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THE ROLE OF INTRAVENOUS TIGECYCLINE MONOTHERAPY IN THE TREATMENT OF SEVERE *CLOSTRIDIOIDES* (FORMERLY *CLOSTRIDIUM*) *DIFFICILE* INFECTION AMONG ADULT HOSPITALIZED PATIENTS

PhD thesis

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„magaslik, nem porlad a megtartó példa”

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I. ABBREVIATIONS

ACG – American College of Gastroenterology

ASCRS – American Society of Colon and Rectal Surgeons

C – cytosine

CCCNA – cell culture cytotoxicity neutralization assay

CCFA – cycloserine and cefoxitin fructose agar

CCMB – cycloserine cefoxitin mannitol broth

ccpA – catabolite control protein A

CDC – Centers for Disease Control and Prevention

CDI – *Clostridioides difficile* infection

CDMN – *Clostridioides difficile* basal agar with moxalactam and norfloxacin

CDT – *Clostridioides difficile* transferase (binary toxin)

CdtLoc – binary toxin locus

CA-CDI community-associated *Clostridioides difficile* infection

CRP – C-reactive protein

CWP – cell wall protein

DNA – deoxyribonucleic acid

DPA – dipicolinic acid

EIA – enzyme immuno-assay

ESCMID – European Society of Clinical Microbiology and Infectious Diseases

FMT – fecal microbiota transplantation

G – guanine

GDH – glutamate dehydrogenase

GTP – guanosine-5'-triphosphate

HA-CDI – healthcare-associated *Clostridioides difficile* infection

IDSA – Infectious Disease Society of America

MALDI-TOF/MS – matrix-assisted laser desorption/ionization time-of-flight mass spectrometer

MLST – multilocus sequence typing

MLVA – multiple locus variable number of tandem repeats analysis

NAAT – nucleic acid amplicfication testing

NET – neutrophil extracellular trap

PaLoc – pathogenicity locus

PCR – polymerase chain reaction

PFGE – pulsed field gel electrophoresis

ppm – parts-per-million

REA – restriction endonuclease analysis

RNA – ribonucleic acid

rRNA – ribosomal ribonucleic acid

SLP – surface layer protein

TcdA – *Clostridioides difficile* (entero)toxin A

TcdB – *Clostridioides difficile* (cyto)toxin B

TSA – trypticase soy agar

WBC – white blood cell

WGS – whole genome sequencing

WSES – World Society of Emergency Surgery

II. INTRODUCTION

1. Clinical microbiology of *Clostridioides* (formerly *Clostridium) difficile*

1.1. History, classification and genomic structure

The genus *Clostridioides* belongs to the bacterial family *Peptostreptococcaceae* (formally *Clostridiaceae*). Based on 16S rRNA gene sequence analysis, the bacterium formerly known as *Clostridium difficile* (*C. difficile*) was transferred from the genus *Clostridium* to *Clostridioides* in 2016, and was renamed *Clostridioides difficile* to more adequately reflect its microbiological differences from the type strain of the original genus, *Clostridium butyricum* (1). *C. difficile* was first described and named *Bacillus difficilis* by *Hall* and *O'Toole* in 1935 after isolation from a stool sample of a healthy newborn (1). The bacterium was not identified as a cause of human disease until 1977, when it has been established as one of the causative agents of antibiotic-associated diarrhea (2).

The first genomic sequencing of *C. difficile* strain 630 (epidemic type X) was completed by using next generation sequencing technology, and published by the Sanger Institute in 2005. Its genome has a single circular chromosome of 4290252 base pairs with a G+C content of 29.1%, and a circular plasmid with 7881 base pairs with a G+C content of 27.9% (3). Further sequence analysis revealed that the genome of *C. difficile* consists of many integrated and extrachromosomal genetic elements. In addition, mobile elements make up 11% of the genome, and mostly reside on the chromosome as conjugative or non-conjugative transposons, and integrated bacteriophages. Presence of these elements suggests that horizontal gene transfer might have played an evolutionary role of *C. difficile,* as they are mostly responsible for antimicrobial resistance, adhesion, virulence and host interaction. Of importance, the chromosomal PaLoc region, responsible for the regulation of toxin expression (see later), is mobilisable, and can be transferred to a non-toxigenic recipient thus converting it to a toxigenic organism (4).

1.2. Cell morphology and metabolism

Under light microscopy with Gram staining, vegetative cells of *C. difficile* could be visualized as Gram positive, long irregular (drumstick shaped) rods measuring 2,5–5,9 to 0,3–1,5 (length to diameter) micrometers in pairs or short chains, forming subterminal endospores and motile flagellata (*Figure 1*). *C. difficile* is unique as it expresses two S-layer proteins on its surface, one of which is highly conserved among strains, and one showing appreciable sequence diversity. Both proteins are derived from a single gene product, and pose amidase activity (5).

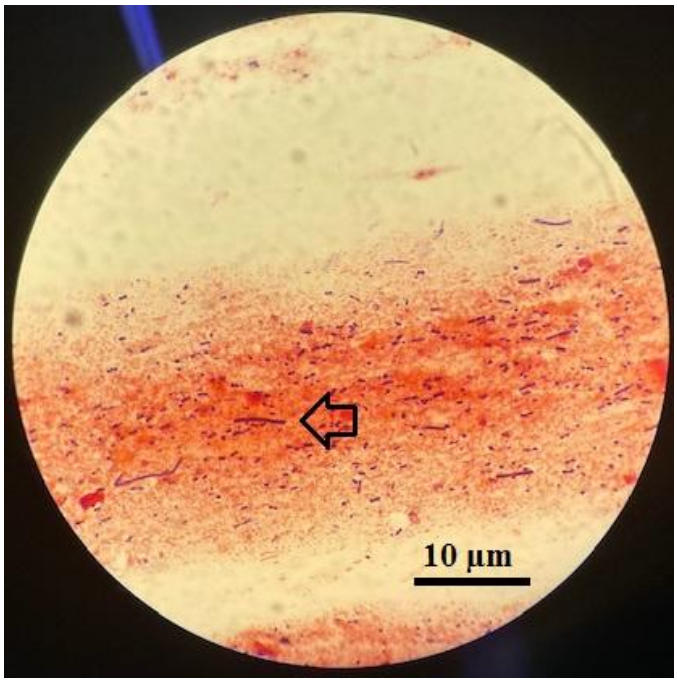

Figure 1. Gram stained smear under light microscopy (40x magnification), showing vegetative *C. difficile* as Gram positive rods (black arrow), among Gram positive cocci (contaminating faecal flora, possibly *Enterococcus sp.*). The smear was prepared from a single *C.difficile* colony, isolated after 48 h of anaerobic culturing on ChromID *C. difficile* Agar (bioMérieux, France) of a stool sample. *Image was kindly provided by Dr. Eszter Vad (DPC-OHII, Budapest, Hungary).*

C. difficile maintains an obligate anaerobic metabolism, as the organism does not possess superoxide dismutase or catalase activity. Under suboptimal conditions, the

bacterium produces endospores which are capable of tolerating extreme environments (see later). *C. difficile* can ferment sugars and amino acids as a means of energy source. *C. difficile* can ferment glucose, fructose, galactose, mannose, raffinose, esculine and mannitol by gas and acid production, while fermentation of maltose, sucrose, glycogen and sorbitol only terminates in acid release. During the exponential growth phase, the Stickland reaction of amino acids is the dominant route for energy production, and the bacterium favors proline and leucine for this biochemical process, while metabolism if shifted towards fermentation pathways associated with the central carbon metabolism in the stationary phase. These fluxes among the metabolic pathways might also govern the toxin producing capacity of *C. difficile*: expression of toxin A and B is repressed during the exponential growth, but shows a significant increase during the stationary phase. In difference to other *Clostridia, C. difficile* is capable of producing *p*-cresol from *p*-hydroxyphenylacetate by decarboxylation, which is toxic to commensal bacteria of the human gut microbiota. *P*-cresol also gives the distinctive „barn" smell of *C. difficile* anaerobic cultures (6).

1.3. Natural ecology, host range and distribution

C. difficile is widely distributed in nature as an ubiquitous bacterium. It inhabits a natural reservoir of soil, sewage and can be found in the faeces and intestinal tract of most mammals, including domestic animals (dogs and cats) and farm animals (swine, calves and cattle). A potential zoonotic transmission of *C. difficile* directly or through the food chain is a possibility, as animals are frequently found to be positive for toxigenic strains without signs of infection, and animals and humans share common bacterial ribotypes (7). This idea is also enforced by the capability of *C. difficile* contaminating retail meat and vegetables: in Europe, prevalence of food products contamination is around 5-8%, whereas is North America, rates are as high as 40-45%, probably due to discrepant procedures utilised for the detection of *C. difficile* in alimentary products (8). Finally, studies have consistently identified healthcare environments, including long-term care facilities and outpatient clinics with surfaces of fomites, such as healthcare devices, clothing or environmental materials serving as reservoirs for endospores of *C. difficile* (8-10).

1.4. Human pathogenesis and virulence

Human virulence of *C. difficile* stands on three possible core mechanisms: endospore, toxin and biofilm forming capability. Steps of pathogenesis in the human host is summarized in *Figure 2*.

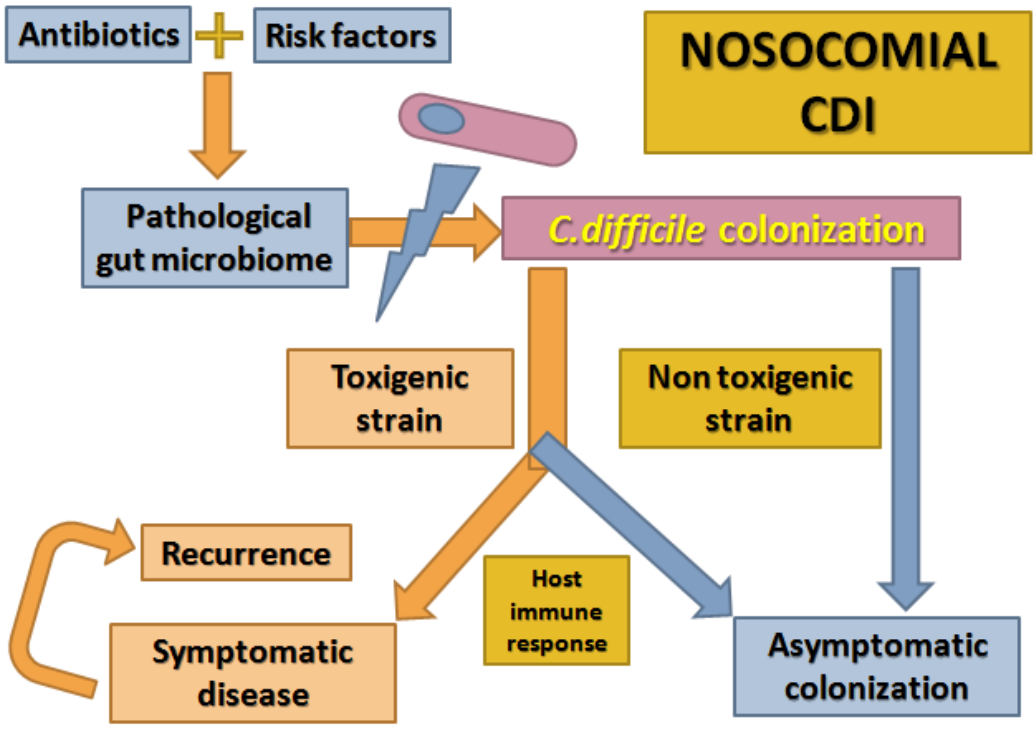

Figure 2. Pathomechanism of *C. difficile* colonization and nosocomial infection in adults. Vulnerable hosts, such as patients with documented risk factors receiving antibiotics, with pathological gut microbiome changes can become colonized with non-toxigenic or toxigenic strains. The former leads to asymptomatic carriage, while the latter may result in asymptomatic colonization or symptomatic infection, depending on the host immune response. Adopted from *Leffler et al*., N Eng J Med, 2015.

