Therapeutic Advances in Gastroenterology

Review

Defining the black box: a narrative review of factors associated with adverse outcomes from severe *Clostridioides difficile* infection

Ther Adv Gastroenterol

2021, Vol. 14: 1–16

DOI: 10.1177/17562848211048127

Adam Ressler, Joyce Wang and Krishna Rao

***Abstract*:** In the United States, *Clostridioides difficile* infection (CDI) is the leading cause of healthcare-associated infection, affecting nearly half a million people and resulting in more than 20,000 in-hospital deaths every year. It is therefore imperative to better characterize the intricate interplay between *C. difficile* microbial factors, host immunologic signatures, and clinical features that are associated with adverse outcomes of severe CDI. In this narrative review, we discuss the implications of *C. difficile* genetics and virulence factors in the molecular epidemiology of CDI, and the utility of early biomarkers in predicting the clinical trajectory of patients at risk of developing severe CDI. Furthermore, we identify associations between host immune factors and CDI outcomes in both animal models and human studies. Next, we highlight clinical factors including renal dysfunction, aging, blood biomarkers, level of care, and chronic illnesses that can affect severe CDI diagnosis and outcome. Finally, we present our perspectives on two specific treatments pertinent to patient outcomes: metronidazole administration and surgery. Together, this review explores the various venues of CDI research and highlights the importance of integrating microbial, host, and clinical data to help clinicians make optimal treatment decisions based on accurate prediction of disease progression.

Keywords: *Clostridioides difficile* infection, epidemiology, healthcare associated infections, risk factors

Received: 31 March 2021; revised manuscript accepted: 31 August 2021.

Correspondence to:
Krishna Rao
Division of Infectious Diseases, Department of Internal Medicine, University of Michigan Medical School, 1150 West Medical Center Drive, MSRB1 1510B, Ann Arbor, MI 48109, USA
kriraos@med.umich.edu

Adam Ressler
Division of Infectious Diseases, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI, USA

Joyce Wang
Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI, USA

Defining severe *Clostridioides difficile* infection

Clostridioides difficile infection (CDI; previously *Clostridium difficile*) is the most common healthcare associated infection; the most common cause of hospital associated diarrhea;[1] and has been identified by the Centers for Disease Control and Prevention as an urgent antimicrobial resistance threat.[2] It results in considerable mortality with an estimated mortality rate of 8–31%[3–5] – accounting for approximately 20,500 deaths in 2017 alone.[6] CDI also leads to substantial morbidity; in a population of patients with CDI 1–2% of patients will undergo total abdominal colectomy with end ileostomy and stapled rectal stump.[3]

Treatment for CDI is usually guided by the severity of illness. However, to date there is not a well-validated, widely-accepted, and effective severity index using parameters available at the time of diagnosis that can predict a patient's most likely outcome. In this narrative review we will not be proposing a new severity index; rather we will examine the outcomes of severe CDI and the association of various factors with poor outcomes of CDI, which could focus future efforts to develop such a classification scheme.

Severe CDI is not well characterized in the literature, as the same definition is not universally used. A frequent definition uses a combination of clinical and laboratory data to define mild/moderate,

severe, and severe/complicated disease, and is found in the IDSA/SHEA guidelines. The 2010 guidelines defined severe disease as CDI with leukocytosis ⩾ 15,000 cells/μL or serum creatinine ⩾ 1.5× premorbid level; complicated disease included those with hypotension/shock, ileus or megacolon.[7] Updated in 2017, the IDSA/SHEA guidelines modified the renal criteria which now use a serum creatinine ⩾ 1.5 mg/dL regardless of baseline serum creatinine.[8]

Although the IDSA/SHEA definition is often used, studies also create their own definition for severe disease leading to significant variation in how severe CDI is defined across studies. Factors used in definitions have included demographics and elements of history/physical exam (age > 60 y, abdominal distention, abdominal tenderness, ileus, megacolon, and septic shock requiring ICU admission); laboratory parameters (leukocyte count ⩾ 15,000 cells/μL, serum creatinine ⩾ 1.5× the premorbid level, serum creatinine ⩾ 1.5 mg/dL regardless of baseline serum creatinine, albumin < 2.5 g/dL or < 3 g/L, elevated lactate); vital sign dyscrasias (fever > 38.5°C and hypotension requiring pressor support); and findings from imaging/procedural studies (colonic wall thickening, fat stranding, unexplained ascites, peritonitis, pseudomembranous colitis, intestinal perforation, and megacolon).[7–14] Some studies have used the eventual outcome to define severity, defining severe disease as a course which results in ICU admission, colectomy, or death.[14] See Table 1 for a visual comparison of several definitions. The variation in case definition leads to populations which are not comparable across multiple studies. The significance of this variation was explored in a 2016 prospective cohort study where the frequency of severe CDI across the same population varied from 11.6–59.2% depending on which of the four evaluated definitions were used.[9] This study also found that the risk factors for severe disease among that population also differed depending on how severe CDI was defined.[9]

In the absence of a well-validated, widely-accepted and effective severity index, it is difficult to make comparisons of patients with severe CDI across different studies to identify specific factors that are consistently associated with severe disease and adverse outcomes. This is not to say that attempts have not been made to develop a CDI severity index. A number of clinical prediction tools have been developed but none have come into routine use in clinical practice. A 2012 systematic review assessing studies of clinical prediction tools for severity, complications, or mortality found relatively low diagnostic accuracy (around 70%) despite modest performance by area under the receiver-operating characteristic curve (AuROC).[10]

Here we will explore many of the microbiologic, immunologic, and clinical factors associated with adverse outcomes of severe CDI. For the purpose of this review severe CDI also includes complicated and fulminant disease.

Microbiologic factors associated with CDI outcomes

C. difficile strains and CDI severity in human studies

C. difficile consists of genetically diverse isolates inhabiting mammalian hosts and the environment such as water and soil.[23] Several typing methods, including pulse-field gel electrophoresis (PFGE), restriction endonuclease analysis (REA), and multilocus sequence typing (MLST) have been developed to characterize *C. difficile* isolates since the late 1990s. In clinical settings, PCR-ribotyping is currently considered the gold-standard for investigating *C. difficile* epidemiology.[24] This technique relies on the amplification of the 16 S to 23 S intergenic spacer region (ISR), part of the ribosomal RNA (rRNA) operon. The variation in rRNA operon copy number and 16 S to 23 S ISR size results in numerous amplicon combinations across *C. difficile* isolates, and those with identical PCR banding patterns are classified into the same ribotype (RT).[24] This method has been adapted into a fluorescent PCR ribotyping protocol that is portable and validated across centers.[25]

The association between *C. difficile* and pseudomembranous colitis was first discovered in 1978.[26] While small-scale outbreaks had been reported in the United States and Europe in the 1980s,[27–29] the incidence of CDIs increased substantially in North America and Europe in early 2000s, with increasing severity and mortality rates, predominantly caused by RT027 (often designated BI/NAP1/027) isolates in older populations.[30–35] Subsequently, RT078 isolates were found to cause severe community-associated CDI in relatively younger individuals (< 80 years).[36–38] These early reports have sparked an intense interest in identifying 'hypervirulent strains' and their

Table 1. Visual representation of the elements defining severe *Clostridioides difficile* infection across multiple studies.

