment and prevention; however, reaching this goal continues to be challenging [5, 6].

Currently, the biological diagnosis of CDI is based on the detection in the stools of either free toxins (using enzyme immunoassays [EIAs] or a stool cytotoxicity assay) or a

toxigenic strain (using toxigenic culture methods or nucleic acid amplification tests [NAATs]). However, as the EIAs for toxins lack sensitivity, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines recommend that they should not be used as standalone tests for the diagnosis of CDI [7]. Conversely, although NAATs are more sensitive, they are less specific for CDI compared with the direct detection of toxins in stools. Thus, while toxin tests may occasionally produce false-negatives in patients with CDI, tests for toxigenic *C. difficile* may result in over-diagnosis and over-treatment. In particular, the new NAATs raise the crucial question of the clinical significance of the presence of a toxigenic strain without any free toxin in stools. Two large and recent studies from the UK and the USA showed that toxin positivity better correlates with clinical outcome and best defines true cases of CDI [6, 8, 9].

Calprotectin and lactoferrin are two proteins produced by polymorphonuclear leucocytes and released by the gastrointestinal tract in response to infections and mucosal inflammation [10]. Measuring faecal concentrations of these proteins has been used to estimate the level of inflammation in diseases such as inflammatory bowel disease (IBD). Evidence regarding its use in the assessment of CDI remains limited [11–15].

The aim of this study was to evaluate whether faecal levels of calprotectin or lactoferrin could be used to distinguish patients with true CDI from those carrying a toxigenic strain.

Materials and methods

Study population

We conducted a case–control study at a single centre between 1 January 2014 and 31 October 2015. Adult in-patients with a positive diagnosis of CDI (≥3 liquid stools per day plus a positive test for toxins and/or a toxigenic strain) were consecutively recruited and compared to two control groups identified within the same time frame: group 1 consisted of diarrhoeic patients who tested negative for *C. difficile* and matched (1:1) to CDI cases on the ward location and age group (18–45, 45–65 and >65 years); group 2 consisted of diarrhoeic patients with a non-toxigenic (NT) strain of *C. difficile*. Patients with digestive infections due to organisms other than *C. difficile* or with IBD were excluded from the study.

The study protocol was approved by the local Research Ethics Committee and presented to the Infection Control Committee. Information was given to the patients but informed consent was not required by the ethics committee.

Clinical assessment

For each patient included, their clinical chart was reviewed and the following data were recorded: age, gender, co-morbidities, Charlson score, number of stools on the day of the CDI diagnosis, clinical symptoms (abdominal pain, fever, bloating), white blood cell counts, serum albumin, creatinine and C-reactive protein (CRP) levels. The origin of diarrhoea (community-acquired or healthcare-associated) was defined according to the European Centre for Disease Prevention and Control (ECDC)/Centers for Disease Control and Prevention (CDC) criteria [16, 17]. The severity of CDI was defined using the criteria summarised in electronic supplementary Table S1 [18–22]. CDI was considered complicated when patients presented with septic shock, perforation, admission to the intensive care unit (ICU), colectomy or megacolon [23]. Mortality was assessed at days 10, 30 and 90.

Laboratory assays

Stool samples were tested for *C. difficile* within 48 h of collection. Three different strategies were used for each stool sample: (i) the stool cytotoxicity assay on cell culture, (ii) the toxigenic culture on selective medium and (iii) a two-step algorithm. For the quantification of calprotectin and lactoferrin, stool samples were frozen and stored at −80 °C before use.

Stool cytotoxicity assay (CTA)

CTA was performed using MRC-5 cells. Fresh stool specimens were diluted in phosphate-buffered saline (PBS) (1:10 [w/v]) and centrifuged at 2500 × g for 30 min. The supernatant was passed through a 0.45-μm pore size filter and added to confluent monolayers of MRC-5 cells in 96-well microplates. The final stool dilution was 1:100. Plates were incubated at 37 °C in a 6.5% CO_2 atmosphere for 48 h. Samples were considered positive if a characteristic cytopathic effect (cell rounding) was observed for at least 50% of the cells which could be neutralised with anti-*Clostridium sordellii* antiserum (obtained from M. R. Popoff, National Reference Center for Anaerobes, Pasteur Institute, Paris, France) [5].

