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Discordant *Clostridioides difficile* diagnostic assay and treatment practice: a cross-sectional study in a tertiary care hospital, Geneva, Switzerland

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ABSTRACT

Objectives To determine the proportion of patients who received a treatment for *Clostridioides difficile* infection (CDI) among those presenting a discordant *C. difficile* diagnostic assay and to identify patient characteristics associated with the decision to treat CDI.

Design Cross-sectional study.

Setting Monocentric study in a tertiary care hospital, Geneva, Switzerland.

Participants Among 4562 adult patients tested for *C. difficile* between March 2017 and March 2019, 208 patients with discordant tests' results (positive nucleic acid amplification test (NAAT+)/negative enzyme immunoassay (EIA–)) were included.

Main outcome measures Treatment for CDI.

Results CDI treatment was administered in 147 (71%) cases. In multivariate analysis, an abdominal CT scan with signs of colitis (OR 14.7; 95% CI 1.96 to 110.8) was the only factor associated with CDI treatment.

Conclusions The proportion of NAAT+/EIA– patients who received treatment questions the contribution of the EIA for the detection of toxin A/B after NAAT to limit overtreatment. Additional studies are needed to investigate if other factors are associated with the decision to treat.

Strengths and limitations of this study

- Patients were considered as treated for *Clostridioides difficile* infection (CDI) according to predefined criteria, including the appropriateness of the antibiotic treatment for CDI, timing of its introduction and duration, and the absence of any alternative justification for its prescription.
- Parameters investigated in multivariate analysis were limited to a selection of risk factors and clinical characteristics known to be associated with CDI.
- Patients without an indication for *C. difficile* testing were excluded from the study.
- Given the monocentric design of the study, our results may reflect local practice only in terms of the diagnostic algorithm and decision to treat.
- Given the observational design of the study and the routinely-collected origin of the data, some covariates may be missing in the model, thus leading to a risk for a phenomenon of confusion.

INTRODUCTION

Clostridioides difficile (formerly *Clostridium difficile*) infection (CDI) is a toxin-mediated disease and the leading cause of healthcare-associated infection, as well as an increasing cause of community-associated diarrhoea.[1–4] During the past decade, easy-to-perform and low-cost diagnostic tests have been developed, comprising nucleic acid amplification tests (NAATs) for the detection of toxin A/B genes and enzyme immunoassays (EIAs) for the detection of glutamate dehydrogenase (GDH) and toxins A/B in stool specimens. However, these tests are not recommended as stand-alone tests for CDI diagnosis due to their suboptimal sensitivity and specificity.[5 6] European and US guidelines recommend a two-stage or three-stage diagnostic approach.[5 7–9] This includes the use of a highly sensitive assay with a high negative predictive value (NPV), either NAAT or EIA for GDH (NPV of 99%–100% in a typical endemic situation with a prevalence of 5%) and, if positive, a reflex test using a highly specific confirmatory assay with a high positive predictive value (PPV), typically a toxin A/B EIA (PPV of 98.5%).[5]

CDI diagnosis relies on the association of clinical manifestations and microbiological tests documenting the presence of a toxigenic *C. difficile* strain and toxin/s in stools.[10] Symptomatic patients with both tests positive (NAAT+ or GDH+/EIA+) are likely to suffer from CDI. In the presence of discordant results (NAAT+ or GDH+/EIA–), the EIA negative result may be interpreted either as a false-negative or a toxin level below threshold in the case of a patient effectively presenting with CDI or as a true negative in the case of *C. difficile* toxigenic strain carriage. A third-stage test, either NAAT, toxigenic culture or GDH,

if not yet performed, can be performed to exclude a false-positive NAAT/GDH,[5 11] but will not distinguish CDI from toxigenic strain carriage. Therefore, this distinction in patients with discordant results relies on clinical evaluation, but current guidelines do not clearly state which factors should be taken into account.[5 8]

CDI overdiagnosis and subsequent overtreatment are major concerns regarding the emergence of resistance, particularly vancomycin-resistant *Enterococcus* spp.[12] Although multiple-step algorithms have been recently implemented with the aim to avoid CDI overdiagnosis and subsequent overtreatment, the actual proportion of NAAT+/EIA− patients who receive a treatment for CDI remains poorly described, as well as the factors influencing the treatment decision.[13]

In this study, we aimed to identify the proportion of patients that receive a treatment for CDI among those with *C. difficile* discordant tests' results (NAAT+/EIA−) and patient characteristics associated with the decision to treat.