Study[a]	Examination			Vital sign dyscrasias		Lab dyscrasias				Complications						Outcomes		
	Age	Pain	AMS	Fever	HDI	WBC	AKI/CKD	↓Alb	↑Lac	Abnl CT	Ileus	PM	TM	ICU	Abd Comp	Surg	Organ Failure	Death
IDSA/SHEA 2010[7]					X	X	X				X		X					
IDSA/SHEA 2017[8]					X	X	X				X		X					
Khanafer *et al.*[9] – 1[b]					X	X	X				X		X	X				
Khanafer *et al.*[9] – 2				X	X	X	X				X	X	X	X	X	X		X
Khanafer *et al.*[9] – 3					X							X	X	X	X	X		X
Khanafer *et al.*[9] – 4					X	X	X					X	X	X	X	X		
Surawicz *et al.*[11c]		X				X		X										
Surawicz *et al.*[11] SC		X	X	X	X	X			X		X			X			X	
Bishop *et al.*[13]				X	X	X	X	X	X	X	X	X	X		X			
Bishop *et al.*[13]													X	X		X		X
Keller and Kuijper[14]					X									X		X		X
Morgan *et al.*[15]					X						X	X	X					
Abou Chakra *et al.*[16]													X	X	X	X		X
Shaw *et al.*[17]				X		X		X				X						X
See *et al.*[18]						X	X						X					
Sundram *et al.*[19]						X					X	X	X					
Swale *et al.*[20]				X	X	X	X				X	X	X			X		
Kim *et al.*[21]	X					X		X				X		X				
Abou Chakra *et al.*[22]	X					X		X						X				

Abd Comp, Peritonitis/Bowel Perforation/Ascites; Abnl CT, Abnormal CT Imaging; Age, Advanced Age; AKI/CKD, Renal Impairment; ↓Alb, Hypoalbuminemia; AMS, Altered Mental Status; Fever, Fever or Rigors; HDI, Hemodynamic Instability, Including Hypotension & Shock; ICU, intensive care unit Level of Care; IDSA/SHEA, Infectious Diseases Society of America/Society for Healthcare Epidemiology of America; Lac, Elevated Lactate; Pain, Abdominal Pain/Tenderness or Distention; PM, Psedumembranous Colitis or Severe Colitis; Surg, Surgery; TM, Toxic Megacolon; WBC, Leukocytosis.

[a]All studies were in hospitalized adults.

[b]Khanafer *et al.*[9] study includes four separate definitions delineated separately in the chart.

[c]Surawicz *et al.*[11] includes a definition for severe CDI and a separate definition for severe-complicated CDI, delineated as SC.

Table 2. Summary of selected studies.

Ribotype (RT)	Study Country Year	Primary outcome associated with disease severity				
		Host response	Diarrhea	ICU admission	Colectomy	Mortality
RT027	Loo *et al.*[30] Canada 2004	–	22.5 / 1000 admissions	6.5%	1.9%	6.9% (30-d attributable mortality)
RT027	Pépin *et al.*[39] Canada 2003–2004	–	–	9.9%	2.5%	16.7% (1-year attributable mortality)
RT027 vs other	Abou Chakra *et al.*[16] Canada 2005 – 2008	–	–	Adjusted OR (95% CI) 1.6 (0.96–2.70) *Together with colonic perforation and toxic megacolon defined as complications of CDI*		
RT027 vs RT078	Goorhuis *et al.*[36] The Netherlands 2005–2008	–	40.0% vs 38.9%	17.7% vs 9.6% *Together defined as complicated course*		4.0% vs 3.8% (attributable mortality)
RT027 vs RT078 vs other clades	Walker *et al.*[41] UK 2006–2011	Significant differences in neutrophils and white blood cell count across RTs/clades	–	–	–	20% vs 25% vs 12%; $p < .0001$ (14-d mortality)
RT027 vs RT078	Patterson *et al.*[42] UK 2008	–	–	–	–	Adjusted RRR (95% CI) 1.88 (0.49–7.17); $p = .21$ (30-d mortality)
RT027/078 vs other	Walk *et al.*[43] USA (MI) 2010–2012	–	–	Adjusted OR (95% CI) 1.06 (0.49–2.20); $p = .871$ *Together defined as severe CDI*		
RT027 vs RT014-020 vs RT106 vs other	Menon *et al.*[44] USA (MI) 2016	RT014-020 significantly associated with IDSA severity (white blood cell count > 15,000 cells/μL or a 1.5-fold increase in serum creatinine above baseline)	–	Unadjusted OR (95% CI) 0.40; $p = .719$ vs 3.17 (1.28–7.57); $p = .010$ vs 0.84 (0.13–2.98); $p = .818$ *Together defined as disease-related complications*		
RT027 vs RT106	Sundram *et al.*[19] UK 2006–2007	RT027 more commonly associated with complications (colitis with leucocytosis and renal impairment, toxic megacolon)	–	–		22.7% vs 10.8% (crude 28-d mortality) 11.4% vs 2.7% (3-d mortality)
RT027 vs RT017	Goorhuis *et al.*[45] The Netherlands 2005–2007	–	–	–	–	25.9% vs 22.9% (overall 30-d mortality)

CDI, *Clostridioides difficile* infection; CI, confidence interval; ICU, intensive care unit; IDSA, Infectious Diseases Society of America; OR, odds ratio; RRR, relative risk ratio; RT, ribotype.

virulence determinants, as early detection and diagnosis have important treatment and infection prevention implications. Below we will discuss our current understanding of the relationship between ribotypes and disease severity, and microbial factors involved in virulence.

Since its emergence and rapid rise to epidemic prominence, RT027 has been considered a 'hypervirulent' ribotype, associated with particularly severe disease including *C. difficile*-associated colectomies and deaths (Table 2).[18,39] However, this relationship has not been universally demonstrated in endemic, non-outbreak settings. One UK study compared CDI severity for patients with RT027 versus non-RT027, where severe CDI was defined as having ≥ 1 of the following: shock, paralytic ileus, pseudomembranous colitis, or toxic megacolon. After adjusting for sex, recent hospitalization, gastroenteritis on admission, antibiotic use, and admitting hospital, RT027 was not associated with severe disease.[15] A study conducted in the US found that infection with RT027 was more likely to lead to a change in antibiotic treatment, but neither affected the severity of CDI nor the incidence of mortality and recurrent CDI.[40]

A Canada-based multivariable analysis found a trend toward an association between RT027 and complicated CDI (cCDI) [adjusted odds ratio (aOR), 1.6; 95% confidence interval (CI) = 0.96–2.7], defined as ≥ 1 of the following: colonic perforation, toxic megacolon, colectomy, admission to an intensive care unit (ICU) for cCDI, or CDI-associated death within 30 days of enrollment.[16] Interestingly, this study noted that no association was found when less specific but more frequently used definitions of cCDI were used: admission to an ICU for any reason, colonic perforation, toxic megacolon, colectomy or hemicolectomy, or 30-day all-cause mortality. This observation was consistent with a series of studies conducted in one large Michigan hospital, where the association between RT027 and severe outcome varied depending on disease definition, in addition to cohort size and inclusion of RT078 in an aggregate predictor.[41,43] Intriguingly, a recent shift in the molecular epidemiology of CDI has been observed in the same Michigan hospital, where the prevalence of RT027 has been surpassed by RT014-020 and the newly emerged RT106 since 2016. Here RT027 was not associated with clinical outcomes including IDSA severity (white blood cell count more than 15 000 cells/μL or a 1.5-fold increase in serum creatinine above baseline), 30-day all-cause mortality, or CDI-associated complications within 30 days of diagnosis. On the other hand, RT014-020 was associated with both IDSA severity and 30-day mortality in unadjusted analyses.[44] While the clinical significance of such shift is currently unclear, an up-to-date assessment of local molecular epidemiology is critical for studying the association between strains and outcome.