Toxigenic culture

Toxigenic culture was performed with the selective chromogenic medium ChromID *C. difficile* (bioMérieux, Marcy l'Etoile, France). Plates were incubated for 48 h at 37 °C in an anaerobic atmosphere. Black colonies or suspicious non-black colonies (based on the morphological aspect) were analysed using matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (Bruker Daltonik, GmbH). *Clostridium difficile* isolates were incubated in brain heart infusion broth for 5 days and the supernatant was tested using the CTA.

Two-step algorithm

A first screening step detected the glutamate dehydrogenase (GDH) using the C. DIFF CHEK-60 assay (TechLab, Blacksburg, VA), according to the manufacturers' instructions. In a confirmatory step, positive stool samples were tested for the presence of the *tcd*B gene using polymerase chain reaction (PCR) (Xpert *C. difficile*, Cepheid, Sunnyvale, CA).

Faecal calprotectin and lactoferrin quantification

Faecal calprotectin concentrations were determined using a quantitative immunoassay according to the manufacturers' instructions (Quantum Blue, Bühlmann). Briefly, faecal aliquots (≈80 mg) were extracted using a faecal sample preparation kit (Bühlmann Smart-Prep). Extraction was performed in duplicate for each stool sample. Each sample was then diluted 1:10 with extraction buffer and centrifuged. The resulting supernatant was assayed using a lateral flow assay (Quantum Blue Calprotectin Extended quantitative lateral flow assay), with a range between 30 and 1000 μg/g. For values exceeding 1000 μg/g, the Quantum Blue Calprotectin High Range assay was used, with a range between 100 and 1800 μg/g. Samples with values exceeding 1800 μg/g were further diluted 1:5 with Chase Buffer and re-tested according to the manufacturers' instructions. The final concentration for each stool sample was determined by averaging the two values measured using the Quantum Blue Calprotectin extended assay. When values exceeded 1000 μg/g, the concentration measured using the High Range assay was acceptable.

Faecal lactoferrin concentrations were determined with a quantitative enzyme-linked immunosorbent assay (ELISA) according to the manufacturers' instructions (IBD-SCAN, TechLab, Blacksburg, VA). Briefly, 50 μL of stool samples were diluted 1:100 and 1:1000. Diluted specimens and standards were transferred to microplate wells. The lactoferrin concentration was determined by plotting absorbance values on a curve generated by lactoferrin standards ranging from 6.25 to 100 ng/mL. If both sample dilutions had absorbance readings greater than that recorded for the highest concentration of standard, the test was repeated using additional 1:10,000 dilutions. Conversely, any sample having an absorbance reading less than that recorded for the lowest concentration of standard was retested using a 1:10 dilution and, if found negative, recorded as <1 μg/g wet weight. The final concentration for each stool sample was determined by averaging the two values recorded with the same dilution.

Data analysis

Statistical analyses were performed using R software version 3.1 (R Foundation for Statistical Computing, Vienna, Austria) and GraphPad Prism 5.04 (GraphPad Software, Inc., La Jolla, CA). Patient demographic characteristics were described by their median and interquartile range (IQR) for quantitative variables and frequency for categorical variables. Quantitative and categorical variables were compared between cases and matched controls (group 1) with Wilcoxon signed-rank and McNemar tests and between cases and unmatched controls (group 2) with Mann–Whitney–Wilcoxon and Fisher exact tests. We plotted receiver operating characteristic (ROC) curves and estimated the area under curve (AUC) to assess the ability of faecal lactoferrin and calprotectin to distinguish patients with CDI from their matched controls, for both patients with and without free toxins in their stools. Covariates associated with CDI cases with and without detectable toxins were identified with univariate conditional logistic regression modelling and multivariate modelling, after adjustment for the main baseline confounders (age, gender and Charlson score). Normality of quantitative covariates was tested and $\log_2$-transformed when needed. Regression estimates for these covariates should, therefore, be interpreted as a variation per two-fold increase of the value. As lactoferrin and calprotectin quantification was left- and right-censored, we used values beyond these thresholds for the lower or upper limits of detection. A p-value of <0.05 was considered significant.