1.4.1. Routes of transmission

C. difficile is mainly transmitted from person to person via the fecal-oral route, or from the environment to person by ingestion of endospores. The vegetative form of *C. difficile* is unable to spread efficiently outside the colon, due to its extreme sensitivity to oxygen content of room air (11). It is established that under healthcare conditions,

patients with *C. difficile* colonization may act as reservoirs for environmental contamination in the presence or absence of clinical infection (8-10, 7). Interestingly, results of one study suggested that administration of antibiotics to prior bed occupants was associated with increased risk for acquisition of *C. difficile* infection (CDI) in subsequent patients (12). Whether symptomatic or asymptomatic patients are more likely responsible for the emergence of CDI cases in the nosocomial setting is conflicting. In a study by *Curry et al.,* newly identified hospital-associated CDI cases could be connected to symptomatic patients in 30%, and in 29% to asymptomatic carriers by using multilocus variable number of tandem repeats analysis (13). The fecal passing of endospores is high even after 4 weeks of symptom abation of *C. difficile* infection (14). Earlier whole genome sequence analysis studies have shown that in-hospital transmission of *C. difficile* is more likely to occur from patients with CDI related diarrhea than from patients with asymptomatic colonization. Recent data, however, indicate that the majority of hospital-acquired cases are genetically distinct from one another, possibly suggesting the existence of divergent exposure routes in the community, prior to hospitalisation (15, 7). In addition to routes of healthcare transmission, culminating evidence suggests that community reservoirs, mainly arising from alimentary and veterinary sources, also play a significant role in the transmission cycle of *C. difficile* (16). High-resolution genomic sequencing demonstrated the possibility of transmission of *C. difficile* between animals and humans, even when a clear epidemiological link is not evident (7). Some authors even suggest that a shift from a primarily nosocomial infection to the community and environment involving food animals and humans at larger scales should be seeked (10).

1.4.2. Sporulation and germination

Under extreme or detrimental conditions (eg. acidic or osmotic shock, antibiotic exposure etc.), the vegetative form of *C. difficile* is capable of sporulation, a process generating endospores which are resistant to heat, radioactivity, 70% ethanol and quaternary ammonium detergents used in disinfectants. The endospore structure is made up of multiple layers, including an exosporium, a coat, a cortex, a membrane and the bacterial DNA core. Notably, sodium hypochlorite-based solutions are capable of inactivating *C. difficile* endospores: at a concentration of 5000 *ppm* (10% aqueous

solution of household bleach in distilled water) and 10 minutes of contact time, a 6 $\log_{10}$ ($\geq$ 99.9999%) reduction of viability could be achieved. Without any physico-chemical impact, endospores are capable of surviving at room temperature for 5 months on nosocomial surfaces, or as long as 14 months under experimental conditions on steel disks without significant loss of viability (2, 17).

Ingested endopores are passively transmitted to the colon, where they adhere to the luminal surface of enterocytes (18). Germination is initiated by the sensing of specific environmental signals called germinants: in the human intestine, potent substrates for this process are primary bile salts (taurocholate and glycocholate), while secondary bile salts (chenodeoxycholate and lithocholic acid) are associated with germination inhibition. The concentration and proportion of different primary and secondary bile salts is directly related to the pathophysiological state of the gut microbiome. In addition to bile acids, germination also requires amino acids as cogerminant ligands (glycine, L-alanine, L-glutamine and taurine). The *cspBAC* chromosomal locus is the major regulator of *C. difficile* germination, encoding the subtilisin-like pseudoprotease *CspC*, a bile salt germinant receptor, and pseudoprotease *CspA*, the possible amino acid cogerminant receptor. The germination signal is further transduced by the activated *CspB* protease, resulting in cortex degradation. Finally, endospore germination is enhanced by exogenous calcium, an alternative cogerminant for bile salts, and endogenous calcium, released from the core. Accumulating intracellular calcium forms complexes with dipicolinic acid (DPA), also released from the core. DPA further activates cortex hydrolases, thereby facilitating core rehydration and completing spore outgrow (19, 20).

In the human colon during physiologial conditions, *C. difficile* is outcompeted for adhesion sites and nutrients by competitors from the microbiome, and therefore kept at non-pathological densities by colonization resistance. Colonisation resistance given by bacterial and fungal members of the intestinal microbiome prevents *C. difficile* from colonising and germinating by different mechanisms, including generation of nutritional niches, production of antimicrobial peptides and metabolites and quorum sensing (21). After gut microbiome disruption mainly by antibiotic usage, *C. difficile* endospores are

able to germinate to full metabolic activity and vegetative forms, as the competitive abundance is reduced (22).

1.4.3. Toxin formation

Vegetative forms of pathogenic *C. difficile* strains can produce multiple toxins, which are key factors of human virulence. Clinical presentation of CDI is attributable to the effects of two distinct, large clostridial toxins: toxin A (TcdA) and toxin B (TcdB). Both toxins are encoded by *tcdA* and *tcdB* genes with three accessory genes (*tcdR, tcdE, tcdC*), which are under a single open reading frame on the pathogenicity locus (*PaLoc*) of toxigenic *C. difficile* strains (23). In contrast, the entire *PaLoc* region is replaced with a shorter, non-coding sequence in non-toxigenic *C. difficile* strains (24). In wild type strains of *C. difficile*, *tcdA* and *tcdB* genes are only expressed in the late logarithmic and stationary growth phases. Toxin production is under the downstream control of two other genes of the *PaLoc* region, *tcdR* and *tcdC*, which serve as positive and negative regulators, respectively. Interestingly, catabolite repression may play a role in toxin transcription regulation, as *in vitro* experimental data suggests that presence of rapidly metabolizable sugars or amino acids (most notably cysteine or proline) in the growth medium inhibits toxin expression in the stationary growth phase. This process might be regulated by the catabolite control protein A (ccpA) (25, 23, 6).

TcdA and TcdB share 63% homology in their amino acid sequences. Both toxin possess four functional domains: 1. glycosyl-transferase domain, 2. autoprotease domain, 3. pore-forming and carrier domain, 4. combined repetitive oligopeptide domain (26). According to earlier research conducted with clinical isolates of *C. difficile*, TcdB is found in all strains, while TcdA is only expressed in 70% (27). Similarly to other large clostridial toxins produced by members of the *Clostridium* genus, TcdA and TcdB both act as glycosyl-transferases on small cytoplasmic GTPases of the Rho and Rac families in their target cells after receptor-mediated endocytosis, ultimately causing actin cytoskeletal degradation, tight junction disruption and cell death through apoptosis and necrosis (28). TcdA is one of the largest known bacterial toxins, with a molecular mass of 308 kDa. According to murine model experiments, it could be described as a potent enterotoxin causing tissue necrosis with infiltration of immunocytes, but it also has some activity as a cytotoxin. In contrast, TcdB is a highly

active *in vivo* cytotoxin with a molecular weight of 270 kDa (29). Prior studies pointed to a synergistic role between TcdB and TcdA, but more recent research demonstrated that TcdB positivity alone is necessary and sufficient to cause CDI in murine models (2).

Another potential virulence factor from the binary toxin locus (*CdtLoc*) region is the *C. difficile* transferase or binary toxin (CDT), encoded by two genes (*cdtA* and *cdtB*). CDT negative *C. difficile* strains harbor a deletion at the *CdtLoc* region. CDT is a binary ADP-ribosyltransfersase with 2 functional domains: CDTa is capable of initiating actin cytoskeleton destruction by ADP-ribosylation, and CDTb, which is responsible for toxin docking and translocation into the target cell. However, TcdA– TcdB– CDT+ *C. difficile* strains are incapable of causing CDI in the murine model, and have not yet been isolated from human infection (30).

1.4.4. Surface associated proteins and biofilm production

Cell surface proteins are essential for *C. difficile* to interact with its own environment. Initially, *C. difficile* attaches to the gut mucosa when the native microbiota is disrupted by broad spectrum antibiotics, by the utilization of surface molecules. The vegetative form of *C. difficile* expresses numerous cell surface associated molecules in the human gut for colonization, adhesion and motility, including surface layer proteins (SLPs), pili and fimbriae, surface polysaccharides, cell wall proteins (CWP), fibronectin-binding proteins, and flagellae for motility (31). It has also been postulated that *in vivo* biofilm production may have a relevant role in the pathogenesis of human *C. difficile* infections (21). Biofilms are well-structured communities of microbes surrounded by an extracellular matrix, which protects them against antimicrobials, stress and host immune responses. The *in vitro* biofilm produced by *C. difficile* is multi-layered and encased in a matrix biopolimer of bacterial exoproteins, extracellular DNA and polysaccharide II., with a possible time-dependent evolution of its members: under experimental conditions with *C. difficile* strain R20291, viable cell counts were higher in 3-day-old compared to 6-day-old biofilms, while the majority of cells are vegetative in 3-day-old and spore-forming in 6-day-old biofilms (32). The biofilm formed by *C. difficile* displays resistance to vancomycin and metronidazole, as reduction of viable cell counts are markedly delayed and spore counts are left unchanged, compared to

planktonic cultures *in vitro* and gut models (33). Moreover, subinhibitory concentrations of metronidazole and vancomycin might induce biofilm formation. In contrast, fidaxomicin, administered at 25x minimal inhibitory concentrations (MIC), is able to reduce viability of planktonic bacteria and spores 2.5- and 1.5-fold. During infection, biofilms may serve as reservoirs of *C. difficile*, which allow bacterial persistence, possibly reestablishing recurrent disease (33, 34).