Although the hypervirulence of RT027 remains contentious, it has been used as a reference to gain insight into the virulence of emerging ribotypes. One Dutch study showed that RT027 and RT078 isolates collected between 2005 and 2008 caused similar proportions of severe diarrhea and attributable mortality, although RT078 tended to affect younger patients than RT027 (67.4 vs 73.5 years; $p = .01$).[36] A study examining isolates collected in 2008 in Northern Ireland found that RT027 was associated with higher, albeit statistically insignificant, 30-day mortality than RT078 in a covariate adjusted analysis.[42] Another ribotype of interest is RT017, hypothesized to have first emerged in Asia, has since disseminated globally and caused outbreaks.[46] RT017 and RT027 are both associated with high mortality rates after 30 days according to a Dutch study (22.9% and 25.9%), significantly higher than other ribotypes.[45] RT023, a recently emerging ribotype in Europe, has been associated with similar disease severity (fever, leukocytosis, diarrhea with hypoalbuminemia and/or dehydration, pseudomembranous colitis and/or bloody diarrhea) and CDI-attributable mortality as RT027, although RT027 remained to be the more common cause for complicated CDI (surgical procedure, admission to ICU and/or overall mortality within 30 days after diagnosis).[17] Finally, RT106 (NAP11) has become one of the most prevalent ribotypes around the globe, although is less likely to cause severe disease than RT027.[4,47] In one UK study, RT106 was associated with lower crude mortality compared to RT027 (28-day mortality: 10.8% and 22.7%; 3-day mortality: 2.7% and 11.4%), less severe CDI (defined as colitis with leukocytosis, renal impairment and toxic megacolon), consistent with a multicenter study conducted in the US finding RT106 to be significantly less associated with severe disease than RT027 (ileus, toxic megacolon, pseudomembranous colitis within 5 days, or white

blood cell count $\geq$ 15,000/mm^3 within one day of positive test).[19]

A recent US single center study examining 386 unique isolates belonging to 21 ribotypes found no correlations between ribotypes and CDI severity.[48] Notably, this lack of association held true when isolates were grouped by core genome multilocus typing utilizing 2270 genes conserved across isolates,[49] suggesting that any putative genetic variations associated with disease severity lie outside the core genome, including the 16 S to 23 S intergenic region used for ribotyping. In fact, a recent study examining $>$ 12,000 *C. difficile* genomes by whole-genome sequencing (WGS) reported that the core genome only constituted 12.8% of the total gene repertoire, highlighting the need to investigate the pathogenic potential encoded by the accessory genome.[50] WGS is changing the landscape of how we think about *C. difficile* strains by grouping ribotypes into different clades based on phylogenetic relationships. WGS is also showing areas where ribotyping or other molecular methods could misidentify relationships. As WGS and other omics methods gain prominence, how we think about strains and severity risk may evolve. While a full overview of studies identifying *C. difficile* genomic features associated with virulence is outside the scope of this review, they are ongoing and may provide important insights into CDI pathogenesis.[51–53] Furthermore, the association between ribotypes and CDI severity needs to be scrutinized in the context of host features including their immune status, comorbidities, and the gut microbiota, a critical player in CDI susceptibility and progression.[54] Together, caution must be used when interpreting individual studies given that pathogen epidemiology, host characteristics, clinical practices (e.g. severity criteria and diagnostic tests), and statistical models can vary over time and geographical regions.

C. difficile toxins and host response

Toxins and disease severity. The pathogenesis of, and host response to CDI has been reviewed previously.[55–57] Briefly, *C. difficile* can be ingested in its spore form, germinate into vegetative cells and proliferate in the colon of susceptible hosts, such as those with an antibiotic-perturbed gut microbiota. Following germination, *C. difficile* strains producing toxins can cause disease by damaging colonic epithelial cells, disrupting tight junctions, and activating both innate and adaptive immune response. While it is critical to appreciate the diverse virulence factors employed by *C. difficile*, such as surface layer proteins that modulate host-pathogen interaction and disease severity,[58] we will focus the following sections on the role of *C. difficile* toxins in disease outcome.

Three clostridial protein toxins, namely toxin A, toxin B, and binary toxin, are believed to be major determinants of *C. difficile* virulence. For example, the hyperproduction of toxins A and B has been proposed to underlie the virulence of RT027, and immunoassay-based toxin detection is more common in patients infected with RT078.[59,60] A Swedish study grouping patients into different severities of *C. difficile*-associated diarrhea ($<$3 loose stools per day, 3–10 per day, $>$ 10 per day) found that fecal toxin level was associated with diarrhea frequency.[61] In an Israeli study defining CDI severity according to the SHEA/IDSA guidelines, patients with severe CDI had significantly higher toxin levels compared to those with mild/moderate disease, although the immunoassay-based detection kits in used these studies did not measure levels of toxins A and B separately.[62]

Due to their clinical relevance, toxins A and B have been characterized extensively.[63] While they are structurally similar, they mediate cytotoxic effects via distinct mechanisms.[64] Toxin A induces apoptosis, whereas toxin B results in apoptosis at lower concentrations and necrosis at higher concentrations.[65] Supported by animal models and identification of toxin A-negative/toxin B-positive clinical isolates around the globe,[66–68] toxin B is currently thought to be the main virulence determinant of toxigenic *C. difficile*.[69,70] Notably, one study found that CDI detected by toxin enzyme immunoassay (EIA) was more severe than those detected by nucleic acid amplification tests (NAAT) targeting the gene encoding toxin B. Toxin-positive cases were more likely to have pseudomembranous colitis, white blood cell count $\geq$ 15 000 cells/μL, albumin $\leq$ 2.5 g/dL, and higher recurrence rates. However, CDI-related complication (colectomy, ileus, or admission to the ICU) and 30-day all-cause mortality rates were not different between toxin-positive and NAAT-positive cases by multivariable analyses adjusting for demographic information, comorbidities, and antibiotic exposure.[71] These results are an important reminder that diagnostic testing

sensitivity, among other factors such as amino acid variations in toxin sequences, could affect the association between toxin presence and disease severity.[71–73]

The role of binary toxin in CDI pathogenesis in humans is more elusive.[74,75] First reported to be produced by RT027 and RT078 isolates, binary toxin has been suggested to contribute to the virulence of these epidemic lineages.[31] Based on murine models this increased severity and poorer outcomes are believed to be, at least in part, secondary to the depletion of peripheral blood eosinophils caused by binary toxin.[76] A Belgian study comparing patients with toxin B+/binary toxin+ and toxin B-/binary toxin+ in non-RT027/078 ribotypes, however, showed no differences between these groups in CDI severity, including minimal albuminemia, maximal serum C-reactive protein, colitis, ileus, diarrhea duration, 30-day mortality, or recurrence rates.[77] A British study showed that patients infected with *C. difficile* encoding binary toxin genes had higher total peripheral white cell count (17×10^9/L, 95% CI = 14–20 vs 13×10^9/L, 95% CI = 12–15, $p < .01$) and 30-day all-cause mortality (31% vs 14%, $p = .02$). Nonetheless, this study did not assess the presence of toxin A or B in isolates.[78] A study conducted in Denmark found that patients infected with *C. difficile* isolates positive for toxins A/B and binary toxin had higher 30-day case-fatality rates compared to those positive for toxins A/B but negative for binary toxin, irrespective of ribotypes.[79] Intriguingly, RT033, a recently emerged ribotype that only produces binary toxin has been isolated in six French patients diagnosed with CDI. While the prevalence of RT033 is likely to be low in this region, CDI caused by RT033 may not be detected by conventional diagnostic methods that are mostly based on the detection of toxins A and B.[74]

To experimentally determine the contribution of A, B and binary toxins, seminal work by Kuehne and colleagues assessed their virulence by generating a set of isogenic RT027 strains in a hamster infection model.[80] All three toxins were found to cause pathology. All hamsters infected and colonized with wild-type strain and an isogenic strain producing toxin B alone succumbed to the disease at time points (3.7 ± 1.97 days after infection vs 2.3 ± 0.52 days, respectively) significantly earlier than those infected with an isogenic strain producing toxin A only (5.9 ± 1.98 days, $p < .05$), but equivalent to a strain that produced both toxin A and binary toxin (3.0 days). Intriguingly, only 33% of hamsters succumbed to the disease when the infecting strain produced binary toxin alone. Intriguing, these animals did not present typical symptoms of CDI such as loose stool and diffuse hemorrhage in the cecum, but instead had hemorrhage and inflammation in their small intestines. Together, it is likely that each of these toxins contributed synergistically to enhance virulence during CDI.

Host immune response in severe CDI. CDI is characterized by intestinal inflammation. The capacity of innate and adaptive immunity to confer proinflammatory damage, anti-inflammatory protection, and antibody-mediated toxin neutralization has been reviewed in detail previously.[81] Here we focus on the role of innate immune response in disease severity. Briefly, the invasion of *C. difficile* toxins into the intestinal epithelia triggers the release of proinflammatory mediators by macrophages, monocytes, and dendritic cells. Major inflammatory cytokines produced include IL-8, IL-1α, IL-1β, IL-6, and TNF-α. In addition, neutrophils and monocytes are recruited to the site of infection by IL-8 and other chemokines. This recruitment is accompanied by increased permeability of the blood vessel, collaterally resulting in fluid leakage into the intestinal lumen and subsequently watery diarrhea. In the case of severe CDI, extensive local damage can lead to pseudomembranous colitis, perforation of the colonic submucosa, systematic symptoms manifesting as organ dysfunction or sepsis, and death.

