P0740

# Clinical outcome and biological features of patients according to *Clostridium difficile* testing methods.

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## Background

Diagnosis of *Clostridium difficile* infection (CDI) is based on detection of free toxins in the stools (by the stool cytotoxicity assay or by EIA tests) or on detection of a toxigenic strain (by toxigenic culture or by nucleic acid amplification technique [NAAT]). Debate still persists as whether toxin detection is correlated to more severe disease.

## Objectives

To compare the clinical presentation and outcome (mortality) of patients with CDI based on the presence or absence of free toxins in the stools at the time of diagnosis.

## Methods

- A prospective study including all patients with CDI hospitalized in a university hospital (Paris, France) was conducted from December 2010 to April 2013.
- Patients were classified into two groups:
  - those who had free toxins in the stools (defined by a positive stool cytotoxicity assay) and
  - those whose diagnosis was based only on the presence of a toxigenic strain (defined as patients with a negative stool cytotoxicity assay but a positive toxigenic culture).

For each patient, a standardized questionnaire including demographic, clinical (severity, recurrence during the 2 months following the initial episode, mortality at Day 30) and biological data (leukocyte count, CRP, albumin concentration, serum creatinin concentration) was fulfilled. Severity was defined by at least one of the following criteria : intestinal perforation or toxic megacolon or signs of septic shock, or neutrophil count > 20,000/mm$^3$ or admission in ICU or surgical ward or CDI-associated death within 30 days.

- A multivariate logistic regression model was used to identify factors associated with the presence of free toxins and severity of CDI. Factors associated with death were determined by a Cox model.

## Results

312 patients (168 males, 148 female; mean age 61.2 ± 20.3 years) with CDI were included: 191 (61.2%) patients had free detectable toxins in the stools whereas 121 (38.2%) only have a toxigenic strain (without any free toxin).

Among these patients:
- 13.5% (n=42) had a CDI recurrence within the 2 months following the initial episode,
- 6.1% (n=19), 12.5% (n=39) et 14.7% (n=46) died at D+10, D+30, D+60, respectively.
- 11.5% (n=42) had a severe/complicated form of the disease

## References:

*Planche TD, Davies KA, Coen PG, Finney JM, Monahan IM, Morris KA, O'Connor L, Oakley SJ, Pope CF, Wren MW, Shetty NP, Crook DW, Wilcox MH.*
Differences in outcome according to *Clostridium difficile* testing method: a prospective multicentre diagnostic validation study of *C difficile* infection
Lancet Infectious Diseases, September 3, 2013 http://dx.doi.org/10.1016/S1473-3099(13)70200-7

## Results (cont'd)

**Table 1.** Factors associated with free toxins (multivariate analysis)

| Variables | Odds ratio | 95% CI | p |
|---|---|---|---|
| IBD | 0,5 | 0,28 – 0,87 | 0,013 |
| Antibiotics in the previous 60 days | 1,94 | 1,06 – 3,57 | 0,03 |
| Leuckcytes (log) | 1,48 | 1,02 – 2,15 | 0,04 |

IBD : inflammatory bowel diesease

**Table 2** . Factors associated with the CDI severity (multivariate analysis)

| Variables | Odds ratio | 95% CI | p |
|---|---|---|---|
| CRP (increase of 10 units) | 1,08 | 1,02 – 1,14 | 0,006 |
| Leukocytes (>10 000 /mm$^3$) | 6,34 | 1,25 – 32,28 | 0,03 |
| PPI or/and anti-acids | 0,22 | 0,04 – 1,10 | 0,07 |
| Free toxin in stools | 1,99 | 0,44 – 9,10 | 0,37 |

**Table 3**. : Factors associated with death at D+30 (Cox model, multivariate analysis)

| Variables | Hazard ratio | 95% CI | p |
|---|---|---|---|
| Age > 65 years | 3,76 | 1,50 – 9,40 | 0,005 |
| Creatinin (increase of 10 units) | 1,02 | 1,0 – 1,04 | 0,08 |
| Severe CDI | 5,19 | 1,98 – 13,62 | 0,001 |
| Specific treatment | 0,30 | 0,12 – 0,73 | 0,008 |
| Leukocytes (4000-10000/mm$^3$) | reference | | |
| Leukopenia (< 4 000/mm$^3$) | 6,05 | 1,56 – 23,50 | 0,009 |
| Hyperleukocytosis (> 10 000/mm$^3$) | 4,52 | 1,29 – 15,86 | 0,02 |

**Factors associated with the presence of free toxins (univariate and multivariate analysis)**
Comparison of CDI patients with or without free toxins indicated that patients with free toxins had a higher CRP count (p=0.015) and received more frequently antibiotic treatment in the previous 2 months (p=0.01). We showed that patients with free toxins were more often treated by vancomycin or metronidazole (89.5%,162/181) than patients without free toxin (78.8%, 82/104) (p=0.0014). Conversely, CDI patients without free toxins were more likely patients with IBD (inflamatory bowel disease) (p=0.009), and were more likely treated by nasogastric tube in the previous month (p=0 .02).

In a multivariate analysis (**Table 1**), factors significantly associated with the presence of free toxins were the leukocyte count (OR=1.48, 95%CI 1.02-2.15, p= 0.04), antibiotics in the previous 2 months (OR=1.94, 95% CI 1.06-3.57, p=0.03) and diagnosis of inflammatory bowel disease (OR=0.50, 95%CI 0.28-0.87, p=0.013).

**Factors associated with severity and death at D+30**
Factors significantly associated with severity (logistic regression) and death at D+30 (Cox model) are described in the **tables 2 and 3,** respectively.
Independent factors associated with severe CDI were elevated CRP (OR=1.08, 95% CI 1.02-1.14, p= 0.006) and leukocytes > 10,000/mm$^3$ (OR=6.34, 95% CI 1.25 - 32.28, p = 0.03). Mortality at Day 30 was significantly higher in elderly patients (> 65 years) (HR=3.76 , 95% CI 1.5-9.40 ) with severe CDI (HR=5.19, 95% CI 1.98-13.62 ), a leukocyte count > 10,000 /mm$^3$ (HR=4.52 , 95%CI 1.56-22.50 ) or leukopenia (HR=6.05, 95%CI 1.29-15.86 ) and in patients without specific *C. difficile* treatment (HR = 0.3, 95%CI 0.12-0.73).

## Conclusions

The presence of free toxins at the time of diagnosis was not significantly associated with a severe form of CDI or higher mortality rate. However, free toxins in stools were significantly associated with biological markers of inflammation or risk factors for infection. Our hypothesis is that the group of patients without free toxin is composed of a mixed population of patients truly infected and simply colonized by a toxigenic strain. These results are in line with those recently reported by T. Planche *et al*. In this multicenter study, they compared 435 patients with free toxins to 207 patients for whom the diagnosis of CDI was only based on toxigenic culture. They showed that patients with free toxins had a higher mortality rate at D+28 (26% versus 17%) and a higher leukocyte count >15,000/mm3 (15% vs 10%) than patients without free toxins.