1.5. Host immunity and asymptomatic carriage

In the immunocompetent host, both innate and adaptive immune responses are generated against the vegetative form and toxins of *C. difficile*, and besides the virulence of the invading strain, this process also contributes to the outcome of infection with *C. difficile* (*Figure 2*.) (35, 36). When the host encounters *C. difficile*, antigen presenting cells of the colonic mucosa stimulate T cells and B lymphocytes, which results in anti-toxin antibody production. This response provides humoral immunity with toxin neutralization capacity. A randomized, placebo controlled trial showed that monoclonal antibodies against TcdA and TcdB administered with standard anti-CDI antibiotics reduced CDI recurrence, indicating the improtance of humoral immunity (37). Approximately 60% of healthy adults have detectable serum IgG and IgA against TcdA and TcdB, despite only 2-3% having colonization with *C. difficile*, while asymptomatic carriers of *C. difficile* bear significantly higher serum neutralizing IgG levels against TcdA than patients with clinically manifest CDI. Moreover, following symptomatic infection, many patients develop circulating and mucosal anti-toxin antibodies in serum and stool associating with recurrence protection, while patients with multiple CDI recurrences usually fail to mount an anti-toxin IgG response (38, 39). Severe *C. difficile* associated colitis with pseudomembrane formation is characterized by mucosal and submucosal infiltration of neutrophil granulocytes, which in turn increases the circulating pool of these cells, resulting in the characteristic neutrophil leukocytosis during laboratory evaluation. The inflammation provided by the effector cells of the innate immune system include phagocytosis and NET (neutrophil extracellular trap) formation (36). Furthermore, several cytokines play a crucial role in the pathogenesis of CDI, including IL-8, IL-1β, IL-6, TNFα, INFγ and leukotriene B4 (40).

Colonization with *C. difficile* may result in asymptomatic intestinal carriage, which can be transient or persistent. Prevalence estimates of asymptomatic colonization vary considerably between different patient groups. Among healthy adults from the general population with no risk factors for CDI (see later), prevalence dispersed between 0% and 15%, among hospital inpatients, prevalence was 5–30%, while among elderly people of long-term care facilities, prevalence was 0 to 50%. Interestingly, carriage is between 10 to 90% among healthy newborns and infants. Asymptomatic carriage of healthy adults is often transient, while patients with significant gut microbiome disruption and immuno-incompetency may become persistently colonized (41, 42). Epidemiological models suggest that in-hospital transmission only from symptomatic patients with CDI does not account for sustained endemic transmission within hospitals, and the contribution of asymptomatic carriers is estimated to be significant (2).

1.6. Molecular epidemiology and strain typing

Strains of *C. difficile* could be typed by several laboratory systems (*Table 1.*). Historically, toxinotyping was the first method used, which was replaced by restriction endonuclease analysis (REA) and pulsed field gel electrophoresis (PFGE), both enabling the differentiation between *C. difficile* lineages. As of today, PCR ribotyping of the 16S–23S intergenetic spacer sequences is the most widely accessible method for molecular epidemiology surveillance programs, whereas more objective, costly and technically demanding methods based on Sanger sequencing, such as multilocus sequence typing (MLST) and whole genome sequencing (WGS) are utilized for research purposes (2).

Table 1. Molecular techniques for strain typing of *C. difficile*. Adopted from *Curry et al.*, Clin Lab Med, 2010.

Method	High-throughput	Lineage determination	Discriminatory capacity
Toxinotyping	No	No	Low
Pulsed field gel electrophoresis (PFGE)	No	Yes	Moderate
Restriction endonuclease analysis (REA)	No	Yes	Moderate
PCR ribotyping	Yes	Yes	Low
Multilocus sequence typing (MLST)	Yes	Yes	Low
TcdC genotyping	Yes	Yes	Low
Multiple locus variable number of tandem repeats analysis (MLVA)	Yes	No	High
Whole genome sequencing (WGS)	No	Yes	High

In the USA, circulating PCR ribotypes of *C. difficile* are under active surveillance by the *Centers for Disease Control and Prevention* (CDC). According to recent data obtained in 2017, cases of community acquired CDI were mostly caused by PCR ribotypes 106 (12%), 002 (10%), 020 (6%), 027 (6%), 014 (5%), while healthcare-acquired CDI cases were mostly caused by 027 (15%), 106 (10%), 002 (7%), 014 (7%), 076 (5%) and 020 (4%) (43). In Europe, multiple studies have documented the dynamical changes of circulating strains during the last decade. According to one of the first comprehensive studies done in 2008 among 106 laboratories of 34 countries, the most prevalent PCR ribotypes were 014 (16%), 020 (16%), 001 (9%), 078 (8%) and 027 (5%) from 389 clinical samples (44). Another study, conducted with 1196 clinical samples collected in 482 hospitals of 19 countries between 2012 and 2013, documented that PCR ribotype 027 (19%) rose to dominance, while the relative percentage of PCR ribotypes 001 (11%), 072 (11%) 014 (10%), and 020 (10%) somewhat decreased. Ribotype distribution showed a relevant dispersion between geographical locations and age groups (45). More recently from 3499 clinical samples collected between 2011 to 2016 in 28 countries, it was estimated that PCR ribotype 027 (12,2%) upheld its significance, in contrast to ribotypes 001 (9,1%), 078 (8,1%), 014 (7,8%) and 020 (4,0%) (46).

Since 2011, the emergence of PCR ribotype strain 027, or NAP1 by PFGE and BI by REA (usually referred to as the NAP1/BI/027 *C. difficile* strain), is evident.

NAP1/BI/027 *C. difficile* was extremely rare before 2000. Since then, the NAP1/BI/027 strain became epidemic, causing as high as 30-50% of all adult healthcare-associated CDI cases worldwide (2). NAP1/BI/027 *C. difficile* strains carry nonsense mutations in the tcdC gene region which leads to a dysfunctional TcdC, causing disinhibition of TcdB production at all phases of bacterial growth. It was estimated by *in vitro* experiments that NAP1/BI/027 strains are capable of toxin production at 20-25x higher concentrations than wild-type ones, creating a hypervirulent phenotype. It is also suggested that the strain could possibly participate in more intensive spore production *in vivo*. Consequently, NAP1/BI/027 *C. difficile* strains are associated with more severe clinical forms and recurrence of CDI among adult patients (40).

1.7. Antibiotic resistance mechanisms

The vegetative form of *C. difficile* could possess intrinsic and acquired resistance to a wide array of commonly prescribed antibiotics, which contributes to the successful pathogenesis of the bacterium during or after administration of these drugs. The widespread resistance to penicillins and cephalosporins is not fully understood, but some *C. difficile* strains produce beta-lactamases, mostly belonging to Ambler class D group, rendering target beta-lactams inactive, or express efflux pumps. Resistance to fluoroquinolones is mostly driven by drug target alteration, arising from point mutations of the quinolone resistance-determining regions (QRDR) of *gyrA* and/or *gyrB* genes. *In vitro* suboptimal concentrations of fluoroquinolones select for GyrA and/or GyrB mutant fluoroquinolone-resistant isolates without relevant fitness cost. This resistance induction in thought to be the highest for ciprofloxacin, followed by levofloacin, moxifloxacin and gatifloxacin. In addition, efflux pump mechanisms might also contribute to clinically relevant fluoroquinolone resistance to some extent. In spite of its *in vitro* fitness cost, ribosomal methylase genes such as *ermB* are considered to mediate resistance to antibiotics of the macrolide-lincosamide-streptogramin B (MLS_B) family, such as clindamycin. The enzyme encoded by this gene, ErmB, alters the drug binding site on the ribosome by methylation, thereby preventing successful binding of antibiotics. It is noteworthy that ermB is coded on a mobile genetic element, and therefore could be a substrate of horizontal gene transfer. Genes that encode the 23S rRNA methyltransferase Cfr (*cfrB, cfrC* and *cfrE),* can also mediate resistance against

MLS_B antibiotics, as well as linezolid. Tetracycline resistance is mostly driven by ribosomal protectant proteins (TetM, TetW and Tet44), which work by preventing drug binding to the bacterial ribosome 30S subunit. Similarly to *ermB*, genes of these proteins are usually located on mobile or conjugative elements. However, these mechanisms are unable to mediate resistance against new-generation tetracyclines such as tigecycline, eravacycline or omadacycline in *C. difficile* (34, 47).

The cornerstones of CDI treatment are vancomycin and metronidazole. Vancomycin resistance is mediated by point mutations in the $vanG_{CD}$ operon. *VanG* is an inducible chromosomal operon which is able to confer vancomycin resistance in enterococci by co-expression of two gene sets to produce an altered peptidoglycan precursor of the bacterial cell wall. A *vanG*-like gene cluster named $vanG_{CD}$ has been detected in 85% of *C. difficile* clinical isolates. Its presence in wild-type strains does not immediately mediate vancomycin resistance, but when activating point mutations arise at specific sites of the regulator gene set, constitutive expression of the $vanG_{CD}$ operon starts in *C. difficile* clinical isolates. Acquired metronidazole resistance of *C. difficile* strains is probably due to inhibition of its intracellular reductive activation from prodrug to active drug, by point mutations in key enzymes of the oxidoreductive metabolic pathway, and possibly by intracellular iron level reductions, shifting the cell towards flavodoxin-mediated oxidoreductase reactions. Interestingly, the presence of a high-copy number plasmid called pCD-METRO in clinical isolates of *C. difficile* is also associated with metronidazole resistance, but the mechanism is unknown. Finally, the existence of an efflux pump targeting metronidazol is also well established (34, 47).

During the last decade, slowly growing rates of antibiotic resistance were documented among clinical isolates of *C. difficile* worldwide. Most *in vitro* antibiotic susceptibility testings were performed using the E-test method. According to recent data, vancomycin resistance rates move between 13.2% to 58%, while resistance rates to metronidazole are between 15.2% and 20.2%, out of all tested clinical isolates of *C. difficile*, depending on geographical location (48). Between 2011 and 2014 in Europe, resistance to metronidazole (0.2%), vancomycin (0.1%) and tigecycline (0%) were overall scarse, while moxifloxacin (35.8%) and clindamycin (56.6%) resistance was high. It was also noted that the NAP1/BI/027 strain frequently associated with multiple

antimicrobial resistance, and the lack of PCR ribotype diversity at a given geographical location correlated with greater antimicrobial resistance rates (46). In contrast to European data, two microbiological studies conducted with clinical isolates of *C. difficile* collected at several centers of Hungary found that between 2010 and 2014, antimicrobial resistance for vancomycin increased from 0% to 29.5% – a phenomenon which came under light by the rise of the NAP1/BI/027 *C. difficile* strain (49, 50).