Since host response plays a critical role in CDI pathogenesis, the utility of host biomarkers as a proxy for infection status and predictors of disease severity is under active investigation, and fecal biomarkers have been pursued extensively. In a prospective cohort study with 48 severe (white cell count $> 20 \times 10^9$/L, $> 50\%$ increase in blood creatinine above baseline, fever > 38.5°C, severe colitis, hypotension, ileus, toxic megacolon, colectomy) and 116 non-severe cases, it was found that the median levels of fecal lactoferrin, primarily derived from activated neutrophils, were significantly higher in severe compared to non-severe cases (104.6 vs 40.1 ng/μL, $p = .02$). However, the authors noted high inter-individual variability and questioned the utility of fecal lactoferrin as a biomarker of CDI disease.[20] This study also measured fecal calprotectin, another

neutrophil-associated molecule, to be higher in severe compared to non-severe cases, but this did not reach statistical significance (969.3 vs 512.7 mg/kg, $p = .09$). Fecal calprotectin was found to reflect disease severity in another study including 30 severe cases (defined by two or more of the following: age of > 60 years, fever > 38.3°C, albumin level < 25 g/L, peripheral white blood cell count $> 15 \times 10^9$/L, or one of the following: endoscopic evidence of pseudomembranous colitis or treatment in the ICU), 50 mild cases, and 71 healthy controls. Using unadjusted analysis, this study found that fecal calprotectin levels were highest in severe patients compared to mild cases and healthy controls (1391.5 μg/g, 188.2 μg/g, and 35.6 μg/g; $p < .001$ between severe and mild, $p = .019$ between mild and healthy).[21] In a recent endeavor to identify novel CDI-specific biomarkers, researchers employed untargeted proteomics to assess molecules differentially abundant in CDI-positive ($N = 54$) individuals, with severity based on the SHEA/IDSA scoring system (0, 1, 2 corresponding to non-severe, severe, and fulminant cases, respectively). The relative abundance of alpha-1-antitrypsin was significantly higher in non-severe than severe patients ($p = .028$), identifying it as a putative biomarker of severity.[82]

Another strategy to profile the association between immune response and CDI severity is to measure systemic biomarkers in circulation. One of the early studies (78 cases; 8 of them met CDC severity criteria) found that severe patients had significantly elevated levels of IL-8 [odds ratio (OR) = 5.92; 95% CI = 1.13–31.1] and IL-6 (OR = 3.12; 95% CI = 1.05–9.28).[83] More recently the same group used two larger cohorts ($N = 156$ and 272) to comprehensively examine the utility of serum biomarkers near the time of diagnosis (within 48 hours) and CDI severity. This updated study found six biomarkers (hepatocyte growth factor, procalcitonin, IL-6, IL-2R α, IL-8, and TNF-α) indicative of epithelial disruption, inflammation and neutrophilic recruitment to be significantly associated with IDSA severity, 30-day mortality and complications including ICU admission, colectomy, and/or death attributed to CDI. Based on these results, this study further identified a panel including only four biomarkers (IL-8, procalcitonin, hepatocyte growth factor, and IL-2Rα) without compromising accuracy of the prediction model. Remarkably, the authors noted that biomarker-based models performed better than models including basic clinical variables such as Elixhauser comorbidity index in predicting 30-day mortality and attributable complications. The inclusion of biomarkers, Elixhauser comorbidity index and IDSA severity only marginally increased predictive ability. For example, a three-factor model (biomarkers + Elixhauser + IDSA severity) had an AuROC of 0.909, compared to 0.892 with biomarkers alone in predicting 30-day mortality. The AuROCs of these models in predicting attributable complications were 0.874 and 0.84, respectively.[84]

A recent study measured 17 plasma cytokines in 341 CDI patients. After adjusting for demographic and clinical characteristics, the researchers found that patients in the top 25th percentile for TNF-α (hazard ratio (HR) = 8.35, $p = .005$) and IL-6 (HR = 4.45, $p = .01$) were at a higher risk of 90-day mortality, whereas those with higher abundance of CCL5 were protected (HR = 0.18, $p \leq .008$). Compared to using clinical features (age and while blood cell count) alone in basic models, the inclusion of TNF-α, IL-8, and CCL5 significantly improved the model (AUC = 0.69 vs 0.83, respectively).[85] Taken together, the integration of biomarker information and host features likely will help better predict the clinical trajectory of CDI patients, and implement targeted interventions to prevent severe outcomes.

Host immune factors associated with CDI outcomes

In this section we discuss the implications of two host immune factors for the outcome of CDI. Immune compromise does appear to be associated with CDI risk. However, in-depth discussion of the interplay among various immunodeficiencies and CDI risk is beyond the scope of this review and will not be addressed.

Eosinopenia

Preclinical murine models have suggested a protective effect of eosinophilia on mortality from CDI.[76,86,87] A human study published in 2018 found that admission eosinophil count was associated with inpatient mortality risk; in their validation cohort they found in-patient mortality of 14.2% of patients with admission eosinophil counts of 0 cells/μL compared to 6.6% of patients with admission eosinophil counts > 0 cells/μL

($p < .001$).[76] This association was only seen when comparing eosinophil count as a binary variable of 0 cells/μL vs > 0 cells/μL and was not observed when eosinophil count was observed as a continuous variable.[76] Secondary outcomes from this study also found that patients with an admission eosinophils count of 0 cells/μL required more frequent admission to monitored care settings (ICU type settings), use of vasopressors, and were more likely to require total colectomy for severe, medically refractory disease.[76]

Antitoxin A IgG levels

Low systemic antitoxin A IgG levels were associated with mortality in a prospective cohort study which primarily included elderly subjects.[88,89]

Clinical features associated with CDI outcomes

Renal dysfunction

Acute kidney injury has been associated with the development of complicated CDI.[22] A study developing a severity score for CDI identified that acute kidney injury was associated with an increased risk of in hospital mortality; there was also a similar finding in another study which identified an association between acute kidney injury and increased perioperative mortality for patients undergoing total abdominal colectomy for treatment of CDI.[3,4]

It is not just acute kidney injury that is associated with outcome in the setting of severe CDI, but also absolute renal function at the time of diagnosis, irrespective of the baseline. Renal disease is not only associated with increased risk of severe CDI, but also has been associated with adverse outcomes, including lower odds of cure and increased probability of recurrence in patients with stage 3 or higher chronic kidney disease.[90] A meta-analysis showed a pooled relative risk of mortality attributed to CDI in pts with chronic kidney disease was 1.73; for those with end-stage renal disease it was 2.15.[90]

As noted above, the 2017 SHEA/IDSA guidelines modified their criteria for severe CDI to include a creatinine of > 1.5 mg/dL rather than a 1.5-fold increase from the baseline creatinine.[7,8] A study published in 2020 validated this change in how renal dysfunction was defined by taking a cohort of patients with CDI and comparing their severity classification using both the 2010 and 2017 IDSA/SHEA severity criteria.[91] They found that around 10% of episodes in their cohort had discordant classification, however the new criteria classified more patients with baseline kidney disease as severe, which better correlated with all-cause mortality.[91]

A systematic review completed in 2012 identified several factors which seemed to have an association with increased risk for mortality including impaired renal function.[92] They identified that in 6 out of 13 included cohorts there was an association between serum creatinine and mortality with this association found primarily in studies with a serum creatinine cut-off of > 200 μmol/L (2.26 mg/dL).[92] This study did not distinguish chronic from acute renal dysfunction.[92]

Age

Advanced age is a significant risk factor for CDI. Older adults also tend to fare worse with CDI – those older than 65 years account for a significant portion of the morbidity and mortality seen from CDI.[88] Older patients are more frequently hospitalized, more likely to experience a lack of improvement in symptoms with therapy, and are more likely to develop severe disease or recurrence.[22,88] One proffered mechanism for this association is the phenomenon of immuno-senescence, or the impaired immune response in older adults that occurs naturally with aging, leading to lessened ability to respond to infection.[88,93]