Results

A total of 135 patients with CDI (81 females and 54 males, median age 64 [IQR: 51–78]) were included: 87 (64.4%) had a positive CTA (free toxin) and 48 (35.6%) had a negative CTA (absence of detectable toxins in the stools) but a positive toxigenic culture or PCR result. Among patients without detectable toxins in the stools, one had a positive toxigenic culture and a negative PCR result, whereas three other patients had a negative toxigenic culture and a positive PCR result. Among the CDI cases, 113 (83.7%) were primary episodes and 22 (16.3%) were recurrences. Patients were hospitalised in medical wards (n = 116; 85.9%), surgical wards (n = 9; 6.7%) or the ICU (n = 10; 7.4%). CDI cases were categorised as healthcare-associated (n = 115; 85.1%), community-acquired (n = 19; 14.1%) or of indeterminate origin (n = 1; 0.7%). In the previous 3 months, 104 (77.0%) patients had received antibiotic treatment. The frequency of moderate/severe cases ranged from 19.3% to 80.0%, depending on the definition used (Table 1). There were five complicated cases (defined by septic shock, perforation, admission to the ICU, colectomy or megacolon; 3.7%). Six deaths (4.4%) were CDI-related.

The median lactoferrin values were significantly higher in patients with CDI (26.8 μg/g; IQR: 4.5–79.8) than in the matched controls who were CDI-negative (8.0 μg/g, IQR: 2.1–32.7) (p = 0.0017) (Table 1). Similarly, the median calprotectin values were higher in patients with CDI (218.0 μg/g, IQR: 67.2–

Table 1 Comparison of *Clostridium difficile* infection (CDI) patients with their matched controls (group 1) and unmatched controls harbouring a non-toxigenic strain of *C. difficile* (group 2)

Variables	CDI (cases) (n = 135)	Matched controls (group 1) (n = 135)		Unmatched controls with a non-toxigenic *C. difficile* strain (group 2) (n = 50)	
Biological and clinical features			p-Value		p-Value (versus CDI cases)
Age (years)	64 [51–78]	63 [49.5–73.5]	0.1322	58 [45.8–66.0]	**0.0136**
Gender (female), n (%)	81 (60%)	66 (48.9%)	0.0548	21 (42.0%)	**0.0318**
Charlson index	2 [2–3]	2 [2–3]	0.1952	2 [2–4]	0.2396
Immunosuppression	67 (49.6%)	76 (56.3%)	0.1172	40 (80.0%)	**0.0002**
No. of stools/day	4 [3–6]	3 [2–5]	**0.0019**	3 [2–4]	**0.0061**
Temperature (°C)	37.3 [37.1–38.2]	37.2 [37.0–37.95]	0.1163	37.3 [37.3–38.0]	0.4421
Abdominal distension, n (%)	24 (17.8%)	10 (7.4%)	0.0082	0 (0.0%)	**0.0004**
Rectal bleeding, n (%)	5 (3.7%)	3 (2.2%)	0.4795	0 (0.0%)	0.3258
Abdominal pain, n (%)	86 (63.7%)	63 (46.7%)	**0.0019**	25 (50.0%)	0.0950
Leucocytes (/mm^3)	7580 [3105–14,250]	7395 [2482–10,430]	**0.0362**	4560 [2000–7572]	**0.0059**
Polynuclear neutrophil count (/mm^3)	7030 [2510–13,790]	5020 [2100–7820]	0.0016	3685 [2008–6002]	**0.0025**
Creatinine (μmol/L)	73 [58.25–95]	79 [65–106.50]	**0.0102**	77 [56–104]	0.7205
Albuminemia (mg/dL)	32 [26–35]	32 [26–36]	0.1889	31 [27–34]	0.4580
C-reactive protein (mg/L)	45 [17–109.5]	39 [16–104]	0.6492	47 [9–108]	0.5394
Calprotectin (μg/g)	218.0 [67.2–795.5]	111.5 [34.8–374.5]	**0.0010**	111.3 [43.9–374.8]	**0.0113**
Lactoferrin (μg/g)	26.8 [4.5–79.8]	8.0 [2.1–32.7]	**0.0017**	15.9 [2.5–51.6]	0.0634
Outcome, n (%)					
Death at D10	5 (3.7%)	4 (3.0%)	0.7389	2 (4.0%)	1
Death at D30	18 (14.5%)	10 (9.3%)	0.2253	5 (10.9%)	0.6221
Death at D90	31 (27.4%)	15 (15.6%)	**0.0343**	8 (19.6%)	0.3064
Scores of diarrhoea severity, n (%)					
Surawicz et al.			0.3173		1
Non-severe	67 (80.7%)	90 (80.4%)		35 (81.4%)	
Severe	16 (19.3%)	22 (19.6%)		8 (18.6%)	
Zar et al.			0.2498		**0.0402**
Non-severe	103 (76.3%)	110 (81.5%)		45 (90.0%)	
Severe	32 (23.7%)	25 (18.5%)		5 (10%)	
Johnson et al.			**0.0006**		**0.0042**
Mild	27 (20.0%)	51 (38.1%)		21 (42.0%)	
Moderate/severe	108 (80.0%)	83 (61.9%)		29 (58.0%)	
Louie et al.			**0.0004**		**0.0005**
Mild	64 (47.4%)	90 (67.2%)		38 (76.0%)	
Moderate/severe	71 (52.6%)	44 (32.8%)		12 (24%)	
Cornely et al.			0.8946		0.1505
Non-severe	88 (65.7%)	90 (67.2%)		38 (77.5%)	
Severe	46 (34.3%)	44 (32.8%)		11 (22.4%)	