METHODS

Study design, setting and population

We conducted a cross-sectional study at Geneva University Hospitals, a 2000-bed Swiss tertiary care centre. Clinical and biological data (results of NAAT/EIA assays performed on stool samples) were collected from electronic medical records (EMR) and the hospital bacteriology laboratory, respectively. Inclusion criteria were all adult patients (≥18 years) hospitalised or not, with *C. difficile* toxin assays performed on stool samples between 1 March 2017 and 1 March 2019 that yielded discordant results (NAAT+/EIA−). Exclusion criteria were asymptomatic patients (without diarrhoea, ileus or toxic megacolon), paediatric patients, patients with a treatment against *C. difficile* introduced ≥48 hours before the results of tests, or without clinical data available in EMR form. In patients presenting several tests with discordant results over the study period, only the first test was considered for analysis.

Outcomes and definitions

The primary objective was to determine the proportion of adult patients with a first discordant test result who received a treatment for CDI and to identify patient characteristics and risk factors for CDI (if any) associated with CDI treatment.[5]

Patients were considered as treated for CDI if they fulfilled all of the following criteria: (1) an appropriate antibiotic treatment administered for CDI according to published guidelines;[5 8 14] (2) treatment introduced less than 48 hours before the results of tests; (3) treatment duration of ≥10 days or still under treatment at the time of death and (4) treatment prescribed with a written decision in the EMR for CDI treatment, or without an alternative indication for its prescription. Of note, as faecal microbiota transplantation is not performed at our centre, it was not retained in the outcome definition.

In patients with a previous positive test (NAAT+ or EIA+ or both), only those who had received a treatment for CDI were considered as having a history of CDI. Abdominal CT scans were considered if they were performed less than 48 hours before and less than 10 days after the test result. Definitions of other characteristics and risks factors are described in the web-only online supplementary table S1.

Laboratory methods

Since 16 January 2017, the hospital bacteriology laboratory has implemented a two-step diagnostic algorithm comprising the use of a NAAT for *C. difficile* toxin B (*TcdB*; BD MAX, Becton-Dickinson, Sparks, Maryland, USA), followed by an EIA for both toxins (A/B; XPect *C. difficile* Toxin A/B EIA, Remel, San Diego, California, USA) as a reflex confirmatory test if the NAAT is positive. Fresh stool samples collected in Cary-Blair tubes are delivered to the laboratory and processed immediately without restrictions related to stool consistency. Samples drawn at night or during the weekend are stored at 4°C in the laboratory before analysis. NAAT and EIA assays are performed daily from Monday to Saturday inclusive.

Statistical analysis

The decision was made to include all eligible patients, and no formal sample size calculation was performed. Instead, we restricted the number of investigated parameters before any confirmatory analysis. Based on the '10 events per variable' rule of thumb, we limited the number of parameters investigated to eight factors selected among known risk factors and clinical characteristics compatible with CDI. Patient characteristics and CDI risk factors were described overall and by treatment for CDI and reported as frequencies and percentages. A multivariate logistic regression model using a backward stepwise method was performed to determine which parameters were independently associated with CDI treatment. At each step, starting from all eight parameters, the variable with the highest p value on the likelihood ratio test was removed from the model until all remaining factors were statistically significantly associated with CDI treatment at a two-sided level of 5%. Sensitivity analyses were performed to assess the robustness of the results when deceased patients were (a) excluded from the analysis and (b) considered as not treated. Missing data were systematically removed from analyses. Statistical significance was assessed at a two-sided 0.05 level for all analyses. All statistical analyses were performed using Stata software, V. 15 (StataCorp, College Station, Texas, USA).

Patient and public involvement

No patients were involved in the design, or conduct, or reporting, or dissemination of our research. The dissemination of the results to the included patients will not be performed.