1.8. Laboratory isolation and detection

While historically, culture of *C. difficile* was the gold standard of microbiological confirmation of diagnosis, nowadays it is primarily used for outbreak investigation and molecular epidemiology, and in ambiguous clinical cases (toxigenic culture). As *C. difficile* is aero-intolerant during its logarithmic growth phases, anaerobic jar systems are needed to isolate the organism after a minimum of 48 hours of anaerobic culturing since inoculation to avoid oxygen intoxication of fresh cultures. Culturing of *C. difficile* can be done by using a wide array of techniques, including non-selective agar plates, such as trypticase soy agar (TSA) with 5% sheep blood, selective agar plates, such as *C. difficile* basal agar with moxalactam and norfloxacin (CDMN) or fructose agar with cycloserine and cefoxitin as selective antibiotics (CCFA), and selective broth media, such as cycloserine cefoxitin mannitol broth (CCMB) with taurocholate and lysozyme. A step of broth enrichment could be utilized before plating onto a solid medium. Once isolated, *C. difficile* can be readily sub-cultured to non-selective media, such as 5% sheep blood agar. *C. difficile* colonies vary in size (from 3-5 mm to 12-15 mm), are irregularly shaped, possess a ground-glass morphology, do not produce hemolysis and fluoresce under UV illumination. Chromogenic commercial media, such as the ChromID *C. difficile* Agar (bioMérieux, France), allow for more rapid isolation of *C. difficile* (*Figure 3.*). No consensus exists on which culture method is the most appropriate for use. In ambiguous cases, biochemical testing and matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF/MS) platforms are able to distinguish *C. difficile* from other clostridia (51, 2, 35).

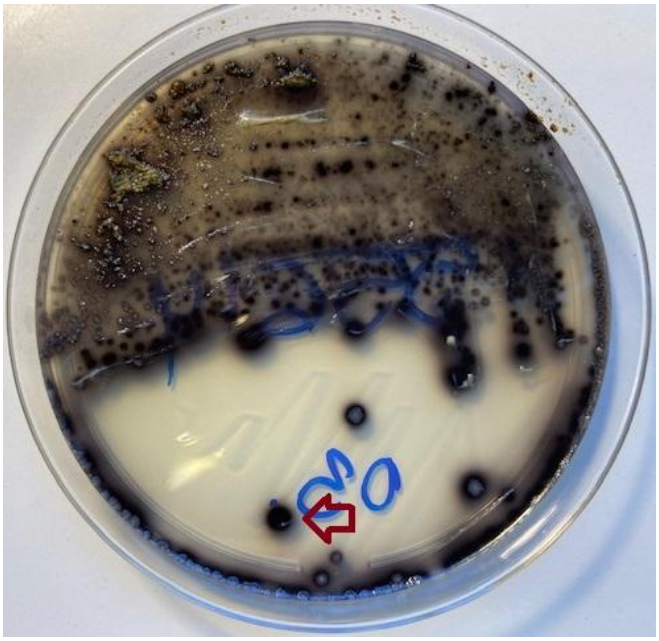

Figure 3. Single *C. difficile* colony (red arrow) growing on ChromID *C. difficile* Agar (bioMérieux, France) after 48 h of anaerobic culturing of a stool sample. *Image was kindly provided by Dr. Eszter Vad (DPC-OHII, Budapest, Hungary).*

As it is essential to differentiate between toxigenic and non-toxigenic strains in clinical practice, culture of the organism should be followed by confirmation of toxin production. Historically, the first, gold standard test used for this purpose was the cell culture cytotoxicity neutralization assays (CCCNA), in which the stool specimen is filtered and incubated with human fibroblast cells with and without *C. difficile* antitoxin for up to 72 hours. If the antitoxin-negative well shows cytopathic effects, whereas the antitoxin-positive well does not, the presence of faecal *C. difficile* toxin is confirmed. CCCNAs were largely replaced by enzyme immuno-assay (EIA) tests detecting the bacterial glutamate dehydrogenase (GDH), TcdA (earlier generations) or TcdA+TcdB (new generations), and nucleic acid amplicfication testing (NAAT), detecting the conserved regions of *tcdB, tcdA* or *cdt* (*Figure 4.*). NAATs include PCR, helicase-dependent amplification and loop-mediated isothermal amplification. The molecular toxin-detection methods consume less human resources and equipment, and provide more rapid turnaround times for clinicians. Most NAAT assays and EIA tests detecting

toxin formation can be used directly on faecal samples, as well as colonies of *C. difficile* from faecal cultures (toxigenic culture) (51, 2, 35).

A) 

B) 

Figure 4. Negative (A) and positive (B) results for *C. difficile* GDH and toxins A+B, detected by C. DIFF Quik Chek Complete EIA (TechLab, USA) from a stool sample. *Image was kindly provided by Dr. Eszter Vad (DPC-OHII, Budapest, Hungary).*

2. *Clostridioides* (formerly *Clostridium) difficile* infection among adults

In the following paragraphs, a brief review of the *state-of-the-art* knowledge considering *C. difficile* infection in the clinical practice will be given, including relevant results from the literature and recently published international guidelines (51-54, 11, 55-57).

2.1. Epidemiology and risk factors for infection and recurrence

In 1978, *C. difficile* was identified as one of the major causative pathogens of antibiotic-associated diarrhea and colitis, with most cases being attributed to the use of clindamycin. Notably, a strain of *C. difficile* highly resistant to clindamycin was implicated in large outbreaks of the early 1990s. In 2004, the hypervirulent NAP1/BI/027 *C. difficile* strain caused major epidemics involving more that 14000 patients in the USA and Canada (58, 59). The CDC documented the steady rise of CDI cases from 2.7 cases per 10000 hospital admission *per annum* to 4.2 cases per 10000 hospital admission *per annum* between 1987 and 2001, respectively. Furthermore, the number of cases have doubled between the period of 1996 and 2003 (60). The rise of *C.*

difficile as a nosocomial pathogen prompted for enforcement of active surveillance measures in state hospitals and clinics worldwide. The USA started its CDI surveillance program in 2009. In its 2018 report from 10 states, a total of 15591 cases were reported, which corresponded to an annual incidence of 130 cases per 100000 patients (*Table 2*.). It was estimated that the female gender and older age cohorts were affected more severely (61).

Table 2. Incidence of reported *C. difficile* infection cases among children and adults in the USA in 2018 (61).

Demographics	Population	CA-CDI[1]		HA-CDI[2]		Total CDI[3]	
		Case numbers[5]	*Incidence*[4]	*Case numbers*[5]	*Incidence*[4]	*Case numbers*[5]	*Incidence*[4]
Gender							
Male	5866907	2905	49.52	3640	62.04	6545	111.56
Female	6116019	4995	81.68	4051	66.23	9046	147.91
Age cohort							
1-17 years	2526903	675	26.70	228	9.03	903	35.74
18-44 years	4691190	1951	41.59	836	17.82	2787	59.41
45-64 years	3088096	2443	79.11	2227	72.12	4670	151.23
≥65 years	1676737	2832	168.91	4399	262.35	7231	431.25
Race							
Caucasian	8053029	6330	78.60	5600	69.54	11930	148.14
Non-caucasian	3929897	1571	39.98	2090	53.18	3661	93.16
SUM	11982926	7901	65.93	7690	64.18	15591	130.11

[1] Community associated CDI
[2] Healthcare associated CDI.
[3] Community and healthcare associated CDI altogether.
[4] Calculated as case numbers per 100000 patients. Including recurrent episodes.
[5] Including recurrent episodes.

In 2016, the European Centre for Disease Prevention and Control (ECDC) initiated its own surveillance program with a standardized protocol across countries of the European Union (EU). In the same year, 556 hospitals from 20 EU countries reported a total of 7711 CDI cases. From these, 5756 (75.6%) were healthcare-associated (HA), and 1955 (25.4%) were community-associated CDI or unknown. Among reported patients, there was a slight tendency for female gender (55.1%), and the median age was 75 years. In addition, 611 cases (7.9%) were recurrent, 921 cases (11.9%) were complicated. Outcomes were documented among 5248 patients, from

which 4160 (79.3%) survived and 1088 (20,7%) died (62). In Hungary, HA-CDI is under surveillance since 2012 (*Table 3.*). State sponsored hospitals and clinics are mandated to report their CDI cases diagnosed at or imported to their premises. Between 2013 and 2018, incidence of CDI seemed to show a bi-modal tendency with a transient decrease in 2016. In the most recent report from 2018, 6153 patients with 6412 episodes of CDI were registered across 94 hospitals. From these, 5549 (86.5%) were new cases with documented healthcare association, and 310 (4.8%) were CDI recurrences (63-65). A recent study conducted between 2010 and 2013 at one academic centre further sheds light on the burden of CDI in Hungary: incidence of CDI was 21.0 per 1000 hospital admissions, corresponding to 4.5% of total inpatient days and accounting for 6.3% of all-inpatient exits. Severe CDI was documented in 12.6%, among 247 infected. Furthermore, the rate of CDI recurrence was 11.3% within 12 weeks post-discharge (66).

Table 3. Incidence of reported healthcare-associated *C. difficile* infection cases among hospitalized adults in Hungary between 2013 and 2018 (63-65).

Year	Number of reporting institutions[1]	Number of hospital admissions[2]	Length of hospital days[2]	Number of CDI cases[3]	Incidence per 10000 patients	Incidence per 100000 hospital days
2013	85	1943941	16859789	6182	31.8	36.7
2014	90	2051141	17476277	6551	31.9	37.5
2015	101	2061443	17564516	5754	27.9	32.8
2016	95	2010385	17293212	4966	24.7	28.7
2017	92	1972926	17045170	5404	27.4	31.7
2018	94	1977696	16935562	5549	28.1	32.8

[1] State sponsored hospitals in Hungary.
[2] Cumulative data, *per annum*.
[3] Including recurrent episodes. Cumulative data, *per annum*.

Perhaps antibiotic usage is the most widely recognized and modifiable risk factor for CDI. Other well validated risk factors for infection are advanced age, prior

and ongoing hospitalization, severe comorbidities, including oncohematological malignancies requiring immuno-chemotherapy, solid organ and hematopoetic stem cell transplantation, obesity, diabetes mellitus, inflammatory bowel diseases, hepatic cirrhosis, major surgeries, including gastrointestinal operations, enteral feeding and gastric acid suppression (51, 2, 40). The duration of risk after cessation of antibiotic administration remains debated. One case-control study of 337 patients with CDI suggested that the infection risk was high during antibiotic therapy and after three months of antibiotic cessation. The risk is perhaps highest during the first month after antibiotic use (67). Systemic perioperative antibiotic prophylaxis may also increase the risk of postoperative CDI. Moreover, a herd effect of antibiotic use has been postulated, during which patients not on antibiotics hospitalized in regions where antibiotic use is high are also at greater risk for CDI, in contrast to patients not on antibiotics hospitalized in regions where antibiotic use is low (68).