795.5) than in the matched controls (111.5 μg/g, IQR: 34.8–374.5, p = 0.001). Forty-three patients had faecal calprotectin concentrations <30 μg/g (the detection threshold) and nine had concentrations above 9000 μg/g; for faecal lactoferrin, six patients had concentrations <1 μg/g and three had levels >950 μg/g. Both markers were highly correlated (Spearman's correlation coefficient 0.76; 95% CI 0.71–0.81; $p < 0.0001$). The median faecal lactoferrin and calprotectin levels were higher in patients with CDI and detectable toxins in stools compared to CDI patients without free toxin (39.0 μg/g, IQR: 6.7–106.0 vs. 10.2 μg/g, IQR: 3.1–37.5, p = 0.003 and 274.0 μg/g, IQR: 85.8–1321.0 vs. 166.0 μg/g, IQR: 47.0–535.0, p = 0.051, respectively).

The calprotectin levels were significantly higher in patients with CDI than the 50 controls with a non-toxigenic strain of *C. difficile* (referred to as control group 2) (218.0 μg/g, IQR: 67.2–795.5 vs. 111.2 μg/g, IQR: 43.9–374.8, $p = 0.011$). The lactoferrin levels were not significantly different between these groups (26.8 μg/g, IQR: 4.5–79.8 vs. 15.9 μg/g, IQR: 2.5–51.6, $p = 0.063$).

Patients with or without detectable toxins were compared separately to their corresponding matched controls. The ROC curves indicated that the AUC varied between 0.59 (95% CI 0.47–0.70) and 0.67 (95% CI 0.59–0.75) (Fig. 1). Using a univariate logistic regression model, the lactoferrin and calprotectin levels (expressed as $\log_2$) were significantly associated with CDI with detectable toxin but not with CDI without detectable toxin (estimates not reported here). Adjustments for age, gender and Charlson score did not provide significantly different results (Fig. 2, Table 2A and B).

We specifically examined the clinical data and outcome of CDI patients without detectable toxin in stools who displayed either high levels of faecal calprotectin (>500 μg/g, $n = 13$) or lactoferrin (> 50 μg/g, $n = 10$). Among the 15 patients, eight had both elevated faecal inflammatory markers. All patients except two (86.7%, 13/15) were treated with oral vancomycin ($n = 3$), metronidazole ($n = 9$) or fidaxomicin ($n = 1$), and were considered clinically cured at day 10.

Discussion

Calprotectin and lactoferrin are two proteins derived from polymorphonuclear neutrophils which are released by the gastrointestinal tract in response to infections and mucosal inflammation. As such, these markers are routinely used to monitor levels of inflammation in patients with IBD. It is proposed that, as CDI is histologically characterised by an infiltration of neutrophils, faecal lactoferrin or calprotectin may represent potential biomarkers for disease activity.