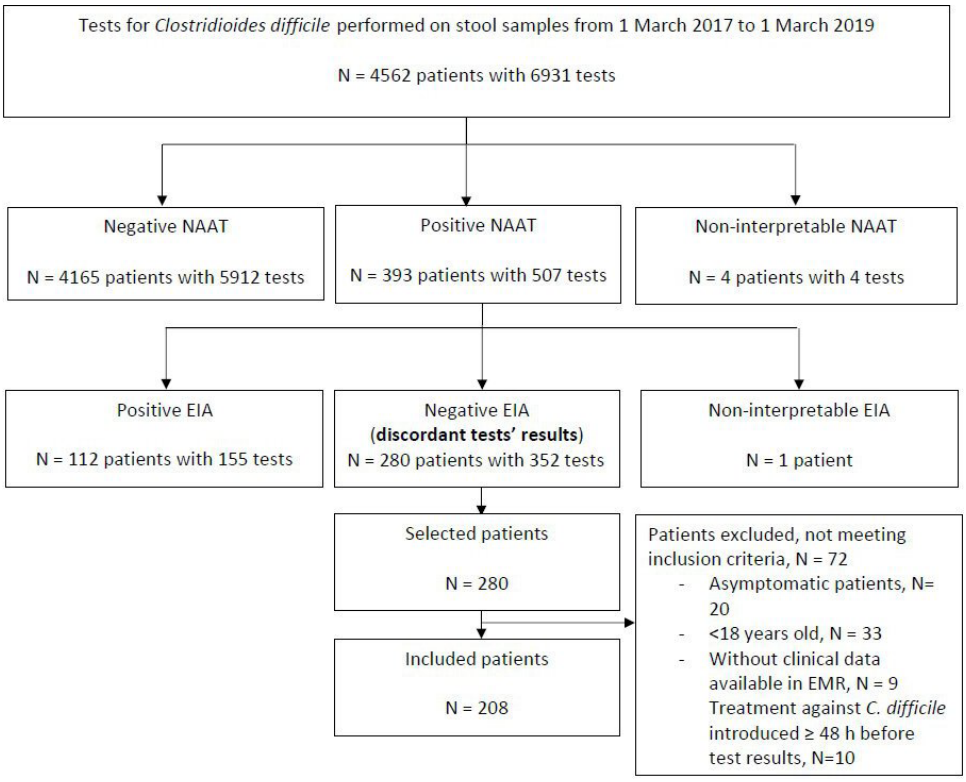

Figure 1 Flowchart of patient selection. EIA, enzyme immunoassay for toxin A/B; EMR, electronic medical records; NAAT, nucleic acid amplification test for toxin B.

RESULTS

Patient characteristics

During the study period, 4562 patients had at least one stool sample tested for *C. difficile* (corresponding to 6931 tests). A total of 393 (8.6%) patients (corresponding to 507 tests) had NAAT+ samples; 280/393 (71.3%; corresponding to 352 tests) had an EIA– for toxin A/B testing (NAAT+/EIA–). Two hundred and eighty (6.1%) patients had 352 (5.1%) discordant test results (figure 1). Among these, 72 (25.7%) were excluded (<18 years (n=33); asymptomatic patients (n=20); without available clinical data in the EMR, apart from demographics (n=9] and with treatment against *C. difficile* introduced 48 hours or more before the results of tests (n=10)). We hereby analysed the first NAAT+/EIA– stool sample of the 208 patients included in the study (figure 1). Baseline patient characteristics are described in table 1. Since the EIA confirmatory test is a reflex test after a NAAT+, the results of the two tests were available simultaneously in the patient's EMR. Median delay from prescription to results validation was 1 day (IQR 0–1).

Among the 208 patients included, none presented with ileus or toxic megacolon, while an alternative diagnosis was reported in the EMR for six patients. One of five patients who underwent recto-sigmoidoscopy had typical endoscopic lesions and was treated. Fifty-nine patients (28%) had an abdominal CT scan and 49 received a treatment for CDI (table 1). A CT scan was performed before the tests' results in 15/59 (25%) patients and after results in 44 patients. The most frequent indications for the CT scan were: investigation for an abdominal infection (40%); signs of colitis (32%) and urological disease (12%). Among patients with signs of colitis, a CT scan was performed to investigate CDI in 16 (53%) patients.