Recurrent CDI is defined by reappearance of CDI specific symptoms within 8 weeks after the end of treatment of a previous episode, provided that symptoms resolved after completion of an appropriate therapy. As high as 25% of patients may experience recurrent disease within 30 days of treatment (11). Additional risk factors for recurrent CDI include the lack of an antibody-mediated immune response to *C. difficile* toxins, and the need for concomittant antimicrobial therapy during treatment for a CDI episode (51, 2, 40). Once patients experience one recurrence, they are at increased risk for further recurrences (multiply recurrent CDI). In one retrospective cohort study including more than 45000 patients with CDI in the USA, the annual incidence of multiple recurrent CDI increased by 189% between 2001 and 2012. Those developing multiple recurrent CDI were older, likely to be female, living with chronic kidney disease, residing in a nursing home, and more likely to have received antibiotics, proton-pump inhibitors or corticosteroids within 90 days of CDI diagnosis. (69). Finally, some risk factors of CDI infection and recurrence were also validated by Hungarian contributors (66, 70).

2.2. Establishment of clinical diagnosis and severity stratification of infection

According to international *C. difficile* guidelines, establishment of diagnosis relies on several key steps, including assessment of symptomatology with physical

examination, findings of imaging, endoscopic and laboratory examinations, in correlation to microbiological evidence. An episode of CDI is defined as a clinical picture compatible with CDI, or pseudomembranous colitis visualized during endoscopy, after colectomy or autopsy, plus microbiological evidence of toxins and the presence of *C. difficile* in stool (EIA, NAAT or toxigenic culture), without evidence for an alternativa cause of diarrhea (51-54, 11, 55-57).

The cardinal symptom of CDI is diarrhea, defined as ≥3 bowel movements under ≤24 hours through a minimum of 2 consecutive days with Bristol 5–7 stool consistency. The Bristol stool scale is a diagnostic scoring system for consistency of human faeces, which is subjectively quantified on a scale of 1 to 7, from severe constipation to severe (watery) diarrhea. It should be noted that the absence of diarrhea does not exclude the diagnosis of CDI, as alternative clinical pictures include toxic megacolon and ileus, usually presenting with an abrupt onset of passage stop. During physical examination, abdominal distension, pain or tenderness, as well as signs of peritonitis, hypovolaemic shock or sepsis should actively be seeked. Upon imaging (abdominal X-ray, ultrasound or computed tomography), colonic distension, wall thickening, pericolonic fat infiltration and ascites, while during endoscopy (sigmoidoscopy or colonoscopy), the presence of a pseudomembrane might be pathognomic for CDI. Laboratory findings include marked leukocytosis with prominent left shift (neutrophilia), a rise in baseline serum creatinine with elevated serum lactate and C-reactive protein (CRP) levels, with decreased serum albumin levels (51-54, 11, 55-57).

In Europe, the two-step microbiological diagnostic workup is recommended by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). In the first step, a highly sensitive test should be performed: either NAAT or GDH EIA, or GDH EIA with TcdA+TcdB EIA. If positive, NAAT or GDH EIA testing should be followed by the highly specific TcdA+TcdB EIA. IF this step is also positive, then CDI is likely to be present. If the sample is GDH EIA positive and TcdA+TcdB EIA negative, a NAAT or a toxigenic culture with re-testing should be performed in clinically suspicious cases. Microbiological testing should only be performed on diarrheal stool samples (or swab samples, if ileus is present) of symptomatic patients.

Re-testing within 4 weeks following a successful treatment of CDI is unnecessary, as asymptomatic carriage is common (51).

After diagnosis establishment, severity of the CDI episode should be determined according to international guidelines, as stratification is necessary for choosing a therapeutic strategy (*Table 4*.). A non-severe episode is characterised by the lack of fever, azotemia or leukocytosis, while patients with severe episodes are likely to develop a fever with clinical and biochemical signs of multi-organ involvement, protein loss and colonic inflammation, such as haemodynamic or respiratory instability and peritonitis. In the last ESCMID guideline, patients requiring intensive care or colectomy, or dying because of CDI were also stratified as having severe CDI (52). A fulminant or severe-complicated episode is characterised by ther presence of at least one of the following potentially fatal complications during diagnosis or treatment: septic shock, paralytic ileus, toxic megacolon or bowel perforation. The progression to fulminant CDI is relatively infrequent (1-3% of all CDI episodes), although the mortality associated with this clinical picture remains the highest (50-90%). In recent years, the significant rise in the incidence of fulminant CDI had been associated with the NAP1/BI/027 *C. difficile* strain (56).

Table 4. Severity assessment of adult *C. difficile* infection according to international guidelines (53-57).

SEVERITY	ESCMID[1]	IDSA[2] and ACG[3]	WSES[4] and ASCRS[5]
Non-severe	Does not satisfy the criteria for severe or severe-complicated / fulminant disease		
Severe	≥1 of the following: - Core body temperature >38.5 °C - Blood leukocyte count >15000 cells/μL - Rise in serum creatinine (>50% above the baseline)	≥1 of the following: - Blood leukocyte count >15000 cells/μL - Rise in serum creatinine (>50% above the baseline)	≥1 of the following: - Core body temperature >38.5°C - Blood leukocyte count >15000 cells/μL - Rise in serum creatinine (≥133 μmol/l or ≥ 1.5 times premorbid level) - Serum albumin <25 g/L
Fulminant / severe-complicated	≥1 of the following: - Elevated serum lactate - Signs of hypotension - Septic shock - Ileus - Toxic megacolon - Bowel perforation - Any fulminant course of disease	≥1 of the following: - Hypotension or shock - Ileus - Toxic megacolon	

[1] European Society of Clinical Microbiology and Infectious Diseases
[2] Infectious Disease Society of America
[3] American College of Gastroenterology
[4] World Society of Emergency Surgery
[5] American Society of Colon and Rectal Surgeons

2.3. Therapy of infection

The following general measures are advised among all patients diagnosed with CDI: (1) discontinuation of unnecessary antimicrobial therapy, (2) adequate replacement of fluid, calories and electrolytes, (3) avoidance of anti-motility drugs and (4) re-evaluating proton pump inhibitor use. After anti-CDI therapy is initiated, a lapse of min. 3-5 days is neccessary before treatment response could be assessed clinically. Treatment response is achieved if (1) the patient has reduction of diarrhea with formation of relatively normal stools for the patient, with maintenance of resolution for ≥48 hours after end of treatment, and no additional anti-CDI therapy is needed, and (2) clinical, laboratory and radiological parameters of disease severity have imporved without novel signs of severe disease. It should be noted that after treatment response, normalization of stool consistency and frequency may take weeks, especially in the elderly. Refractory CDI is defined as a complicated or non-complicated course of CDI not responding to recommended CDI antibiotic treatment after 5-6 days (57).

Pharmacological treatment strategies for CDI are detailed in *Table 5*. In recent years, vancomycin and fidaxomicin became the standard-of-care of pharmacological therapy of non-severe CDI. Vancomycin is a glycopeptide antibiotic administered orally, and is capable of inhibiting cell wall synthesis, while oral fidaxomicin is a macrocyclic antibiotic, inhibiting clostridial RNA polymerase with high selectivity. According to the literature, both antibiotics possess a clinical cure rate of 85-90%, but fidaxomicin is associated with lower recurrence rates, possibly due to lesser disruption of the intestinal microbiome (approx. 15% vs. 25%). Metronizadole, a nitroimidazole derivative interfering with oxidoreductive metabolism, is only recommended if neither vancomycin, nor fidaxomicin is available, as this antibiotic is associated with lower cure (75-80%) and high recurrence rates (15-30%). For severe CDI, if oral administration is not possible, a rectal retention enema with vancomycin, and intravenous metronidazole and/or tigecyclyine could also be used as adjunctives. Tigecycline, a glycylcycline antibiotic, inhibits toxin synthesis and is also probably less disruptive to the instestinal microbiome. In fulminant CDI, high-dose oral vancomycin could be administered with adjunctive metronidazole and rectal enemas. Fecal microbiota transplantation (FMT) may be reserved as rescue therapy for patients with

fulminant or refractory CDI, where a surgical approach is not immediately feasible (53, 54, 56, 57). The treatment of the first recurrence of CDI depends on the therapy of the index episode. If the initial CDI episode was treated with vancomycin or metronidazole, then fidaxomicin the preferred agent to treat a first CDI recurrence. If the initial CDI episode was treated with fidaxomicin, vancomycin or another course of fidaxomicin could be administered, and the addition of bezlotoxumab, an anti-TcdB monoclonal antibody should also be considered. Vancomycin administration is recommended in a pulsed or tapered regimen when recurrent CDI is treated. For a second or further CDI recurrence, FMT or bezlotoxumab should be included in the strategy after anti-CDI therapy. Non-pharmacological strategies involve operative approaches, including total colectomy, or diverting loop ileostomy with colonic lavage as alternative to resection in selected patients. Surgical intervention should be considered in patients with fulminant colitis or patients with severe CDI who progress to systemic toxicity early during the disease course (51-54, 11, 55-57).

Table 5. Pharmacological treatment strategies of *C. difficile* infection by severity, according to international guidelines (53-57).

SEVERITY	ESCMID[1]	IDSA[2]	ACG[3]	WSES[4] and ASCRS[5]
Non-severe CDI	FDX 200 mg bid po. 10 days or VAN 125 mg qid po. 10 days Only if unavailability: MTZ 500 mg tid po. 10-14 days		VAN 125 mg qid po. 10 days or FDX 200 mg bid po. 10 days Only if unavailability: MTZ 500 mg tid po. 10-14 days	
Severe CDI	FDX 200 mg bid po. 10 days or VAN 125 mg qid po. 10 days Oral administration not possible: ± rectal enema ± adjunctive MTZ 500 mg tid iv. or TGC 50 mg bid iv.	FDX 200 mg bid po. 10 days or VAN 125 mg qid po. 10 days	VAN 125 mg qid po. 10 days or FDX 200 mg bid po. 10 days	VAN 125 mg qid po. 10 days or FDX 200 mg bid po. 10 days Oral administration not possible: ± rectal enema
Fulminant / severe-complicated CDI	VAN 125 mg qid po. 10 days or FDX 200 mg bid po. 10 days ± adjunctive TGC 50 mg bid iv. ± FMT	VAN 500 mg qid po. 10 days ± rectal enema ± adjunctive MTZ 500 mg tid iv.	VAN 500 mg qid po. 10 days ± rectal enema ± adjunctive MTZ 500 mg tid iv.	
Refractory CDI		n.a.	FMT	n.a.
First recurrent CDI	FDX 200 mg bid po. 10 days or VAN taper/pulse + BEZ	FDX 200 mg bid po. 10 days or VAN taper/pulse ± BEZ	VAN taper/pulse or FDX 200 mg bid po. 10 days ± BEZ	VAN 125 mg qid po. 10 days or FDX 200 mg bid po. 10 days
Multiple recurrent CDI	FMT or VAN taper/pulse + BEZ	FDX 200 mg bid po. 10 days or VAN taper/pulse or FMT ± BEZ	FMT, suppressive VAN ± BEZ	VAN taper/pulse ±BEZ