Given this association, some criteria for severe disease include age as a factor.[10] One study found an odds ratio of 1.14 for treatment failure per decade increase.[22] In studies which have developed severity scores for CDI, increasing age, in particular age > 80 has been associated with high in-hospital mortality.[7,8] Advanced age was also associated with increased risk of death in patients who underwent emergency surgery.[94]

Leukocytosis

Leukocytosis is widely accepted as a marker for severe CDI as demonstrated by its inclusion in both the SHEA/IDSA as well as guidelines from the *American Journal of Gastroenterology*.[7,8,11] This association has been demonstrated in systematic reviews as well. One systematic review found that

the relative risk of complicated CDI was 2.7–5.5 for those with a leukocyte count $> 20 \times 10^9$/L across the included studies.[22] Other systematic reviews also frequently find that leukocytosis associated with severe disease.[92,95] With a definition of severe CDI that included at least two of age > 60, albumin < 2.5 mg/dL, leukocytosis and ICU admission, the risk of 90 day mortality increased substantially (OR = 1.8).[22]

Serum albumin

Interpretation of a serum albumin concentration in a patient who presents acutely ill can be challenging as a clinician. This challenge arises because the measured serum albumin can result from the interaction of several different causal pathways. Hypoalbuminemia can be chronic, resulting from malnutrition or liver disease. Alternatively, hypoalbuminemia can also occur acutely – albumin is a negative acute phase reactant and its level can drop in the setting of acute inflammation, and it can be excreted after renal injury or with protein-losing enteropathy.[96,97] Therefore, conceptually it makes sense that serum albumin would be useful in predicting outcomes from severe CDI, because it is simultaneously a potential marker of acute inflammation and the baseline health status of an individual.

Although the predictive utility of serum albumin is not well defined, in some studies hypoalbuminemia (defined as < 25–35 g/L) alone or as part of various clinical prediction rules has been found to be associated with an increased risk of mortality.[12,22,92,95]

Level of care

Level of care has been described in several studies, including a systematic review, as a factor that associates with increased risk of mortality, particularly for in-hospital mortality.[4,22] Similar to albumin, however, level of care – specifically ICU level of care – has been used by many studies in the definition for severe CDI.[22] Studies including ICU admission in the definition of severe CDI have demonstrated a significantly increased risk of 90 day mortality.[22] One study found that ICU level of care was associated with an increased risk of treatment failure. However, this finding was confounded by the primary treatment, which was metronidazole, and therefore it is difficult to extrapolate that finding to a population in an ICU receiving the current standard of care.[22] Ultimately, since patients receiving a higher level of care are typically those with more severe illness, it is not surprising that a higher level of care is associated with adverse outcomes. However, care should be taken when interpreting this, because the level of care itself resulted from numerous preceding factors, and including these factors alongside the level of care itself can count the same risk twice and bias results due to this collinearity.

Inflammatory bowel disease, chronic illnesses, and other lesser factors

Chronic illnesses have been linked to an increased risk of CDI. Certain chronic illnesses such as cardiopulmonary or liver disease, malignancy and inflammatory bowel disease (IBD) have been found to have been found to be associated with increased severity of CDI in the development of clinical risk scores.[4] Inflammatory bowel disease in particular has been linked to more frequent and more severe CDI with worsened outcomes compared to the general population.[98] For patients who are admitted for management of their inflammatory bowel disease, their risk of death increases if they also have CDI.[94,99]

There are numerous other associations which have been suggested to be seen with more severe CDI or with poorer outcomes from CDI. These include chronic illnesses, particularly liver disease, cardiopulmonary disease, or malignancy, and chronic use of certain medications such as steroids.[3,4] Mechanical ventilation has also been observed as a risk factor for more severe disease and worse outcomes.[3]

Treatment factors and CDI outcomes

A full discussion of the treatment of severe CDI is beyond the scope of this review. However, we will discuss two specific treatment modalities owing to their complicated relationship specifically with severe CDI. Although not discussed here, tantamount to any antimicrobial therapy or surgical intervention is excellent supportive care. As Vely states in their discussion of the role of surgery in the treatment of CDI: 'Resuscitation to euvolemia, early aggressive treatment with the appropriate antibiotics, and early surgical intervention are pivotal factors for better outcomes'.[100]

Metronidazole

Metronidazole has had a shifting role in the management of CDI in recent years. Previously the standard first line therapy for mild to moderate CDI, it has now taken a backseat to other therapies. In the management of severe CDI in particular, metronidazole is not recommended as primary therapy because it has been associated with higher rates of treatment failure. A recent retrospective study of patients with severe CDI in Singapore found that in 2012 83.9% of patients with severe CDI (by 2010 IDSA/SHEA criteria) and 77.3% of those with severe CDI received treatment with metronidazole monotherapy.[101] This same study also saw more persistent diarrhea, major complications and deaths with increased severity of CDI, where the majority of the severe CDI was treated with metronidazole.[101]

That is not, however, to say that there is no role for metronidazole in the treatment of severe CDI. A 2014 study found that for patients who were critically ill with severe CDI there was higher mortality in those that received monotherapy with PO vancomycin compared to combination therapy with metronidazole.[102] There is, of course, conflicting data with a 2018 study showing higher 30-day mortality in the combination therapy group compared to the monotherapy, although this finding did not reach statistical significance.[103]

Overall, the role of metronidazole in the management of severe CDI, as it relates to outcomes, has yet to be fully established; while monotherapy with metronidazole is associated with worse outcomes further studies are needed to further delineate its role as an adjunctive therapy.

Surgery

Due to its associated morbidity and mortality, surgery is frequently considered an adverse outcome of severe CDI, rather than as a stepping stone to a different outcome such as cure, relapse, refractory disease or death. Surgical intervention for the management of CDI is a potentially lifesaving intervention for certain patients with severe illness, but there are some definite drawbacks to this treatment modality.

The most significant draw back to surgery as a treatment for severe CDI is the mortality which has been associated with surgical intervention. The most common surgical procedure performed is a total abdominal colectomy with end ileostomy.[94,100] Colectomy with end ileostomy has an estimated mortality rate of 34–71% which is significantly influenced by how well the patient is doing leading up to surgery.[14] In one systematic review of patient outcomes following emergency surgery for CDI, the frequency of surgery for patients with severe CDI (as determined by the original authors) was 29.9% (2.2–86%).[92] This same review found a 30-day post-operative mortality of 41.3% (19–71%) and in-hospital mortality of 41.6% (25–80%).[94] The preoperative condition of the patient had a significant influence on their outcome following the surgery, with shock requiring vasopressors, pre-operative intubation, acute renal failure or multiple organ failure predicting mortality.[94] The drawbacks associated with this procedure could lead clinicians to delay surgery in favor of giving medical therapy more time, but delaying surgical intervention can lead to worsening clinical status and then, in turn, worse surgical outcomes.[100]

There has been interest in alternative surgical approaches, which may have less morbidity and have the potential to be performed earlier in the course.[14] Loop ileostomy with colonic lavage is one such procedure but does have limitations – it cannot be used if there is transmural necrosis, perforation or distal blockage.[100] One study comparing diverting loop ileostomy with colonic lavage demonstrated a reduced mortality rate when compared to a historical colectomy cohort.[94,104] A retrospective study did find this procedure was associated with less blood loss, shorter OR time and decreased mortality.[100] However, despite this initial promise, improvements in morbidity and mortality have not been consistently seen across studies. A systematic review, which compared the overall morbidity and mortality for diverting loop ileostomy with colonic lavage to total abdominal colectomy, found no significant difference in post-operative morbidity and mortality.[105]

Conclusion

Management of patients presenting with CDI can be challenging owing to the wide range of clinical presentations and difficulty in predicting, at the early stages of infection, which patients are most likely to progress to have adverse outcomes. Further research needs to be done to create a better predictive model to determine what patients

are most likely to progress to severe disease and also which patients with severe disease will progress to poor outcomes. Incorporating the microbiologic and host factors described above into novel modeling techniques, such as those from the machine learning field, may yield improved predictive ability. This can not only benefit clinicians making decisions today, but also enable research into future therapeutics, because adverse outcomes are rare enough that adequately powering such studies can be challenging. Examples of promising avenues of research that could be enabled by a better understanding of risk for adverse outcomes from CDI include use of novel antimicrobials (e.g. ridinilazole), prostaglandins, defined live biotherapeutics, monoclonal antibodies, fecal transplant in the acute setting, and alternative surgical approaches.[106] In addition, therapies currently used in the treatment and prevention of recurrent CDI, including monoclonal antibodies and fecal material transplant, could be found with additional research to have further benefits in the acute setting. Targeted, evidence-based use of earlier, more aggressive therapeutics in the patients who would benefit the most from them could lead to overall improved outcomes.

