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Tests for Inflammation (Stool and Blood) and Stool Spore Density Correlate Better with *Clostridium difficile* Infection (CDI) than the Number of Stools per Day

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Abstract

Background: Distinguishing between *C. difficile* colonization vs. infection (CDI) is difficult and requires a combination of laboratory tests and clinical criteria. PCR is a highly sensitive test that is commonly used to diagnose toxigenic CDI. However, results from several recent studies raise question of whether PCR is overly sensitive. Our hypothesis was that selected laboratory tests could be used to distinguish between colonized and infected patients who were PCR positive for toxigenic *C. difficile*.

Methods: Patients that submitted an unformed stool sample for CDI testing between 10/1/12 and 9/30/13 and had a positive test by PCR (Cepheid) were included in the study. Interviews were conducted for the number of stools per day (BM/D) and charts were reviewed for relevant clinical data. Stool samples were sent frozen to TechLab for additional testing including cell cytotoxicity for TcdB, quantitative toxigenic bacterial culture, PCR-ribotyping, spore counts, and quantitative fecal lactoferrin and glutamate dehydrogenase (GDH) by immunoassay.

Results: A total of 215 PCR-positive patients were included with a mean age of 71 years. Stools per day separated patients into groups with <3 BM/D (26%), 3 to 10 BM/D (57%), and >10 BM/D (17%). Further testing showed that 55% of patients were cell cytotoxicity positive and 90% were toxigenic culture positive. Spore counts ranged from 10^2 to 10^8 CFU/g stool. Ribotype 027 was detected in 23% of patients. Stool toxin-positive patients had lower Ct (p=1x10^{-19}), higher WBC (p=1x10^{-6}), higher spore counts (p=1.8x10^{-16}), more lactoferrin (p=1x10^{-6}), and higher GDH concentrations (p=4.6x10^{-18}). Interestingly, the number of stools per day did not correlate with microbiological or clinical indicators of CDI even when patients on laxative and antidiarrheal (50%) agents were removed.

Conclusion: Patients who had lower Ct, higher spore counts, and positive stool toxin by cell cytotoxicity were associated with significantly increased levels of fecal lactoferrin and blood WBC; however, the number of stools per day did not correlate with worse inflammatory markers. Additional studies are needed to both determine the utility of stools per day as a criteria in diagnosing CDI and also determine if these markers can help define clinical CDI.

Aim

To determine what testing protocol is optimal by including clinical and lab diagnostic tests for the diagnosis of CDI as a quality of improvement measure.

Patient Characteristics

Table 1 Patient Characteristics N=215	Tissue Culture Positive Patients N=118	Tissue Culture Negative Patients N=97	p-value*	Point estimate (odds ratio)	95% CI for difference (odds ratio) lower	upper
Age in years, median	81	75	0.042	4.000	0.000	9.000
Maximum Temperature °F, median	99.2	98.9	0.195	0.200	-0.100	0.500
Female gender, N (%)	71 (60)	54 (56)	0.579	1.203	0.673	2.149
Apache II, median	14.5	13.0	0.126	1.000	0.000	3.000
Stools per day, N (%)						
'< 3	33 (28)	24 (25)	0.799	1.145	0.593	2.228
'3 - 10	67 (57)	55 (57)	-	1.023	0.573	1.826
'> 10	18 (15)	18 (18)	-	0.798	0.365	1.747
Intensive care unit (ICU), N (%)	26 (22)	26 (27)	0.425	0.388	0.388	1.496
Readmitted, N (%)	17 (14)	24 (25)	0.056	0.505	0.237	1.064
30-day mortality (all cause), N (%)	26 (22)	20 (20)	0.868	1.074	0.530	2.200
100-day mortality (all cause), N (%)	32 (24)	23 (27)	0.734	1.181	0.608	2.314

Continuous variable were compared between tissue culture + (TC+) and tissue culture – (TC-) groups using Wilcoxon rank sum test. 95% confidence intervals for the difference in location parameters are provided. For categorical variables, Fisher's exact test was used to compare proportions between TC+ and TC- groups. Point estimates and 95% confidence intervals for odds ratios are also provided. *P*-values <0.05 were considered significant.

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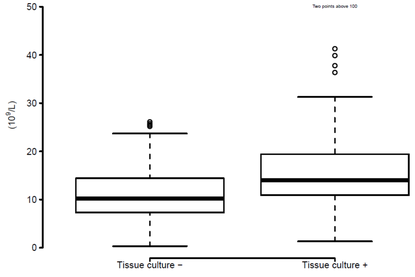
Diagnostic Variables

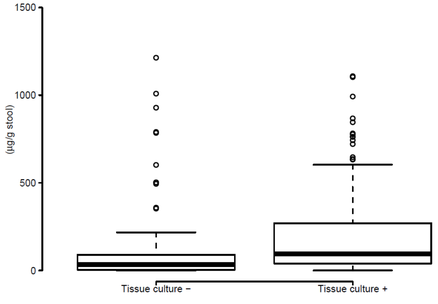
Table 2 Diagnostic Variables N=215	Tissue Culture Positive Patients N=118	Tissue Culture Negative Patients N=97	p-value*	Point estimate (median difference)	95% CI for difference lower	upper
PCR Ct value, median	23	31	<0.0001	-6.400	-7.600	-5.200
Ribotype - 027 , N (%)	42 (37)	7 (9)	<0.0001	5.753	2.334	16.075
White blood cell count, 10E3/mm3, median	14	10	<0.0001	3.800	2.200	5.400
Lactoferrin, µg/g, median	94	34	<0.0001	49.510	28.670	76.240
Total *C. difficile* Count, CFU/g, median	1.0 x 10^6	4.5 x 10^3	<0.0001	7.0 x 10^5	3.6 x 10^5	1.4 x 10^6
Glutamate Dehydrogenase ng/g, median	1675	28	<0.0001	1293.472	692.000	2433.000