Using a case–-control study including 135 patients with CDI, we have shown that both faecal calprotectin and lactoferrin significantly increased during CDI. These two markers were highly correlated with each other ($r = 0.76$), which is unsurprising considering their common cellular origin. These results are consistent with those published by Swale et al. [12], who also compared both markers in a cohort

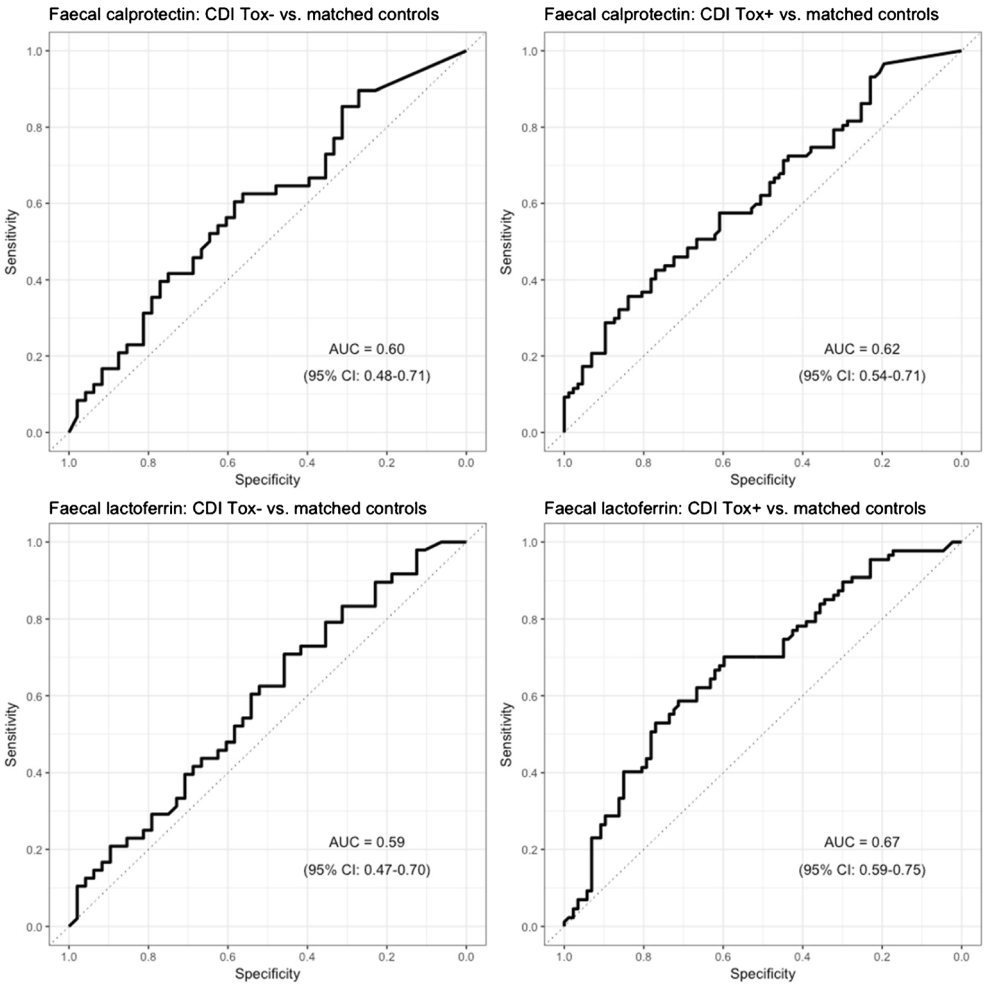

Fig. 1 Receiver operating characteristic (ROC) curves of faecal calprotectin and lactoferrin for predicting *Clostridium difficile* infection (CDI) based on positive free toxin test (*right side*) or positive *C. difficile* toxin gene assay without detectable toxin (*left side*)