BEZ: bezlotoxumab, bid: two times daily, FDX: fidaxomicin, FMT: fecal microbiota transplantation, iv.: intravenously, MTZ: metronidazole, n.a.: not applicable, po.: orally (*per os*), TGC: tigecycline, tid: three times daily, qid: four times daily, VAN: vancomycin.
[1] European Society of Clinical Microbiology and Infectious Diseases
[2] Infectious Disease Society of America
[3] American College of Gastroenterology
[4] World Society of Emergency Surgery
[5] American Society of Colon and Rectal Surgeons

2.4. Prognosis and prevention of infection

According to the literature, some prognostic host factors might adequately identify patients at risk for severe and/or recurrent CDI. Perhaps the most important risk factors for severe CDI are older age (>65 years) and presence of multiple comorbidities (57, 71). Comorbidities investigated in studies include a wide range of risk factors, including oncoheamtological malingancies, cognitive impairment, chronic cardiovascular, respiratory and kidney disease, diabetes mellitus, previous operations, inflammatory bowel disease and immunocompromised states (52). Furhtermore, a „dose dependent" effect could also be observed: the risk of severe CDI is higher with increasing age and/or increasing number of severe comorbidities. The comorbidity burden might be estimated by the Charlson Comorbidity Score, whereas CDI severity may be quantified by the ATLAS score, a validated system predicting the likelihood of patient mortality and clinical responsiveness to anti-CDI antibiotic treatment during hospitalization by taking five parameters into account (age of patient, body temperature, white blood cell [WBC] count, serum albumin and the need for systemic antibiotics) (72, 73). The incidence of single and multiple recurrent CDI is alarming. According to the literature, the relapse rate is 10-35% after a first episode, around 40% after a first recurrence and 50–100% following 2 or more recurrences. Perhaps the most clinically relevant risk factors for recurrence include older age (>65 years), prior CDI episode(s), healthcare-associated CDI and prior hospitalization in the last three months, concomitant non-CDI antibiotic use after the diagnosis establishment of CDI, and ongoing proton pump inhibitor therapy. The „dose dependent" effect is also obvious in this case (52, 57, 71).

On the patient-level, routine administration of probiotics or anti-CDI antibiotics when on systemic antibiotic treatment to prevent CDI is not recommended. One experimental study suggested that oral probiotics might even supress the physiological

regereneration of intestinal microbiota after CDI in patients (54, 74). In contrast, among a very selected subgroups of patients, prophylaxis with microbiota sparing anti-CDI antibiotics may be warranted, after balancing risk and benefits, and after consulting an Infectious Diseases or Clinical Microbiology specialist. Selected patients may include patients with a history of multiple recurrent CDI precipitated by systemic antibiotic use, oncohaematological patients receiving active immuno-chemotherapy, haematopoetic stem cell or solid organ transplant patients. Anti-CDI prophylaxis could be achieved by administering low-dose vancomycin or fidaxomicin during the time of risk exposition (57). Multiple CDI relapses indicate the need for FMT and passive immunization with bezlotoxumab, as detailed earlier. To date, no *C. difficile* toxoid vaccine is licensed for prophylaxis (40).

On the community-level, an implementation of an evidence-based antibiotic stewardship program can decrease rates of CDI. For every hospitalized patient with active signs of CDI, strict infection control measures should be implemented by placing patients in contact (enteric) isolation. Screening or isolation of asymptomatic carriers of *C. difficile* is not warranted at the moment. Hand hygiene with soap and water is the cornerstone for the prevention of *C. difficile* spore trasmission in the nosocomial setting. In all, hand hygiene, contact precautions and good cleaning and disinfection of equipment and environment should be followed by all health-care workers in contact with any patient with known or suspected CDI (2, 40, 55, 56).

III. AIMS

In the Introduction, we have detailed the core microbiological and clinical characteristics of *C. difficile* and the human infection it causes in adult patients. Also, we have shown that in severe and fulminant clinical forms of CDI, the number of alternative therapeutic strategies is scarce, and a negative effect on prognosis is relevant.

With this end in view, our scientific aims during the studies presented in this dissertation were the following:

1) Our primary objective was to analyse the efficacy of intravenous tigecycline monotherapy compared to standard anti-CDI therapy (oral vancomycin + intravenous metronidazole) among adult patients hospitalized with severe CDI.

2) Our secondary objective was to assess characteristics and predictors of treatment failure with intravenous tigecycline monotherapy among adult patients hospitalized with severe CDI.

At our institution during the study period, the 2014 ESCMID guideline was followed. Application of tigecycline as a last-resort drug was considered if the patient with severe CDI deteriorated or improvement of physical, laboratory and imaging findings attributable to ongoing CDI failed during standard anti-CDI therapy, and a surgical approach was not immediately feasible.

IV. METHODS

1. Study design and settings

A retrospective observational cohort study of consecutive adult patients (≥18 years at diagnosis) hospitalized at South Pest Central Hospital, National Institute of Hematology and Infectious Diseases with severe CDI between 2014 and 2018 was carried out. Our institution is a tertiary referral centre with >100 dedicated beds for infectious diseases and national catchment.

The study was carried out in two phases: in the first phase, a *pilot* study aiming the primary objective was executed between 2014 and 2015, while in the second phase, a study was completed with data generated between 2015 and 2018. The study was in accordance with institutional and national ethical standards, as well as the Helsinki Declaration (1975, revised in 2000 and 2008). The institutional review board approved the study protocol, informed consent was not necessary for this type of study.

2. Patient selection and data collection

To overcome selection bias, all eligible patients were identified through an electronic records search and evaluated for inclusion on a case-by-case basis, if a diagnosis of CDI was established at admission or during hospital stay (*International Classification of Diseases*, 10th Edition: A04.70). In the first phase following an *a priori* inclusion criterium, patients receiving anti-CDI treatment for severe CDI with intravenous tigecycline monotherapy for ≥48 hours were included in the tigecycline therapy group, while patients receiving oral vancomycin + intravenous metronidazole (standard therapy) without tigecycline for severe CDI were identified using a computer-generated random selection from the same time frame, and included in the standard therapy group in a 1:1 ratio. Patients were excluded from both groups if anti-CDI antibiotics were administered for <48 hours, for any reason. In the second phase, only one cohort was established from all eligible patients, consisting of included patients receiving intravenous tigecycline monotherapy, according to the same inclusion and exclusion criteria.

A database was established for the purpose of the study aims, by manually extracting data of included patients from hospital records, and anonymously transferring them to a standardized case report form. For both phases, variables extracted were: 1) age, and gender at baseline; 2) comorbidities at baseline (essential hypertension, chronic heart disease [ischaemic heart disease, cardiomyopathies], chronic pulmonary disease [COPD, intersticial lung diseases], chronic kidney disease [chronic renal insufficiency], diabetes mellitus, active oncohematological malignancy, long-term systemic corticosteroid therapy [≥15 mg/day prednisone or dose-equivalent for ≥3 months], chronic immunosuppression [congenital immunodeficiency, asplenia, HIV infection, solid organ or hematopoetic stem cell transplantation, chemotherapy or immunosuppressive therapy within ≤6 months, autoimmune disease, hepatic cirrhosis]); 3) documented risk factors for CDI at baseline (systemic antibiotic use in ≤3 months, hospitalization for ≥3 days in ≤6 months, long-term care facility residency, prior CDI episode or multiple CDI recurrences); 4) number and treatment of preceding CDI episodes at baseline; 5) characteristics of current CDI episode at baseline (first or recurrent appearance, onset time and place, symptoms, physical and laboratory results); 6) imaging and endoscopic findings at baseline; 7) durations and types of anti-CDI antibiotics and supportive therapies initiated after diagnosis; 8) need for intensive care unit (ICU) admittance and hospital length of stay (LOS); 9) clinical outcomes. Baseline variables were assessed on the day of CDI diagnosis, clinical outcomes were assessed at hospital discharge or upon patient death.

3. Assessment of diagnosis and severity

At our institution during the study period, diagnosis and severity of CDI was evaluated by the ESCMID guidelines (75, 51, 52). Each case was retrospectively re-assessed for correct diagnosis and severity classification of CDI. Briefly, a case of CDI was defined by the demonstration of toxigenic *C. difficile* from an unformed stool sample by EIA (C. DIFF Quik Chek Complete EIA, TechLab, USA), detecting glutamate dehydrogenase and toxins A+B during a clinically compatible case. If toxin production by EIA was not proven, a culture was performed (ChromID *C. difficile* Agar, bioMérieux, France), and the isolate was re-checked for toxin production. Laboratory tests and imaging (abdominal X-ray, computed tomography and

ultrasonography) were done in each case at baseline and during follow-up, as deemed clinically necessary by recommendation of expert gastroenterologists and infectious disease specialists. Rectosigmoidoscopy or colonoscopy was only performed in ambiguous cases by recommendation of expert gastroenterologists and infectious disease specialists. At least 4 bottles of blood cultures (BacT/ALERT aerobic and anaerobic Culture Media, bioMérieux, France) were taken from peripheral and/or central veins (if feasible) from patients with fever, or suspicion of sepsis or complicated CDI. At our centre, fresh stool samples were also sent from every patient with acute-onset diarrhea for routine bacterial culturing of *Salmonella sp.*, *Yersinia sp.*, *Campylobacter sp.* and *Shigella sp*. All clinical specimens were processed within 2 hours at the Core Microbiology Laboratory of our institution.

For case evaluation, diarrhea was defined by ≥3 unformed stools (Bristol type 5–7) in ≤24 hours for ≥2 consecutive days. Severe CDI was defined by a CDI episode with ≥2 of the following: fever (core body temperature ≥38.5°C) with or without chills, severe abdominal pain, respiratory failure (need for ventilatory support) or haemodynamic instability (need for circulatory support), peritonitis (muscle defense with rebound sensitivity), blood leukocytosis (WBC count >$15x10^9$/L), marked left-shift (>20% of bands), elevated serum creatinine (≥1.5-fold rise compared to premorbid levels), elevated serum lactate (≥5 mmol/L), reduced serum albumin (<30 g/L), colonic distension with wall thickening, ascites or pseudomembranous colitis. Complications of CDI were ileus, toxic megacolon or CDI associated sepsis. Ileus was defined as bowel passage absence for ≥24 hours and radiological features of abnormal bowel distension. Toxic megacolon was defined as colonic ileus with a transverse width of >6 cm for the ascending or transverse colon upon radiological evaluation. CDI associated sepsis was defined as a confirmed CDI with presence of sepsis according to *American College of Chest Physicians/Society of Critical Care Medicine* (76). Recurrent CDI was defined as ≥1 episode with documented clinical resolution before current disease onset. The ATLAS score and the Charlson Comorbidity Score was calculated at baseline for each case (72, 73). Anti-CDI antibiotics were promptly initiated after establishment of CDI diagnosis, or if the clinical scenario was alarming for complications. Tigecycline was administered intravenously in monotherapy with a loading dose of 100 mg, followed by 50 mg twice daily. Vancomycin was administered orally with 125 mg four times daily.