Treatment, treatment type and duration

Overall, 147 patients (71%) were treated for CDI. Treatment consisted of oral metronidazole for 132 patients (90%) and oral vancomycin for 15 patients (10%) (table 2). Treatment was initiated at the time of test results in 133 patients (90%) and within the 48 hours preceding the results in the remaining 14. Of the 145 treated patients with available data regarding severity criteria, 55 (38 %) presenting with severity criteria were treated for CDI (oral metronidazole (n=46) and oral vancomycin (n=9)). Among untreated patients (n=61), 46 (75%) did not receive any CDI treatment and 15 (25%) received a treatment for CDI during less than 10 days (median duration of treatment, 7 days; IQR, 4.5–8.5).

Associated factors

In univariate and multivariate analyses, abdominal CT scan with signs of colitis was the only associated factor with CDI treatment (OR 14.7; 95% CI 1.96 to 110.8) (table 3).

DISCUSSION

In this study of patients who presented discordant test results (NAAT+/EIA–), 71% received a treatment for CDI, suggesting that most patients with discordant test

Table 1 Baseline characteristics of included patients with NAAT+/EIA– (n=208)

	All patients, no. (%)	Treatment, no. (%)	No treatment, no. (%)	P value
	208	147 (71)	61 (29)	
Age, mean (SD)	66 (19)	67 (19)	64 (20)	0.309
Age ≥65 years old*	133 (64)	93 (63)	66 (30)	0.752
Gender, female n (%)	104 (50)	72 (49)	32 (52)	0.648
Hospitalisation*, n (%)	186 (89)	134 (91)	52 (85)	0.207
► Internal medicine	97 (47)	67 (46)	30 (49)	
► Surgery	39 (19)	25 (17)	14 (23)	
► Intensive care unit	5 (2)	4 (3)	1 (2)	
► Emergency	17 (8)	15 (10)	2 (3)	
► Rehabilitation	13 (6)	13 (9)	0	
► Oncology and haematology	13 (6)	9 (6)	4 (7)	
► Gynaecology and obstetrics	2 (1)	1 (1)	1 (2)	
Symptoms*				
► Diarrhoea†	208	147 (100)	61 (100)	
► Ileus				
► Toxic megacolon				
Presence of an alternative diagnosis in EMR	6 (3)	1 (1)	5 (8)	**0.009**
Any severity criteria‡	72/205 (35)	55/145 (38)	17/60 (28)	0.19
Complicated*§	6/205 (3)	5/145 (3)	1/60 (2)	0.673
► Sepsis	4 (2)	4 (3)	0	
► Hypotension	1 (0.5)	1 (1)	0	
► Septic shock	1 (0.5)	0	1 (2)	
Body mass index ≥30*	29/200 (15)	21/142 (15)	8/58 (14)	0.856
Creatinine clearance £ 60 mL/min*	74/205 (36)	54/146 (37)	20/59 (34)	0.677
Immunosuppression¶	44 (21)	31 (21)	13 (21)	0.971
Abdominal imaging (CT)	59 (28)	49 (33)	10 (16)	**0.014**
► Radiologic signs of colitis	30 (14)	29 (20)	1 (2)	**0.001**
Ongoing PPI treatment*	119/207 (57)	84/146 (58)	35 (57)	0.983
History of hospitalisation***	196 (94)	139 (95)	57 (93)	0.75
History of CDI*††	19 (9)	12 (8)	7 (11)	0.45
History of antibiotic treatment*‡‡	137 (66)	96 (65)	41 (67)	0.792
Infectious disease specialist advice§§, n (%)	64 (31)	43 (29)	21 (34)	0.462

*At the time of testing.
†≥ 3 unformed stools in 24 hours.
‡Blood leucocytes >15 g/L or serum creatinine >133 µmol/L.
§Ileus, toxic megacolon, septic shock or hypotension.
¶Including chemotherapy ≤60 days before test prescription; SOT; HSCT; steroid (minimum 20 mg/day prednisone or equivalent during at least 4 weeks before test prescription).
**Any hospitalisation of ≥48 hours in the last 12 weeks before test prescription.
††History of positive test results in EMR (NAAT+/EIA+ or EIA+ or TC+).
‡‡Any antibiotic treatment of ≥48 hours in the last 4 weeks before test prescription.
§§Any recommendation about treatment.
CDI, *Clostridioides difficile* infection; EIA, enzyme immunoassay; EMR, electronic medical record; HSCT, haematopoietic stem cell transplant; NAAT, nucleic acid amplification test; PPI, proton pump inhibitor; SOT, solid organ transplant; TC, toxigenic culture.