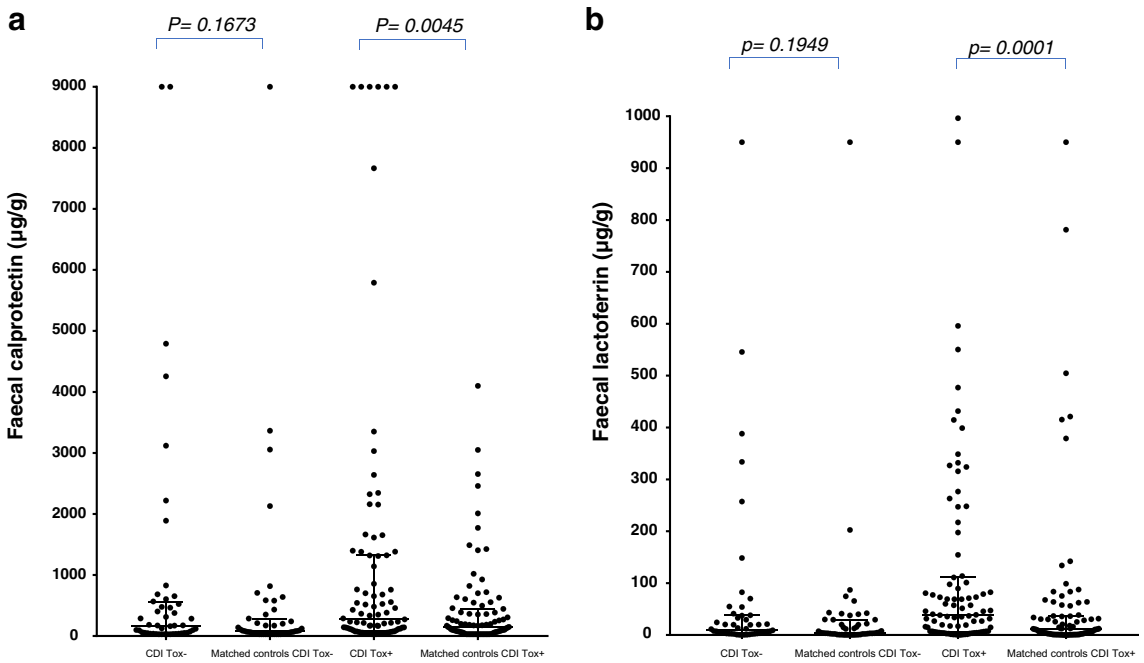

Fig. 2 Comparison of faecal calprotectin levels (**a**) and lactoferrin levels (**b**) in patients with CDI (stratified according to the presence or absence of detectable toxin) and their matched controls (the *bars* represent median values and their interquartile range)

Table 2 Comparison of CDI cases with (A) or without (B) detectable toxins in stools to their matched controls (multivariate logistic regression estimates adjusted for age, gender and Charlson score)

A			
Variables	OR	95% CI	*p*-Value
Lactoferrin (log_2)	1.244	1.097–1.410	**0.0007**
Calprotectin (log_2)	1.274	1.077–1.507	**0.0047**
No. of stools	1.101	0.984–1.231	0.0923
Leucocytes (log_2)	0.970	0.822–1.146	0.7203
Death at D30	2.136	0.527–8.659	0.2879
Death at D90	1.704	0.459–6.323	0.4260
Severe diarrhoea (Surawicz et al.)	0.495	0.136–1.807	0.2875
Severe diarrhoea (Zar et al.)	1.050	0.464–2.374	0.9069
Moderate/severe diarrhoea (Johnson et al.)	2.520	1.157–5.487	**0.0200**
Moderate/severe diarrhoea (Louie et al.)	1.795	0.817–3.943	0.1455
Severe diarrhoea (Cornely et al.)	0.868	0.457–1.652	0.6673
B			
Variables	OR	95% CI	*p*-Value
Lactoferrin (log_2)	1.116	0.968–1.287	0.1303
Calprotectin (log_2)	1.141	0.951–1.370	0.1562
No. of stools	1.157	0.9741.374	0.0960
Leucocytes (log_2)	0.943	0.748–1.189	0.6200
Death at D30	1.434	0.302–6.814	0.6500
Death at D90	5.905	1.212–28.767	0.0200
Severe diarrhoea (Surawicz et al.)	1.041	0.219–4.955	0.9595
Severe diarrhoea (Zar et al.)	4.949	0.551–44.444	0.1534
Moderate/severe diarrhoea (Johnson et al.)	3.504	1.125–10.917	**0.0305**
Moderate/severe diarrhoea (Louie et al.)	6.417	1.775–23.202	**0.0046**
Severe diarrhoea (Cornely et al.)	1.472	0.449–4.827	0.5237

