# Absence of Toxemia in *Clostridioides difficile* infection: Results from Ultrasensitive Toxin Assay of Serum

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## Background

- *Clostridioides difficile* infection (CDI) is the major cause of hospital-acquired bacterial infectious diarrhea, are caused by Toxin A (TcdA) and Toxin B (TcdB), two protein exotoxins secreted by pathogenic strains of the bacteria.
- In fulminant CDI, these toxins lead to systemic complications and extra-colonic manifestations consistent with sepsis or even toxemia. However, identification of toxemia in CDI patients is incredibly rare.
- We hypothesized that the rarity at which toxemia is detected in CDI may be due to very low concentrations of circulating toxin in the blood, below the limit of detection of commercially available toxin assays.

## Methods

- Eligible patients were enrolled in the study if they were NAAT positive and had acute diarrhea.
- Ultrasensitive toxin Single Molecule Array (Simoa) was performed on baseline stool samples, collected within 48 hours of initiating CDI treatment
- Simoa clinical cut-offs were defined by signal (mean plus two standard deviations) observed in sera from patients without CDI or C. difficile carriage:

TcdA = 15.0 pg/ml  TcdB = 26.7 pg/ml

| Variable | n = 169 | |
|---|---|---|
| **Demographic Information** | | |
| Age Median (IQR) | 68 (54 – 78) | |
| Male Gender | 90 | 53.3% |
| **Race** | | |
| White | 126 | 74.6% |
| African American | 21 | 12.4% |
| Other | 18 | 10.7% |
| Unknown | 4 | 2.4% |
| **Ethnicity** | | |
| Hispanic | 9 | 5.3% |
| Not Hispanic | 144 | 85.2% |
| Unknown | 16 | 9.5% |
| **Laboratory Results** | | |
| WBC ($10^3$ cells/µL) Median (IQR) | 11.5 (7.4 – 18.6) | |
| WBC ≥15 x $10^3$ cells/µL | 61 | 36.1% |
| Creatinine (mg/dl) Median (IQR) | 1.1 (0.8 – 1.9) | |
| Creatinine ≥ 1.5 mg/dl | 61 | 36.1% |
| Albumin (g/dl) Median (IQR) | 3 (2.5 – 3.6) n = 152 | |
| Albumin ≤ 3 g/dl | 73 (n = 152) | 43.2% |
| 027 / NAP1 / B1 strain | 17 | 10.1% |
| **Severe Clinical Outcomes - Total** | | |
| ICU admission | 24 | 14.2% |
| Colectomy | 1 | 0.6% |
| Death within 40 days | 14 | 8.3% |
| **Severe Clinical Outcomes – Attributed to CDI** | | |
| ICU admission | 13 | 7.7% |
| Colectomy | 1 | 0.6% |
| Death within 40 days | 2 | 2.4% |
| **Severity Classifications* n = 153** | | |
| IDSA Severe | 90 | 74.4% |
| ESCMID Severe | 93 | 76.9% |
| Zar *et al* Severe | 73 | 60.3% |
| Belmares *et al* Severe | 23 | 19.0% |

**Table 1.** Demographics, Baseline Laboratory Values, and Clinical Outcomes for the cohort.

## Results

- Our cohort included 169 patients with a median age of 68 years (IQR 54-78), most with severe CDI and many with severe clinical outcomes attributed to CDI including ICU admission, colectomy or death.
- No TcdA or TcdB detected in the serum of our patient cohort despite a wide range of toxin concentrations in paired stool.
- In 10.6% of serum samples, an artifact was observed in which a small percentage of beads demonstrated many bound antibody complexes with high enzymatic activity consistent with background signal related to binding of an unknown interfering molecule. Additional testing yielded negative results for the toxins.

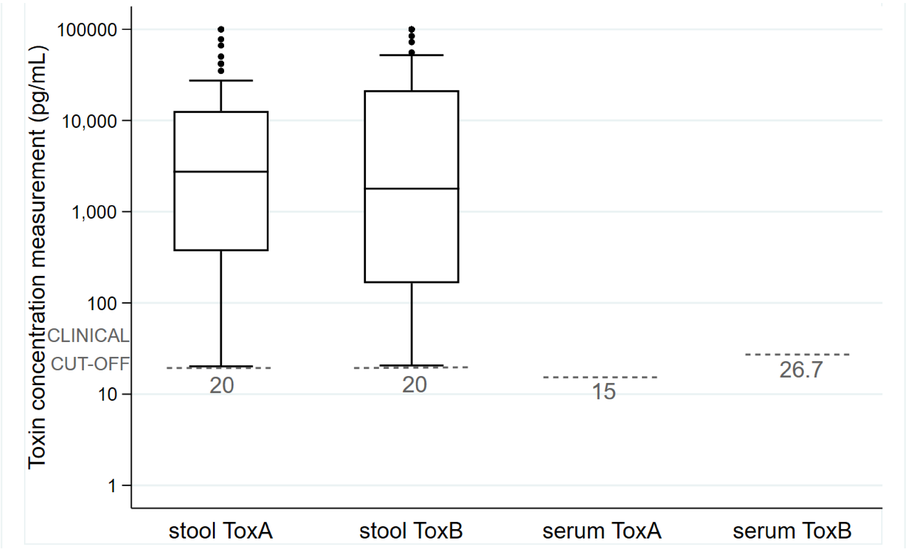


**Figure 1.** Comparison of TcdA and TcdB concentrations, as measured by Simoa, in serum and stool. Clinical cutoffs are shown. Signals below these cut-offs are below backgrounds and considered negative.

## Conclusions

- Circulating anti-toxin antibodies in blood may interfere with TcdA and/or TcdB detection. High anti-toxin antibody concentrations were associated with loss of Simoa signal, suggesting substantial inhibition of toxin measurements.
- Though earlier published findings reported on the presence of detectable toxin in the serum of patients with CDI, our results did not support this observation.
- There were several limitations of this study potentially contributing to these negative results.
  - Storage conditions of serum samples could have caused toxin degradation
  - Fulminant findings related to CDI are rare
  - Plausible that the circulating toxin levels still remain below Simoa's limit of detection.

**Although Simoa is highly sensitive for detection of picogram quantities of TcdA or TcdB, it was unable to detect either toxin in serum during CDI.**

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