results were considered as having a CDI and treated as such. These findings raise the question of the added value of EIA for CDI diagnosis. According to institutional guidelines at the time of the study, oral metronidazole was the most frequently administered antibiotic for patients without any severity criteria.[5] Notably, 84% of treated patients with severity criteria were treated as non-severe CDI, and these results highlight issues in treatment

Table 2 Treatment type and duration

	No. (%)	
CDI treatment, n (%)	147	(70.7)
► Metronidazole (oral)	132	(89.8)
► Vancomycin (oral)	15	(10.2)
Median duration of treatment, days (IQR)	11	(11–15)
Timing of CDI treatment introduction		
► Treatment introduced ≤48 hour prior to test results	14	(9.5)
► Treatment introduced at the time of test results	133	(90.5)

CDI, *Clostridioides difficile* infection.

decisions in patients with discordant results and severity criteria for CDI. Results revealed that an abdominal CT scan with signs of colitis was significantly associated with CDI treatment in NAAT+/EIA– patients. Indeed, radiological signs of colitis are known as a convincing clue for active disease.[15 16]

We did not demonstrate any association between a history of CDI and a past hospitalisation with CDI treatment. The proportion of patients with a history of CDI was lower among treated patients, but this result was not significant. These findings were surprising considering the risk of CDI recurrence after a previous CDI, and the risk of CDI associated with a history of hospitalisation.[17–19] Concerning the presence of any severity criteria, we did not demonstrate any significant association with the decision to treat, although recent data revealed that leukocytosis and acute renal failure at presentation were associated with poor outcomes in patients with discordant results.[13]

Although a positive EIA for toxin A/B has been associated with a more severe outcome,[20 21] data are conflicting regarding the outcomes of patients with NAAT+/EIA– results.[13 21] When considering the suboptimal sensitivity of the currently available EIA tests for toxin A/B, clinicians mostly seemed to base their decision to treat patients with discordant results only on a NAAT+ in order to avoid severe outcomes.

LIMITATIONS

This study has several limitations. First, it was monocentric, possibly reflecting local practice only. Second, the sample size limited the number of variables to investigate, as well as the capacity of the study to detect associations between the investigated factors and the outcome. Despite the fact that some are well-known risk factors associated with CDI, few were associated with the decision to treat, which may be due to a lack of power. Third, given the observational design, some covariates may be missing in the model, thus leading to a substantial risk for a phenomenon of confusion. Missing data may have resulted in information bias. Nevertheless, all main clinical characteristics and known risk factors for CDI according to current knowledge, were selected for univariate and multivariate analyses. Finally, one of the most important factors in the decision to treat that could not be analysed in the present study is human

Table 3 Univariate and multivariate regression models for the association of patient characteristics with CDI treatment (n=208)

	Likelihood of receiving treatment for CDI					
	OR (95% CI)					
	Treatment n=147 (70.7%)	No treatment n=61 (29.3%)	Unadjusted	P value	Adjusted	P value
Characteristics						
Age ≥ 65 years	93 (63.3)	40 (65.6)	0.9 (0.48–1.69)	0.752		
Any severity criteria*	55/145 (37.9)	17/60 (28.3)	1.54 (0.8–2.97)	0.192		
Immunosuppression†	31 (21.1)	13 (21.3)	0.98 (0.47–2.04)	0.971		
Radiologic signs of colitis	29 (19.7)	1 (1.6)	14.7 (1.96–110.8)	0.009	14.7 (1.96–110.8)	0.009
Ongoing PPI treatment	84/146 (57.5)	35 (57.4)	1 (0.54–1.84)	0.983		
History of hospitalisation‡	139 (94.6)	57 (93.4)	1.21 (0.35–4.2)	0.754		
History of CDI¶	12 (8.2)	7 (11.5)	0.68 (0.25–1.83)	0.452		
History of antibiotic treatment[6]	96 (65.3)	41 (67.2)	0.91 (0.48–1.72)	0.792		