of 162 patients and 52 non-matched controls with antibiotic-associated diarrhoea [12]. Consistent with other studies, we observed a great variability of faecal lactoferrin and calprotectin levels, with a significant overlap between cases and controls [14, 23]. This observation actually reduces the predictive accuracy of both markers for CDI and makes it difficult to determine an optimal cut-off value. However, the large inter-individual variability may be due, in part, to the over-diagnosis of CDI when tests for toxigenic *C. difficile* or NAATs are used. Interestingly, when the population with CDI was split into patients with a positive CTA (free toxin) and those positive for PCR or toxigenic culture but negative for the CTA, we found that levels of faecal calprotectin and lactoferrin remained significantly higher only in patients with free toxin compared to their controls. Similarly, the AUCs for both calprotectin and lactoferrin, although low, were slightly higher for CDI patients with free toxin compared to those who were positive for PCR or toxigenic culture but negative for the CTA. A similar observation was reported by LaSala et al., who found higher faecal lactoferrin levels in CDI patients with detectable toxin than in patients with a toxigenic strain without any detectable toxin in the faeces (median 80 [range: 3–124] μg/mL vs. 24 [range: 4–160] μg/mL, respectively) [14]. In another independent study, the median faecal calprotectin levels were higher in patients with a positive stool CTA than in those who were toxin gene positive but negative for the CTA assay (336 μg/g [IQR: 208–536 μg/g] vs. 249 μg/g [IQR: 155–498 μg/g] [24]. These data are consistent with two recent studies, which suggest that the severity of CDI is greater in toxin-positive than in toxin-negative patients [6, 8]. In a large study from the UK, Planche et al. [6, 9] found that patients with free toxins (detected by the stool CTA) had a significantly higher mortality rate within 6 months of diagnosis and higher mean white cells count than patients with positive NAATs and negative CTAs. Of note, a US study showed that virtually all CDI-related complications and deaths occurred in patients with positive toxin immunoassay test results [8]. In this study, patients with positive molecular and negative toxin immunoassay test results had comparable outcomes to patients without *C. difficile*. These data support the idea that *C. difficile* toxin is more closely linked to the infection than the toxin gene. The significance of the presence of toxigenic *C. difficile* strain without free toxin in a patient with diarrhoea remains debatable. This group of patients is likely to include patients with true CDI and patients carrying a toxigenic strain. Indeed, in our study, 15 patients from this group had a high level of either lactoferrin (>50 μg/g) or calprotectin (>500 μg/g). All except two were successfully treated for CDI for 10 to 14 days, suggesting that *C. difficile* might have been the cause of diarrhoea in these patients. This observation suggests that calprotectin or lactoferrin levels, in conjunction with clinical assessment, may help guide the decision to treat PCR-positive patients without detectable toxin in stools.

This study has several limitations. Firstly, it was performed in a single institution and the results might not be transposable to other healthcare settings with different patients and testing. Secondly, the number of patients is relatively low, despite representing one of the largest studies ever performed with these markers. Finally, we did not evaluate the usefulness of faecal lactoferrin and calprotectin for monitoring the efficacy of treatment or predicting the clinical outcome. However, we consider that this point deserves further study.

In summary, this case–control study showed that both faecal calprotectin and lactoferrin levels are higher in patients with CDI, especially those with detectable toxin in faeces, suggesting a correlation between intestinal inflammation and toxins in stools. In patients with a toxigenic strain without detectable toxin in stools, a high level of faecal lactoferrin or calprotectin could help guide the physician's decision to treat the patient.

Compliance with ethical standards

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Astellas had no role in the study design and conduct, and was not involved in the data collection, data management, or data analysis and interpretation. Astellas provided editorial assistance with grammar and English language editing.

Conflict of interest Frédéric Barbut reports grants, personal fees and non-financial support from Astellas, personal fees from Pfizer, grants and personal fees from Sanofi Pasteur, grants and non-financial support from Anios, grants, personal fees and non-financial support from MSD, grants from bioMérieux, grants from Quidel Bühlmann, grants from Diasorin, grants from Cubist, grants from Biosynex and grants from GenePoc; Catherine Eckert reports non-financial support from Astellas and Mobidiag.

Ethical approval The study protocol was approved by the local Research Ethics Committee and presented to the Infection Control Committee.

Informed consent No.

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