Author contributions

Adam Ressler and Joyce Wang have equally contributed to this manuscript.

Conflict of interest statement

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: K.R. has consulted for Bio-K+ International, Inc., Roche Molecular Systems, Inc., and Seres Therapeutics, and he holds an investigator-initiated research grant from Merck & Co., Inc. All other authors report no potential conflicts.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by grants from the National Institute of Allergy and Infectious Diseases at the National Institutes of Health and from the Agency for Healthcare Research and Quality (grant nos: U01AI124255 and R01HS027431). J.W. was supported by the Canadian Institutes of Health Research fellowship (grant no. 201711MFE-396343-165736) and the Michigan Institute for Clinical and Health Research (MICHR) Postdoctoral Translational Scholars Program (PTSP) (grant no. UL1TR002240). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

ORCID iD

Krishna Rao https://orcid.org/0000-0002-9213-7850

References

1. Reigadas E, Alcalá L, Marín M, *et al*. Clinical, immunological and microbiological predictors of poor outcome in *Clostridium difficile* infection. *Diagn Microbiol Infect Dis* 2017; 88: 330–334.
2. Centers for Disease Control and Prevention (CDC). *Antibiotic resistance threats in the United States, 2019*. Atlanta, GA: CDC, U.S. Department of Health and Human Services, 2019.
3. Kulaylat AS, Kassam Z, Hollenbeak CS, *et al*. A surgical clostridium-associated risk of death score predicts mortality after colectomy for *Clostridium difficile*. *Dis Colon Rectum* 2017; 60: 1285–1290.
4. Kassam Z, Cribb Fabersunne C, Smith MB, *et al*. *Clostridium difficile* associated risk of death score (CARDS): a novel severity score to predict mortality among hospitalised patients with *C. difficile* infection. *Aliment Pharmacol Ther* 2016; 43: 725–733.
5. Napolitano LM and Edmiston CE Jr. *Clostridium difficile* disease: diagnosis, pathogenesis, and treatment update. *Surgery* 2017; 162: 325–348.
6. Guh AY, Mu Y, Winston LG, *et al*. Trends in U.S. burden of *Clostridioides difficile* infection and outcomes. *N Engl J Med* 2020; 382: 1320–1330.
7. Cohen SH, Gerding DN, Johnson S, *et al*. Clinical practice guidelines for *Clostridium difficile* infection in adults: 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). *Infect Control Hosp Epidemiol* 2010; 31: 431–455.
8. McDonald LC, Gerding DN, Johnson S, *et al*. Clinical practice guidelines for *Clostridium difficile* infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). *Clin Infect Dis off Publ Infect Dis Soc Am* 2018; 66: e1–e48.

9. Khanafer N, Barbut F, Eckert C, *et al*. Factors predictive of severe *Clostridium difficile* infection depend on the definition used. *Anaerobe* 2016; 37: 43–48.
10. Abou Chakra CN, Pepin J and Valiquette L. Prediction tools for unfavourable outcomes in *Clostridium difficile* infection: a systematic review. *PLoS ONE* 2012; 7: e30258.
11. Surawicz CM, Brandt LJ, Binion DG, *et al*. Guidelines for diagnosis, treatment, and prevention of *Clostridium difficile* infections. *Am J Gastroenterol* 2013; 108: 478–498.
12. Belmares J, Gerding DN, Tillotson G, *et al*. Measuring the severity of *Clostridium difficile* infection: implications for management and drug development. *Expert Rev Anti Infect Ther* 2008; 6: 897–908.
13. Bishop EJ, Tiruvoipati R, Metcalfe J, *et al*. The outcome of patients with severe and severe-complicated *Clostridium difficile* infection treated with tigecycline combination therapy: a retrospective observational study: Tigecycline combination therapy for CDI. *Intern Med J* 2018; 48: 651–660.
14. Keller JJ and Kuijper EJ. Treatment of recurrent and severe *Clostridium difficile* infection. *Annu Rev Med* 2015; 66: 373–386.
15. Morgan OW, Rodrigues B, Elston T, *et al*. Clinical Severity of *Clostridium difficile* PCR Ribotype 027: a case-case study. *PLoS ONE* 2008; 3: e1812.
16. Abou Chakra CN, McGeer A, Labbé A-C, *et al*. Factors associated with complications of *Clostridium difficile* infection in a multicenter prospective cohort. *Clin Infect Dis off Publ Infect Dis Soc Am* 2015; 61: 1781–1788.
17. Shaw HA, Preston MD, Vendrik KEW, *et al*. The recent emergence of a highly related virulent *Clostridium difficile* clade with unique characteristics. *Clin Microbiol Infect* 2020; 26: 492–498.
18. See I, Mu Y, Cohen J, *et al*. NAP1 strain type predicts outcomes from *Clostridium difficile* infection. *Clin Infect Dis* 2014; 58: 1394–1400.
19. Sundram F, Guyot A, Carboo I, *et al*. *Clostridium difficile* ribotypes 027 and 106: clinical outcomes and risk factors. *J Hosp Infect* 2009; 72: 111–118.
20. Swale A, Miyajima F, Roberts P, *et al*. Calprotectin and Lactoferrin Faecal levels in patients with *Clostridium difficile* infection (CDI): a prospective cohort study. *PLoS ONE* 2014; 9: e106118.
21. Kim J, Kim H, Oh HJ, *et al*. Fecal calprotectin level reflects the severity of *clostridium difficile* infection. *Ann Lab Med* 2017; 37: 53–57.
22. Abou Chakra CN, Pepin J, Sirard S, *et al*. Risk factors for recurrence, complications and mortality in *Clostridium difficile* infection: a systematic review. *PLoS ONE* 2014; 9: e98400.
23. Janezic S, Potocnik M, Zidaric V, *et al*. Highly divergent *Clostridium difficile* strains isolated from the environment. *PLoS ONE* 2016; 11: e0167101.
24. Bouchet V, Huot H and Goldstein R. Molecular genetic basis of ribotyping. *Clin Microbiol Rev* 2008; 21: 262–273.
25. Martinson JNV, Broadaway S, Lohman E, *et al*. Evaluation of portability and cost of a fluorescent PCR Ribotyping protocol for *Clostridium difficile* epidemiology. *J Clin Microbiol* 2015; 53: 1192–1197.
26. Bartlett JG, Chang TW, Gurwith M, *et al*. Antibiotic-associated pseudomembranous colitis due to toxin-producing clostridia. *N Engl J Med* 1978; 298: 531–534.
27. Pierce PF Jr, Wilson R, Silva J Jr, *et al*. Antibiotic-associated pseudomembranous colitis: an epidemiologic investigation of a cluster of cases. *J Infect Dis* 1982; 145: 269–274.
28. Johnson S, Clabots CR, Linn FV, *et al*. Nosocomial *Clostridium difficile* colonisation and disease. *Lancet* 1990; 336: 97–100.
29. Nolan NP, Kelly CP, Humphreys JF, *et al*. An epidemic of pseudomembranous colitis: importance of person to person spread. *Gut* 1987; 28: 1467–1473.
30. Loo VG, Poirier L, Miller MA, *et al*. A Predominantly clonal multi-institutional outbreak of *Clostridium difficile*–associated diarrhea with high morbidity and mortality. *N Engl J Med* 2005; 353: 2442–2449.
31. McDonald LC, Killgore GE, Thompson A, *et al*. An epidemic, toxin gene–variant strain of *Clostridium difficile*. *N Engl J Med* 2005; 353: 2433–2441.
32. McDonald LC, Owings M and Jernigan DB. *Clostridium difficile* infection in patients discharged from US Short-stay Hospitals, 1996–2003. *Emerg Infect Dis* 2006; 12: 409–415.
33. Redelings MD, Sorvillo F and Mascola L. Increase in *Clostridium difficile*–related mortality rates, United States, 1999–2004. *Emerg Infect Dis* 2007; 13: 1417–1419.