Table 3 Diagnostic Variables N=115 Laxatives/Antidiarrheal removed	Tissue Culture Positive Patients N=66	Tissue Culture Negative Patients N=49	p-value*
PCR Ct value, median	23	31	<0.0001
Maximum white blood cell count, 10E3/mm	14	10	0.0003
Lactoferrin, µg/g, median	100	61	0.006
Total *C. difficile* Count, CFU/g, median	1.1 x 10^6	3.2 x 10^3	<0.0001
Glutamate Dehydrogenase ng/g, median	1643	28	<0.0001

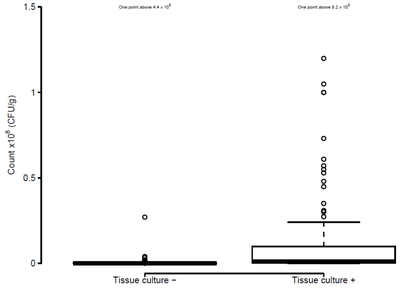
White blood cell count



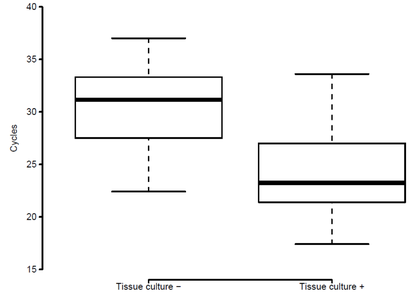
Lactoferrin



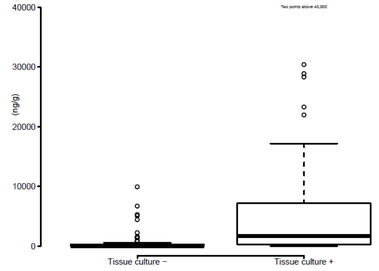
Total C. difficile count



Ct value



Glutamate dehydrogenase



Glutamate dehydrogenase (GDH) enzyme is constitutively expressed by actively growing cells. GDH levels were determined by ELISA using a standard curve generated with recombinant GDH and an OD value that fell on the linear portion of the graph.

Table 3 N=215	N	Median Lactoferrin	Median GDH	027=%	Total count Median	Mean Temp	Mean WBC	Mean AGE	100death all cause	Mean ApacheII	Mean PCR Ct
TC-POS-027	42	101	3060	100%	1.86E+06	99.4	24.9	76	28.60%	16	24.1
TC-POS-Non027	72	84	1342	0%	9.45E+05	99.5	12.1	71	26.40%	13	24.1
Group A (<3 stools/day)	57	63	548	21.10%	4.15E+05	99.0	12.6	75	31.50%	15	27.2
Group B (3 - 10 stools/day)	122	65	334	26.20%	1.16E+05	99.6	16.3	72	25.00%	14	26.7
Group C (10> stools/day)	36	62	273	13.90%	1.50E+04	100.3	12.2	65	18.90%	13	27.2
*TC-POS GDH>75ng/g	105	101	2476	37.10%	1.33E+06	98.4	17.6	74	26.70%	15	24
*TC-POS GDH<75ng/g	11	54	24	18.20%	4.91E+04	100.3	13.7	70	18.20%	14	28
*TC-NEG GDH >75ng/g	28	21	681	0.00%	6.41E+04	99.4	12.5	70	28.60%	13	27.3
*TC-NEG GDH <75ng/g	64	30	20	7.80%	1.11E+03	100	11.4	71	23.40%	13	31.9
*7 IBD Patients removed											

What is known

- Patients may be colonized with toxigenic *C. difficile* and not suffer from disease.
- Symptoms are not specific for *C. difficile* disease thus requiring that other diarrheal diseases must be considered.
- Current guidelines, U.S. and European, include the ≥3 stools per day criteria or <u>any unformed stool</u>, respectively, as a testing criteria for patients suspected of CDI.

What is new

- Increased WBC count and fecal lactoferrin are associated with higher bacterial counts, increased GDH levels, lower Ct values for *tcdB*, and presence of stool toxin.
- Stools per day rating is not correlated with microbiological or clinical indicators of CDI, even when patients on antidiarrheal medications and laxatives were removed.
- Stool per day < 3, 3-10 and >10 categories are not correlated with indicators of more severe disease.
- Our results support the ESCMID guidelines for using any unformed stool as a testing criteria for CDI.
- Tissue culture cell cytotoxicity detection did not separate <u>potential</u> CDI patients for ICU stay, 30 or 100 day mortality, readmission, or disease severity based on frequency of diarrhea.

References

(1) Crobach M.J., *et al* (2016) European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for *Clostridium difficile* infection. Clinical Microbiology and Infection. Aug 22 Suppl 4: S63-81
(2) Kelly S.G. , *et al* (2016) Inappropriate *C. difficile* testing and Consequent Overtreatment and Inaccurate Publicly Reported Metrics. Infect Control Hosp Epidemiol. Sep 26:1-6.