*Blood leucocytes count >15 g/L or serum creatinine >133 µmol/L.
†Including chemotherapy ≤60 days before test prescription; SOT; HSCT; steroid (minimum 20 mg/day prednisone or equivalent during at least 4 weeks before test prescription).
‡Any hospitalisation of ≥48 hours in the last 12 weeks before test prescription.
§History of positive test results in EMR (NAAT+/EIA+ or EIA+ or TC+).
¶Any antibiotic treatment of ≥48 hour in the last 4 weeks before test prescription.
CDI, *Clostridioides difficile* infection ; EIA, enzyme immunoassay; EMR, electronic medical record; HSCT, hematopoietic stem cell transplant; NAAT, nucleic acid amplification test; PPI, proton pump inhibitor; SOT, solid organ transplant; TC, toxigenic culture.

behaviour, which depends on the clinician's experience and each individual clinical situation.

Recent studies have questioned current algorithms for CDI diagnosis. Pollock *et al* revealed that the concentration of toxins A, B and A/B tested by a single molecule array were not significantly different in symptomatic (CDI) and asymptomatic (carriage) individuals selected on the basis of a positive NAAT for toxin gene, thus questioning the use of an EIA for toxin A/B after NAAT.[22] By contrast, in patients selected on the basis of a positive toxin test, the concentrations were significantly higher in symptomatic patients, highlighting the possibility to prioritise toxin detection over toxin gene.[22] *C. difficile* toxin gene real-time PCR cycle threshold values have been associated in some studies with toxin-EIA positive results and adverse outcomes. However, data are conflicting, and the accuracy of cycle threshold values for toxin-positive prediction remains low with currently available EIA assays.[23] The use of a single ultrasensitive assay has been shown to be more sensitive and specific compared with a multistep algorithm using NAAT and EIA for toxin A/B.[24]

Regarding the missed opportunity of EIA to avoid overdiagnosis and CDI treatment as revealed by the proportion of treated patients with a negative EIA in our study, similar to Origuen *et al*,[13] further investigations should be performed to assess the use of ultrasensitive and quantitative immunoassays for toxin A/B detection as stand-alone tests for CDI diagnosis as evoked by recent studies described above.

CONCLUSIONS

In conclusion, 5.2% of patients tested for *C. difficile* harboured discordant *C. difficile* test results (NAAT+/EIA–), with 71% receiving a treatment for CDI. An abdominal CT scan with signs of colitis was the only factor associated with the decision to treat. Nevertheless, additional studies are needed to assess whether other factors are associated with the decision to treat these patients. The proportion of NAAT+/EIA– patients that did not receive any treatment for CDI (29%) questions the contribution of the EIA for the detection toxin A/B after NAAT to limit CDI overdiagnosis and overtreatment.

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Contributors LL, M-CZ, AP and JS contributed to the conception and design of the study, advised on all statistical aspects and interpreted the data. LL and M-CZ performed the statistical analysis, assisted by AP. LL, M-CZ, AP, LK and JS drafted and reviewed the manuscript and approved the final version to be published. LL, M-CZ and JS had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. LL and M-CZ contributed equally to this work and are joint first authors. LL, M-CZ and JS are the guarantors. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. The corresponding author has the right to grant on behalf of all authors and does grant on behalf of all authors, a worldwide licence to the publishers and its licensees in perpetuity, in all forms, formats and media (whether known now or created in the future), to (1) publish, reproduce, distribute, display and store the contribution, (2) translate the contribution into other languages, create adaptations, reprints, include within collections and create summaries, extracts and/or, abstracts of the contribution, (3) create any other derivative work(s) based on the contribution, (4) to exploit all subsidiary rights in the contribution, (5) the inclusion of electronic links from the contribution to third party material where-ever it may be located and (6) licence any third party to do any or all of the above.

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Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval The study was approved by the Geneva ethics commission and a waiver of informed consent was granted due to its retrospective nature (study number 2018-02012).

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