Abstract #40610

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Stool Cytotoxicity Activity Correlates with Immune Competency in Patients with *C. difficile* Infection

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Background

- *C. difficile* infection (CDI) may present with a wide spectrum of illness, from mild diarrhea to life-threatening toxic megacolon, but current prediction models for poor outcomes are suboptimal[1]. In particular, there is little data on prediction tools in immunocompromised patients or the effect of immune status on the course of disease.[2]
- Highly virulent strains of *C. difficile* characterized by increased cytotoxin production have been associated with an increased risk of complications and mortality, indicating the amount of toxin may be an important disease determinant[3].
- The final cytotoxicity activity in stool represents the sum of organism load, toxin production, and host response.
- We hypothesize that cytotoxin activity in stool may correlate with disease severity and outcome.

Methods

- Goal: To determine stool cytotoxicity titers In 100 subjects, including 50 immunocompetent normal hosts (NH) and 50 immunocompromised (IC) patients, with acute symptoms of CDI and a positive stool *C. diff* PCR test consistent with CDI (first episode or first recurrence).
- IC subjects are defined as patients with neutropenia, stem cell or solid organ transplant recipients, advanced HIV infection, and/or patients on immune suppressive drugs, including high-dose corticosteroids.
- Filtrates and serial dilutions of CDI positive stool samples were prepared as described using the Bartels® Cytotoxicity Assay for Clostridium difficile Toxin (Trinity Biotech, Carlsbad, CA). Cytotoxicity activity was determined by assessment of cytopathic effect (CPE) under microscopic analysis. (Figure 2)
- ATLAS scores were calculated for each subject using the age, temperature, WBC count, albumin and systemic concomitant antibiotics. Serum creatinine and number of bowel movements per day were also recorded.

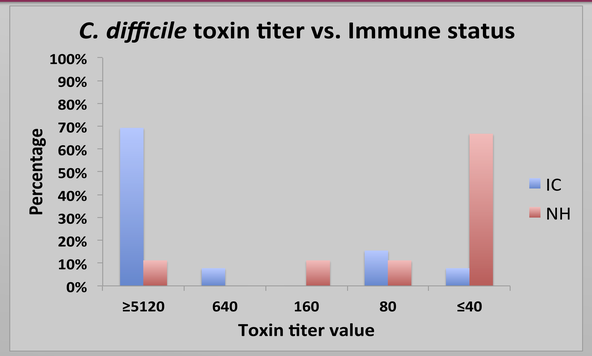

Figure 1. *C. difficile* toxin titers and Immune status. A significant difference can be appreciated in both high and low titers groups based on immune status (p=0.014). IC denotes immunocompromised host, NH normal host.

IMMUNE DEFICIT	CDIFF TOXIN TITER	ATLAS SCORE
YES	> 5120	3
YES	> 5120	2
YES	5120	3
YES	> 5120	5
YES	> 5120	4
YES	> 5120	4
YES	> 5120	5
YES	>5120	0
YES	<40	N/A
YES	80	2
YES	640	3
YES	5120	2
YES	80	5
NO	80	1
NO	160	4
NO	<40	2
NO	> 5120	0
NO	<40	3
NO	<40	2
NO	<40	6
NO	<40	4
NO	<40	N/A

Table 1. Immune status and toxin titers of the participants. ATLAS score calculated based on age, temperature, WBC count, albumin and systemic concomitant antibiotics. N/A denotes not available.

GROUPS AND *C diff* TOX TITER	IC	NH	Mean Difference 95% CI	p value
Mean	3609.23	622.22	2987.01 (1070.55, 4903.47)	0.004

Table 2. *C difficile* toxin titers mean differences between both groups. IC denotes Immunocompromised host, NH normal host, CI confidence interval. Analyzed using t-test.

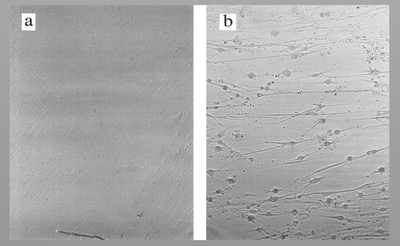

Figure 2. Cytopathic effect (CPE) in monolayer of human fibroblasts induced by C diff toxin. Note the rounding and clumping of the cells.

Results

- 22 patients have been enrolled, 9 NH (40.9%) and 13 IC (59.0%) (Table 1).
- Patients in the IC group had higher *C. difficile* toxin mean titers (3609) compared to NH patients (622) (p=0.0040 T-Test)(Figure 1 and Table2).
- A non-parametric analysis where we defined a titer ≥ 5120 as "high" and , < 5120 as "low" demonstrated an association between IC status and high toxin titers (p=0.01, Fisher exact test).
- High titers did not correlate with number of BMs, ATLAS score or other clinical parameters that have been associated disease severity or mortality.

Conclusions

- The *C. difficile* cytotoxicity titers in stool of CDI patients are significantly higher in IC compared to NH patients.
- The host response to *C. difficile* may be important in the course of CDI and responses to therapy.
- We plan to determine serum anti-toxin titers for our cohort and correlate these titers with stool cytotoxicity.
- Further study is needed to evaluate if high cytotoxin titers correlate with disease severity, clinical outcomes, or recurrences.
- Stool cytotoxin titers could help guide clinical management and/or clinical trial design.

References

1. Chakra CAN, et al. Prediction tools for unfavourable outcomes in Clostridium difficile infection: As systematic review. PLoS One. 2012;7(1):e330258.
2. Schaier M, et al. C lostridium difficile diarrhea in the immunosuppressed patient – Update on prevention and management. Nephrol Dial Transplant. 2004 Oct;19(10):2432-6.
3. O'Connor JR, et al. Clostridium difficile infection caused by epidemic BI/NAP1/027 strain. Gastroenterol. 2009;136(6);1913-24.

Acknowledgments

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