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

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

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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; and has been identified by the Centers for Disease Control and Prevention as an urgent antimicrobial resistance threat. It results in considerable mortality with an estimated mortality rate of 8–31%—accounting for approximately 20,500 deaths in 2017 alone. 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. 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 > 60y, 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 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                                          | Examination | Vital sign dyscrasias | Lab dyscrasias | Complications | Outcomes |
|------------------------------------------------|-------------|-----------------------|----------------|---------------|----------|
| IDSA/SHEA 2010[7]                             | X           | X                     | X              | X             | X        |
| IDSA/SHEA 2017[8]                             | X           | X                     | X              | X             | X        |
| Khanafar et al.[9] – 1[b]                     | X           | X                     | X              | X             | X        |
| Khanafar et al.[9] – 2                        | X           | X                     | X              | X             | X        |
| Khanafar et al.[9] – 3                        | X           |                       |                | X             | X        |
| Khanafar et al.[9] – 4                        | X           | X                     |                | X             | X        |
| Surawicz et al.[11c]                          | X           |                       |                | X             | X        |
| Surawicz et al.[11] SC                        | X           | X                     | X              | X             | X        |
| Bishop et al.[13]                             | X           | X                     | X              | X             | X        |
| Bishop et al.[13]                             | X           | X                     |                | X             | X        |
| Keller and Kuijer[14]                         | X           |                       |                | X             | X        |
| Morgan et al.[15]                             | X           |                       |                | X             | X        |
| Abou Chakra et al.[16]                        | X           | X                     | X              | X             | X        |
| Shaw et al.[17]                               | X           | X                     |                | X             | X        |
| See et al.[18]                                | X           |                       |                | X             | X        |
| Sundram et al.[19]                            | X           |                       |                | X             | X        |
| Swale et al.[20]                              | X           | X                     | X              | X             | X        |
| Kim et al.[21]                                | X           |                       |                | X             | X        |
| Abou Chakra et al. 22                         | 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, Pseudomembranous Colitis or Severe Colitis; Surg, Surgery; TM, Toxic Megacolon; WBC, Leukocytosis.

[a] All studies were in hospitalized adults.

[b] Khanafar 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.
| Ribotype (RT) | Study Country Year | Primary outcome associated with disease severity | Host response | Diarrhea | ICU admission | Colectomy | Mortality |
|---------------|--------------------|-------------------------------------------------|---------------|----------|-------------|-----------|-----------|
| RT027         | Loo et al. 2004    | 22.5 / 1000 admissions                           | –             | 6.5%     | 1.9%        | 6.9%      | (30-d attributable mortality) |
| RT027         | Pépin et al. 2003| –                                                | –             | 9.9%     | 2.5%        | 16.7%     | (1-year attributable mortality) |
| RT027 vs other | Abou Chakra et al. 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. 2005–2008 | 40.0% vs 38.9%                                  | –             | 17.7% vs 9.6% | 4.0% vs 3.8% | (attributable mortality) |
| RT027 vs RT078 vs other clades | Walker et al. 2006–2011 | Significant differences in neutrophils and white blood cell count across RTs/clades | –             | –         | –           | 20% vs 25% vs 12%; < .0001 (14-d mortality) |
| RT027 vs RT078 | Patterson et al. 2008 | –                                                | –             | –         | –           | Adjusted RRR [95% CI] | 1.88 (0.49–7.17); = .21 (30-d mortality) |
| RT027/078 vs other | Walk et al. 2010–2012 | –                                                | –             | Adjusted OR [95% CI] | 1.06 (0.49–2.20); = .871 | Together defined as severe CDI |
| RT027 vs RT014-020 vs RT106 vs other | Menon et al. 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; = .719 vs 3.17 (1.28–7.57); = .010 vs 0.84 (0.13–2.98); = .818 | Together defined as disease-related complications |
| RT027 vs RT106 | Sundram et al. 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) |
| RT027 vs RT017 | Goorhuis et al. 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). 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.

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. 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 hemicolecotomy, 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 aggregated predictor. 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. 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). 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. Another ribotype of interest is RT017, hypothesized to have first emerged in Asia, has since disseminated globally and caused outbreaks. 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. 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). Finally, RT016 (NAP11) has become one of the most prevalent ribotypes around the globe, although is less likely to cause severe disease than RT027. In one UK study, RT016 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 RT016 to be significantly less associated with severe disease than RT027 (ileus, toxic megacolon, pseudomembranous colitis within 5 days, or white
blood cell count ≥ 15,000/mm³ 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.53 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 ≥ 15,000 cells/µL, albumin ≤ 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 × 10⁹/L, 95% CI = 14–20 vs 13 × 10⁹/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, systemic 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 × 10⁹/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 \( \times 10^9/\text{L} \), 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 \( \alpha \), IL-8, and TNF-\( \alpha \)) 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-2Ra) 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-\( \alpha \) (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, \( p = .008 \)). Compared to using clinical features (age and while blood cell count) alone in basic models, the inclusion of TNF-\( \alpha \), 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.
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. 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.

Antitoxin A IgG levels
Low systemic antitoxin A IgG levels were associated with mortality in a prospective cohort study which primarily included elderly subjects.

Clinical features associated with CDI outcomes
Renal dysfunction
Acute kidney injury has been associated with the development of complicated CDI. 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.

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. 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.

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. 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. 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.

A systematic review completed in 2012 identified several factors which seemed to have an association with increased risk for mortality including impaired renal function. 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). This study did not distinguish chronic from acute renal dysfunction.

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. 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. 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.

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

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. 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 × 10⁹/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

**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

**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 preceding factors twice and bias results due to this collinearity.

**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. 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.

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. 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.

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 life-saving intervention for certain patients with severe illness, but there are some definite drawbacks to this treatment modality.

The most common surgical procedure performed is a total abdominal colectomy with end ileostomy. 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. 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%). This same review found a 30-day post-operative mortality of 41.3% (19–71%) and in-hospital mortality of 41.6% (25–80%). 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. 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.

There has been interest in alternative surgical approaches, which may have less morbidity and have the potential to be performed earlier in the course. 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. One study comparing diverting loop ileostomy with colonic lavage demonstrated a reduced mortality rate when compared to a historical colectomy cohort. A retrospective study did find this procedure was associated with less blood loss, shorter OR time and decreased mortality. 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.

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. 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.

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

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