Metronidazole was administered intravenously with 500 mg three times daily. Efficacy and adverse reactions were assessed during daily visits of attending physicians.

4. Outcome measures

The primary outcome was clinical cure, defined by patient survival and complete resolution of all following characteristics of CDI at the end of treatment without the need for addition of a different anti-CDI therapy: 1) diarrhea; 2) abdominal pain; 3) fever; 4) leukocytosis. Treatment failure was established as persistence of any of the mentioned CDI characteristics, need for introduction of additional anti-CDI therapy or patient death occuring during anti-CDI therapy. For the first phase, clinical cure, and for the second phase, treatment failure was assessed.

Secondary outcomes were mortality, relapse, colectomy and complication rates. In the first phase, in-hospital outcomes were assessed by final clinical diagnoses at discharge (autopsy reports were not collected at this phase), while 90-day outcomes of discharged patients were ascertained through focused electronic record searches and telephone interviews up for 90 days. In the second phase, only in-hospital outcomes were assessed by final clinical diagnoses at discharge or autopsy reports. Relapse was defined as re-occurrence of diarrhea and any of the other CDI specific characteristics after completion of cure, without evidence for alternative causes. Colectomy was registered if surgical intervention done on the colon was performed during hospitalization due to CDI. Complicated disease course was counted if any complication occured during anti-CDI treatment.

5. Statistical analysis

Continuous variables are expressed as mean±standard deviation (SD) or median±interquartile range (IQR) with minimum–maximum ranges, comparison was done with Student's *t*-test or Mann–Whitney *U*-test, depending on distribution. Normality of continuous variables was checked using the D'Agostino–Pearson omnibus test. Categorical values are reported as absolute numbers (n) with percentages (%). For statistical comparison, *Fisher*'s exact test and *Pearson*'s χ^2 test were used.

In the first phase, the probability of occurrence of one of the binary outcomes was modelled. Due to the probability being constrained between values of 0 and 1, their logit transformation was used instead: $logit(p) = ln\frac{p}{1-p}$. For stratified analysis along the ATLAS scores, the regression model of $logit(p) = \beta + Ax + \beta Ax$ was employed, where β was the effect of treatment (tigecycline vs. standard therapy), A is the effect of the ATLAS score (x being the actual ATLAS score of the patient), and βA is the interaction effect between them. Model goodnes-of-fit was checked using deviance and Pearson residuals.

In the second phase, predictors of clinical failure were identified by uni- and multivariate binomial logistic regression. Univariate analysis was planned *a priori* to include patient demographics, comorbidities and case severity characteristics, temporal parameters of hospitalization and tigecycline therapy as covariates. Parameters with a p value of ≤0.1 were loaded into forward stepwise multivariate logistic regression (entry criterion p=0.05, removal criterion p=0.1). The maximal number of independent predictors was approximated with a common rule-of-thumb (77). Linearity in the logit was tested by *Box–Tidwell* test, model goodness-of-fit was tested by *Hosmer–Lemeshow* test.

A two-tailed p-value of <0.05 was considered statistically significant for all tests. When reported, odds ratios (OR) and their 95% confidence intervals (95%CI) are given for positive outcomes (clinical cure, survival, no relapse, no need for colectomy, uncomplicated disease course) with the treatment under investigation. Statistical analysis was done using IBM SPSS Statistics 23 (New York, USA). Results are reported by following the *Strengthening the Reporting of Observational Studies in Epidemiology* (STROBE) statement (78).

V. RESULTS

1. First phase: efficacy of intravenous tigecycline monotherapy compared to standard anti-CDI therapy

1.1. Baseline and clinical characteristics

After reviewing 602 patients hospitalized with CDI during the study period, 359 (59.6%) cases of severe CDI were found. Of these, 90 (25.1%) patients met study criteria, and 45–45 (12.5–12.5%) were assigned to the tigecycline and standard treatment groups, respectively.

Baseline patient characteristics are shown in *Table 6.* There was no difference in age and gender. Patients receiving tigecycline were more likely to suffer from chronic immunosuppression (53.3% vs. 28.9%; p=0.02), while chronic renal disease was more prevalent in the standard therapy group (22.2% vs. 53.3%; p=0.002). Altogether 75 (83.3%) patients received systemic antibiotics ≤3 months before disease onset. More patients had recurrent episodes in the tigecycline therapy group. However, previous administration of oral vancomycin for CDI was higher in this group (24.4% vs. 6.7%; p=0.02).

Table 6. Baseline characteristics of adult patients with severe *Clostridium difficile* infection included in the first phase, subgrouped by disease treatment

Characteristics	Tigecycline therapy group (n=45)	Standard therapy group (n=45)	*p* value
Age (years, mean±SD, min–max)	75.2±10.1 (51.4–94.6)	78.0±10.0 (55.4–94.1)	0.17
Male gender (n, %)	25 (55.6)	13 (28.9)	0.1
Comorbidities (n, %):			
- Arterial hypertension	38 (84.4)	44 (97.8)	0.05
- Chronic heart disease	33 (73.3)	36 (80.0)	0.45
- Chronic pulmonary disease	9 (20.0)	12 (26.7)	0.45
- Chronic renal disease	10 (22.2)	24 (53.3)	**<0.02**
- Diabetes mellitus	13 (28.9)	14 (31.1)	0.81
- Active malignancy	15 (33.3)	8 (17.8)	0.09
- Chronic corticosteroid use	12 (26.7)	6 (13.3)	0.11
- Chronic immunosuppression	24 (53.3)	13 (28.9)	**0.02**
Charlson Comorbidity Score (mean±SD, min–max)	4.6±2.0 (1–11)	5.0±1.9 (1–9)	0.33
Risk factors for CDI (n, %):			
- Antibiotic use within 3 months	37 (82.2)	38 (84.4)	0.78
- Hospitalization for ≥3 days within 6 months	42 (93.3)	42 (93.3)	1.0
- Long-term care facility resident	13 (28.9)	17 (37.8)	0.37
Recurrent CDI episode (n, %)	17 (37.8)	13 (28.9)	0.37
No. of previous CDI episodes (mean±SD, min–max)	1.5±0.8 (1–4)	1.5±0.9 (1–4)	1.0
Treatment for for previous CDI episode (n, %):			
- Metronidazole	17 (37.8)	11 (24.4)	0.17
- Vancomycin	11 (24.4)	3 (6.7)	**0.02**
- Tigecycline	1 (2.2)	0 (0)	0.31

Clinical characteristics at diagnosis are shown in *Table 7*. Median ATLAS score was 8 in both groups. Significantly longer LOS (25.4±13.7 days vs. 13.5±11.5 days; p=0.001) and higher frequency of hospital-onset CDI (64.4% vs. 28.9%; p=0.001) were observed in the tigecycline treatment group, but rates of ICU admissions and ICU LOS were similar. Patients receiving tigecycline had more prolonged symptoms before treatment initiation (15.9±12.7 days vs. 9.6±10.1 days; p=0.01). Initial physical and laboratory signs of severe CDI did not show any statistically significant difference. Imaging diagnostics more often detected signs of severity among recipients of tigecycline (91.1% vs. 66.7%; p=0.004). Although more endoscopic examinations were performed on patients treated with tigecycline (48.9% vs. 11.1%; p=0.004), and more blood cultures were taken from them (84.4% vs. 62.2.%; p=0.02), the rate of demonstrated pseudomembranous colitis and true bacteraemia did not differ between groups.

Table 7. Clinical characteristics of adult patients with severe *Clostridium difficile* infection included in the first phase, subgrouped by disease treatment

Characteristics	Tigecycline therapy group (n=45)	Standard therapy group (n=45)	*p* value
CDI onset (n, %):			
- Hospital	29 (64.4)	13 (28.9)	**<0.01**
- Long-term care facility	8 (18.8)	14 (31.1)	0.21
- Community	8 (18.8)	19 (42.2)	**0.01**
LOS at ward (days; median±IQR, min–max)	25.0±17.5 (2–60)	10.5±11.5 (2–51)	**<0.01**
ICU admission (n, %)	10 (22.2)	12 (26.7)	0.62
LOS at ICU (days; median±IQR, min–max)	6.0±12.0 (1–25)	6.5±10.3 (1–34)	0.76
Symptom duration before admission (days; median±IQR, min–max)	14.0±13.0 (5–60)	6.0±10.0 (1–60)	**0.01**
Bristol stool score (median±IQR, min–max)	7.0±1.0 (6–7)	7.0±0.5 (6–7)	0.1
No. of stools per day (median±IQR, min–max)	6.0±4.0 (3–20)	7.0±3.0 (3–20)	0.83
ATLAS score (mean±SD, min–max)	7.8±1.3 (5–10)	8.0±1.1 (5–10)	0.25
Physical signs of CDI (n, %):			
- Fever (≥38.5 °C)	37 (82.2)	42 (93.3)	0.11
- Chills	18 (40.0)	19 (42.2)	0.83
- Abdominal pain	12 (26.7)	13 (28.9)	0.81
- Meteorism (tympanites)	9 (20.0)	14 (31.1)	0.23
- Respiratory failure	16 (35.6)	21 (46.7)	0.28
- Haemodynamic instability	36 (80.0)	31 (68.9)	0.23
- Peritonitis	4 (8.9)	6 (13.3)	0.5
Laboratory signs of CDI (n, %):			
- White blood cell count >15x10^9/L	38 (84.4)	43 (95.6)	0.08
- Band neutrophil cells >20%	36 (80.0)	39 (86.7)	0.39
- Serum creatinine ≥1.5x premorbid level	20 (44.4)	25 (55.6)	0.29
- Serum lactate >5 mmol/L	19 (42.2)	19 (42.2)	1.0
- Serum albumin <30 g/L	36 (80.0)	41 (91.1)	0.14
Imaging signs of CDI (n, %):			
- Dystension of colon (>6 cm)	30 (66.7)	25 (55.6)	0.28
- Mural thickening of colon	31 (68.9)	23 (51.1)	0.08
- Ascites	29 (64.4)	15 (33.3)	**<0.01**
Patients with performed endoscopy[2] (n, %)	22 (48.9)	5 (11.1)	**<0.01**
No. of pseudomembranous colitis (n, %)	19 (86.4)	4 (80.0)	0.71
Patients with performed blood cultures (n, %)	38 (84.4)	28 (62.2)	**0.02**
Patients with detected bloodstream-infections (n, %)	10 (26.3)	6 (21.4)	0.64

[1] Abdominal X-ray and ultrasonography for all patients.
[2] Sigmoidoscopy or total colonoscopy for all patients.