34. Smith A. Outbreak of *Clostridium difficile* infection in an English hospital linked to hypertoxin-producing strains in Canada and the US. *Wkly Releases* 2005; 10: 2735.

35. Hubert B, Loo VG, Bourgault A-M, *et al*. A portrait of the geographic dissemination of the *Clostridium difficile* North American pulsed-field type 1 strain and the epidemiology of *C. difficile*-associated disease in Québec. *Clin Infect Dis off Publ Infect Dis Soc Am* 2007; 44: 238–244.

36. Goorhuis A, Bakker D, Corver J, *et al*. Emergence of *Clostridium difficile* Infection due to a new hypervirulent strain, polymerase chain reaction Ribotype 078. *Clin Infect Dis* 2008; 47: 1162–1170.

37. van Dorp SM, Smajlović E, Knetsch CW, *et al*. Clinical and microbiological characteristics of *Clostridium difficile* infection among hospitalized children in the Netherlands. *Clin Infect Dis* 2017; 64: 192–198.

38. Keessen EC, Hensgens MP, Spigaglia P, *et al*. Antimicrobial susceptibility profiles of human and piglet *Clostridium difficile* PCR-ribotype 078. *Antimicrob Resist Infect Control* 2013; 2: 14.

39. Pépin J, Valiquette L and Cossette B. Mortality attributable to nosocomial *Clostridium difficile*–associated disease during an epidemic caused by a hypervirulent strain in Quebec. *CMAJ* 2005; 173: 1037–1042.

40. Scardina T, Labuszewski L, Pacheco SM, *et al*. *Clostridium difficile* infection (CDI) severity and outcome among patients infected with the NAP1/BI/027 strain in a non-epidemic setting. *Infect Control Hosp Epidemiol* 2015; 36: 280–286.

41. Walker AS, Eyre DW, Wyllie DH, *et al*. Relationship between bacterial strain type, host biomarkers, and mortality in *Clostridium difficile* infection. *Clin Infect Dis* 2013; 56: 1589–1600.

42. Patterson L, Wilcox MH, Fawley WN, *et al*. Morbidity and mortality associated with *Clostridium difficile* ribotype 078: a case–case study. *J Hosp Infect* 2012; 82: 125–128.

43. Walk ST, Micic D, Jain R, *et al*. *Clostridium difficile* Ribotype does not predict severe infection. *Clin Infect Dis* 2012; 55: 1661–1668.

44. Menon A, Perry DA, Motyka J, *et al*. Changes in the association between diagnostic testing method, polymerase chain reaction Ribotype, and clinical outcomes from Clostridioides difficile infection: one institution's experience. *Clin Infect Dis* 2020: ciaa1395.

45. Goorhuis A, Debast SB, Dutilh JC, *et al*. Type-specific risk factors and outcome in an outbreak with 2 different *Clostridium difficile* types simultaneously in 1 hospital. *Clin Infect Dis* 2011; 53: 860–869.

46. Imwattana K, Knight DR, Kullin B, *et al*. *Clostridium difficile* ribotype 017 – characterization, evolution and epidemiology of the dominant strain in Asia. *Emerg Microbes Infect* 2019; 8: 796–807.

47. Carlson TJ, Blasingame D, Gonzales-Luna AJ, *et al*. *Clostridium difficile* ribotype 106: a systematic review of the antimicrobial susceptibility, genetics, and clinical outcomes of this common worldwide strain. *Anaerobe* 2020; 62: 102142.

48. Cho J, Cunningham S, Pu M, *et al*. *Clostridium difficile* whole-genome sequencing differentiates relapse with the same strain from reinfection with a new strain. *Clin Infect Dis* 2020: ciaa159.

49. Bletz S, Janezic S, Harmsen D, *et al*. Defining and evaluating a core genome multilocus sequence typing scheme for genome-wide typing of *Clostridium difficile*. *J Clin Microbiol* 2018; 56: e01987-17.

50. Knight DR, Imwattana K, Kullin B, *et al*. Major genetic discontinuity and novel toxigenic species in *Clostridium difficile* taxonomy. *Elife* 2021; 10: e64325.

51. Lewis BB, Carter RA, Ling L, *et al*. Pathogenicity locus, core genome, and accessory gene contributions to *Clostridium difficile* virulence. *mBio* 2017; 8: e00885-17.

52. Knight DR, Elliott B, Chang BJ, *et al*. Diversity and evolution in the genome of *Clostridium difficile*. *Clin Microbiol Rev* 2015; 28: 721–741.

53. Saund K, Rao K, Young VB, *et al*. Genetic Determinants of trehalose utilization are not associated with severe *Clostridium difficile* infection outcome. *Open Forum Infect Dis* 2020; 7: ofz548.

54. Theriot CM and Young VB. Interactions between the gastrointestinal microbiome and *Clostridium difficile*. *Annu Rev Microbiol* 2015; 69: 445–461.

55. Madan R and Petri WA. Immune responses to *Clostridium difficile* infection. *Trends Mol Med* 2012; 18: 658–666.

56. Rupnik M, Wilcox MH and Gerding DN. *Clostridium difficile* infection: new developments in epidemiology and pathogenesis. *Nat Rev Microbiol* 2009; 7: 526–536.

57. Abt MC, McKenney PT and Pamer EG. *Clostridium difficile* colitis: pathogenesis and host defence. *Nat Rev Microbiol* 2016; 14: 609–620.

58. Lynch M, Walsh TA, Marszalowska I, *et al*. Surface layer proteins from virulent *Clostridium*

difficile ribotypes exhibit signatures of positive selection with consequences for innate immune response. *BMC Evol Biol* 2017; 17: 90.

59. Warny M, Pepin J, Fang A, *et al*. Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. *Lancet* 2005; 366: 1079–1084.
60. Fairley DJ, McKenna JP, Stevenson M, *et al*. Association of *Clostridium difficile* ribotype 078 with detectable toxin in human stool specimens. *J Med Microbiol* 2015; 64: 1341–1345.
61. Akerlund T, Svenungsson B, Lagergren A, *et al*. Correlation of disease severity with fecal toxin levels in patients with *Clostridium difficile*-associated diarrhea and distribution of PCR ribotypes and toxin yields in vitro of corresponding isolates. *J Clin Microbiol* 2006; 44: 353–358.
62. Cohen NA, Miller T, Na'aminh W, *et al*. *Clostridium difficile* fecal toxin level is associated with disease severity and prognosis. *United Eur Gastroenterol J* 2018; 6: 773–780.
63. Chandrasekaran R and Lacy DB. The role of toxins in *Clostridium difficile* infection. *FEMS Microbiol Rev* 2017; 41: 723–750.
64. Pruitt RN, Chambers MG, Ng KK-S, *et al*. Structural organization of the functional domains of *Clostridium difficile* toxins A and B. *Proc Natl Acad Sci USA* 2010; 107: 13467–13472.
65. Chumbler NM, Farrow MA, Lapierre LA, *et al*. *Clostridium difficile* Toxins TcdA and TcdB cause colonic tissue damage by distinct mechanisms. *Infect Immun* 2016; 84: 2871–2877.
66. Drudy D, Harnedy N, Fanning S, *et al*. Isolation and characterisation of toxin A-negative, toxin B-positive *Clostridium difficile* in Dublin, Ireland. *Clin Microbiol Infect* 2007; 13: 298–304.
67. Samra Z, Talmor S and Bahar J. High prevalence of toxin A-negative toxin B-positive *Clostridium difficile* in hospitalized patients with gastrointestinal disease. *Diagn Microbiol Infect Dis* 2002; 43: 189–192.
68. Kim H, Riley TV, Kim M, *et al*. Increasing prevalence of toxin A-negative, toxin B-positive isolates of *Clostridium difficile* in Korea: impact on laboratory diagnosis. *J Clin Microbiol* 2008; 46: 1116–1117.
69. Di Bella S, Ascenzi P, Siarakas S, *et al*. *Clostridium difficile* toxins A and B: insights into pathogenic properties and extraintestinal effects. *Toxins* 2016; 8: 134.
70. Lyras D, O'Connor JR, Howarth PM, *et al*. Toxin B is essential for virulence of *Clostridium difficile*. *Nature* 2009; 458: 1176–1179.
71. Guh AY, Hatfield KM, Winston LG, *et al*. Toxin enzyme immunoassays detect *Clostridium difficile* infection with greater severity and higher recurrence rates. *Clin Infect Dis* 2019; 69: 1667–1674.
72. Johnson S. The rise and fall and rise again of toxin testing for the diagnosis of *Clostridium difficile* infection. *Clin Infect Dis* 2019; 69: 1675–1677.
73. Li Z, Lee K, Rajyaguru U, *et al*. Ribotype classification of *Clostridioides difficile* isolates is not predictive of the amino acid sequence diversity of the toxin virulence factors TcdA and TcdB. *Front Microbiol* 2020; 11: 1310.
74. Eckert C, Emirian A, Le Monnier A, *et al*. Prevalence and pathogenicity of binary toxin–positive *Clostridium difficile* strains that do not produce toxins A and B. *New Microbes New Infect* 2015; 3: 12–17.
75. Androga GO, Hart J, Foster NF, *et al*. Infection with toxin A-negative, toxin B-negative, binary Toxin-positive *Clostridium difficile* in a young patient with ulcerative colitis. *J Clin Microbiol* 2015; 53: 3702–3704.
76. Kulaylat AS, Buonomo EL, Scully KW, *et al*. Development and validation of a prediction model for mortality and adverse outcomes among patients with peripheral eosinopenia on admission for *Clostridium difficile* infection. *JAMA Surg* 2018; 153: 1127.
77. Pilate T, Verhaegen J, Van Ranst M, *et al*. Binary toxin and its clinical importance in *Clostridium difficile* infection, Belgium. *Eur J Clin Microbiol Infect Dis* 2016; 35: 1741–1747.
78. Goldenberg SD and French GL. Lack of association of tcdC type and binary toxin status with disease severity and outcome in toxigenic *Clostridium difficile*. *J Infect* 2011; 62: 355–362.
79. Bacci S, Mølbak K, Kjeldsen MK, *et al*. Binary toxin and death after *Clostridium difficile* infection. *Emerg Infect Dis* 2011; 17: 976–982.
80. Kuehne SA, Collery MM, Kelly ML, *et al*. Importance of Toxin A, Toxin B, and CDT in virulence of an epidemic *Clostridium difficile* strain. *J Infect Dis* 2014; 209: 83–86.
81. Solomon K. The host immune response to *Clostridium difficile* infection. *Ther Adv Infect Dis* 2013; 1: 19–35.
82. Golizeh M, Winter K, Roussel L, *et al*. Fecal host biomarkers predicting severity of *Clostridium difficile* infection. *JCI Insight* 2021; 6: e142976.
83. Rao K, Erb-Downward JR, Walk ST, *et al*. The systemic inflammatory response to *Clostridium difficile* infection. *PLoS ONE* 2014; 9: e92578.

84. Dieterle MG, Putler R, Perry DA, *et al*. Systemic inflammatory mediators are effective biomarkers for predicting adverse outcomes in *Clostridium difficile* infection. *mBio* 2020; 11: e00180-20.

85. Abhyankar MM, Ma JZ, Scully KW, *et al*. Immune profiling to predict outcome of *Clostridium difficile* infection. *mBio* 2020; 11: e00905-20.

86. Cowardin CA, Buonomo EL, Saleh MM, *et al*. The binary toxin CDT enhances *Clostridium difficile* virulence by suppressing protective colonic eosinophilia. *Nat Microbiol* 2016; 1: 16108.

87. Buonomo EL, Cowardin CA, Wilson MG, *et al*. Microbiota-regulated IL-25 increases eosinophil number to provide protection during *Clostridium difficile* infection. *Cell Rep* 2016; 16: 432–443.

88. Keller JM and Surawicz CM. *Clostridium difficile* infection in the elderly. *Clin Geriatr Med* 2014; 30: 79–93.

89. Solomon K, Martin AJ, O'Donoghue C, *et al*. Mortality in patients with *Clostridium difficile* infection correlates with host pro-inflammatory and humoral immune responses. *J Med Microbiol* 2013; 62: 1453–1460.

90. Ramesh MS and Yee J. *Clostridium difficile* infection in chronic kidney disease/end-stage renal disease. *Adv Chronic Kidney Dis* 2019; 26: 30–34.

91. Carlson TJ, Gonzales-Luna AJ, Nebo K, *et al*. Assessment of kidney injury as a severity criteria for *Clostridium difficile* infection. *Open Forum Infect Dis* 2020; 7: ofaa476.

92. Bloomfield MG, Sherwin JC and Gkrania-Klotsas E. Risk factors for mortality in *Clostridium difficile* infection in the general hospital population: a systematic review. *J Hosp Infect* 2012; 82: 1–12.

93. Hakim FT and Gress RE. Immunosenescence: deficits in adaptive immunity in the elderly. *Tissue Antigens* 2007; 70: 179–189.

94. Bhangu A, Nepogodiev D, Gupta A, *et al*. West Midlands research collaborative. Systematic review and meta-analysis of outcomes following emergency surgery for *Clostridium difficile* colitis. *Br J Surg* 2012; 99: 1501–1513.

95. Beauregard-Paultre C, Abou Chakra CN, McGeer A, *et al*. External validation of clinical prediction rules for complications and mortality following *Clostridium difficile* infection. *PLoS ONE* 2019; 14: e0226672.

96. Rybolt Ann H, Laughon Barbara E, Greenough William B, *et al*. Protein-losing enteropathy associated with *clostridium difficile* infection. *Lancet* 1989; 333: 1353–1355.

97. Dansinger ML, Johnson S, Jansen PC, *et al*. Protein-losing enteropathy is associated with *Clostridium difficile* diarrhea but not with asymptomatic colonization: a prospective, case-control study. *Clin Infect Dis* 1996; 22: 932–937.

98. Rao K and Higgins PDR. Epidemiology, diagnosis, and management of *Clostridium difficile* infection in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2016; 22: 1744–1754.

99. Ananthakrishnan AN, McGinley EL, Saeian K, *et al*. Temporal trends in disease outcomes related to *Clostridium difficile* infection in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2011; 17: 976–983.

100. Vely A and Ferrada P. Role of Surgery in *Clostridium difficile* Infection. *Clin Colon Rectal Surg* 2020; 33: 87–91.

101. Tay HL, Chow A, Ng TM, *et al*. Risk factors and treatment outcomes of severe *Clostridium difficile* infection in Singapore. *Sci Rep* 2019; 9: 13440.

102. Rokas KEE, Johnson JW, Beardsley JR, *et al*. The addition of intravenous metronidazole to oral vancomycin is associated with improved mortality in critically ill patients with *Clostridium difficile* infection. *Clin Infect Dis* 2015; 61: 934–941.

103. Vega A, Hopkins T, Heil E, *et al*. 488. Oral vancomycin plus intravenous metronidazole for severe *Clostridium difficile* infection in critically ill patients. *Open Forum Infect Dis* 2018; 5: S181.

104. Neal MD, Alverdy JC, Hall DE, *et al*. Diverting loop ileostomy and colonic lavage: an alternative to total abdominal colectomy for the treatment of severe, complicated *Clostridium difficile* associated disease. *Ann Surg* 2011; 254: 423–427.

105. McKechnie T, Lee Y, Springer JE, *et al*. Diverting loop ileostomy with colonic lavage as an alternative to colectomy for fulminant *Clostridium difficile*: a systematic review and meta-analysis. *Int J Colorectal Dis* 2020; 35: 1–8.

106. Dieterle MG, Rao K and Young VB. Novel therapies and preventative strategies for primary and recurrent *Clostridium difficile* infections. *Ann NY Acad Sci* 2019; 1435: 110–138.