1.2. Clinical outcomes

Clinical outcomes are displayed in *Table 8*. Clinical cure was significantly higher in the tigecycline treatment group: 34 (75.6%) patients met the *a priori* defined criteria for recovery, compared to 24 (53.3%) patients in the standard treatment group (p=0.02). No significant differences were detected among mortality and relapse rates between the observed groups. Colectomy was not performed on any tigecycline recipients; 2 (4.4%) patients had colectomies in the standard treatment group (p=0.15). In total, complicated disease course occured in 13 (28.9%) patients treated with

tigecycline, compared to 24 (53.3%) patients receiving standard treatment (p=0.02). CDI sepsis was more frequent among patients with standard treatment (15.6% vs. 40.0%, p=0.009). Rates of ileus and toxic megacolon showed no statistically significant difference. In the *logit* regression model, outcomes of clinical cure, complicated disease course and CDI sepsis remained significantly different between treatment groups (*Table 8*.).

Table 8. Clinical outcomes of adult patients with severe *Clostridium difficile* infection included in the first phase, subgrouped by disease treatment and stratified by *logit* regression analysis

Outcome measures (n, %)	**Tigecycline therapy group (n=45)**	**Standard therapy group (n=45)**	***p* value, nonstratified**	**OR, nonstratified (95%CI) [1]**	***p* value, stratified**	**OR, stratified (95%CI) [1]**
Clinical cure	34 (75.6)	24 (53.3)	**0.02**	2.7 (1.1–6.6)	**0.02**	11.9 (0.01–999<)
Mortality:						
- In-hospital	15 (33.3)	16 (35.6)	0.82	0.9 (0.4–2.2)	0.83	0.03 (0.001–42.66)
- 90-day	17 (37.8)	21 (46.7)	0.39	0.7 (0.3–1.6)	0.40	0.01 (0.001–12.46)
Relapse:						
- In-hospital	3 (6.7)	4 (8.9)	0.69	0.73 (0.2–3.5)	0.72	0.58 (0.001–31.55)
- 90-day	7 (15.6)	8 (17.8)	0.78	0.8 (0.3–2.6)	0.75	0.52 (0.001–999<)
Colectomy rate	0	2 (4.4)	0.15	0.2 (0.01–4.1)	NA	NA
Complicated disease:						
- Any manifestation	13 (28.9)	24 (53.3)	**0.02**	0.4 (0.2–0.8)	**0.04**	0.001 (0–36.24)
- Sepsis	7 (15.6)	18 (40.0)	**<0.01**	0.3 (0.1–0.8)	**<0.01**	0.01 (0–191.79)
- Ileus	5 (11.1)	4 (8.9)	0.72	1.3 (0.3–5.1)	0.73	9.28 (0.001–999<)
- Toxic megacolon	3 (6.7)	3 (6.7)	1.0	1.0 (0.2–5.2)	1.0	1.0 (0.01–999<)

[1] ORs and 95%CIs are reported for positive outcomes with tigecycline treatment.

Time to different outcomes are shown in *Table 9*. Only time to cure and in-hospital mortality from admission was significantly longer in the tigecycline treatment group.

Table 9. Time to different outcomes of adult patients with severe *Clostridium difficile* infection included in the first phase, subgrouped by disease treatment

Time elapsed (days; median±IQR, min–max)	**Tigecycline therapy group (n=45)**	**Standard therapy group (n=45)**	***p* value**
To cure from admission	19.5±8.8 (8–45)	10.5±3.5 (7–20)	**<0.01**
To cure from treatment initiation	10.0±4.8 (5–24)	10.0±1.8 (7–18)	0.5
To in-hospital mortality from admission	13.0±12.5 (2–60)	3.0±9.0 (2–21)	**<0.01**
To mortality from hospital discharge	36.0±29.0 (7–65)	25.0±9.0 (22–55)	0.75
To in-hospital relapse from admission	20.0±7.0 (11–25)	19.5±3.3 (12–22)	1.0
To relapse from hospital discharge	22.0±7.0 (10–30)	38.5± 26.8 (12–80)	0.19

1.3. Characteristics of anti-CDI antibiotic regimens

Course of tigecycline therapy was started 8.2±7.1 (0–38) days after hospital admittance with a mean treatment duration of 10.3±4.4 (2–22) days. Seven (15.6%) patients received tigecycline as first-line treatment without initial standard therapy. Tigecycline was given to 38 (84.4%) patients as an alternative after clinical failure of standard treatment was acknowledged. Among them, initial vancomycin was given for 8.9±4.7 (2–23) days compared to 9.0±5.4 (2–21) days to patients receiving standard therapy alone (p=0.79), and metronidazole was administered for 6.2±4.6 (2–18) days compared to 6.5±4.8 (2–21) days (p=0.92).

Time to cure from treatment initiation was equal between observed groups (10.7±4.2 days vs. 10.7±2.7 days; p=0.97), while time to in-hospital mortality from admission was longer among patients receiving tigecycline (18.7±14.4 days vs. 7.2±6.6 days; p=0.007). Adverse drug reactions attributable to tigecycline treatment were not observed. In contrast, 6 (13.3%) patients complained of nausea after initiation of metronidazole from the standard therapy group (p=0.02). Discontinuation of therapy was not necessary due to spontaneous resolution of complaints.

In a limited subgroup analysis of 38 patients who received tigecycline as salvage treatment (excluding those who received tigecycline without initial standard therapy

[n=7]), selected characteristics measured on the day of standard therapy initiation and tigecycline initiation were compared (*Table 10.*). It was demonstrated that on the day tigecycline initiation was decided upon, most clinical and laboratory parameters corresponded to severe ongoing *C. difficile* infection.

Table 10. Clinical characteristics of study patients who received tigecycline as salvage treatment after failure of standard therapy by treatment initiation days. Characteristics represent paired observations of same patients. Patients receiving tigecycline first without initial standard therapy (n = 7) were not included in the analysis.

Characteristics	Day of tigecycline initiation (n = 38)	Day of standard therapy initiation (n = 38)	*p* value
Duration of symptoms (days; median±IQR, min–max)	24.6±14.5 (9–68)	15.4±13.2 (5–60)	**<0.01**
Patients with symptoms for ≥14 days (n, %)	29 (76.3)	12 (31.6)	**<0.01**
Bristol stool score (median±IQR, min–max)	6.5±0.5 (6–7)	6.6±0.5 (6–7)	0.3
No. of stools per day (median±IQR, min–max)	9.3±4.0 (2–16)	6.9±3.2 (3–15)	**<0.01**
Physical signs of sCDI (n, %):			
- total	38 (100)	36 (94.7)	0.15
- abdominal pain	27 (71.1)	30 (78.9)	0.42
- fever (≥38.5 °C)	28 (73.7)	14 (36.8)	**<0.01**
- chills	14 (36.8)	8 (21.1)	0.12
- respiratory failure	11 (28.9)	6 (15.8)	0.13
- haemodynamic instability	23 (60.5)	13 (34.2)	**0.02**
- meteorism (tympanites)	33 (86.8)	29 (76.3)	0.23
- peritonitis	4 (10.5)	3 (7.9)	0.69
Laboratory signs of sCDI (n, %):			
- total	38 (100)	36 (94.7)	0.49
- WBC >15x10^9/L	38 (100)	34 (89.5)	**0.04**
- band neutrophils >20%	38 (100)	31 (81.6)	**<0.01**
- serum creatinine ≥1.5x premorbid level	32 (84.2)	18 (47.3)	**<0.01**
- serum lactate >5 mmol/L	27 (71.1)	15 (39.5)	**<0.01**
- serum albumin <30 g/L	34 (89.5)	32 (84.2)	0.49
WBC (x10^9/L; median±IQR, min–max)	34.0±7.3 (21.0–45.5)	24.1±10.3 (2.1–52.0)	**<0.01**
Relative neutrophilia (%;median±IQR, min–max)	92.7±3.6 (88–98)	85.6±12.1 (29–98)	**<0.01**
CRP (mg/L; median±IQR, min–max)	307.5±110.3 (101–550)	206.8±103.4 (62–520)	**<0.01**
Serum creatinine (μmol/l/1.73 m^2; median±IQR, min–max)	361.0±179.4 (117–729)	215.1±155.9 (44–653)	**<0.01**
Serum lactate (mmol/L; median±IQR, min–max)	6.3±1.8 (3.0–9.5)	5.3±3.1 (2.1–16.9)	0.09
Serum albumin (g/L; median±IQR, min–max)	21.9± 5.1 (14–30)	25.3±3.4 (19–33)	**<0.01**

2. Second phase: characteristics and predictors of treatment failure with intravenous tigecycline monotherapy

2.1. Baseline and clinical characteristics

During the study period, 2718 CDI episodes were found (with any severity), and from these, 1148 (42.2%) severe CDI cases were identified. Of these, 110 cases met study criteria and were included in the cohort. Altogether, 62.7% (69/110) had treatment success and 37.3% (41/110) had treatment failure.

Baseline and clinical characteristics are shown in *Table 11*. Median age was 75.0±14.4 years, and there was a tendency for treatment success among males (38/69, 55.1% vs. 18/41, 43.9%, p=0.32). Among patients with treatment failure, chronic heart (50/69, 72.5% vs. 38/41, 92.7%, p=0.01) and pulmonary diseases (13/69, 18.8% vs. 17/41, 41.5%, p=0.01) were more common, other comorbidities were balanced. Risk factors for CDI and ATLAS scores were statistically comparable between the two groups.

Table 11. Baseline and clinical characteristics of adult patients with severe *Clostridium difficile* infection included in the second phase, subgrouped by response to tigecycline treatment

Parameter	Total (n=110)	Treatment success (n=69)	Treatment failure (n=41)	*p* value
Age (years, median±IQR, min–max)	75.0±14.4 (40–94)	73.3±15.2 (45–94)	76.9±11.9 (40–93)	0.71
Male gender (n, %)	56 (50.9)	38 (55.1)	18 (43.9)	0.32
Comorbidities (n, %)				
- Arterial hypertension	99 (90.0)	61 (88.4)	38 (92.7)	0.53
- Chronic heart disease	88 (80.0)	50 (72.5)	38 (92.7)	**0.01**
- Chronic pulmonary disease	30 (27.3)	13 (18.8)	17 (41.5)	**0.01**
- Chronic renal disease	43 (39.1)	24 (34.8)	19 (46.3)	0.31
- Diabetes mellitus	43 (39.1)	26 (37.7)	17 (41.5)	0.84
- Active malignancy	32 (29.1)	22 (31.9)	10 (24.4)	0.51
- Chronic corticosteroid use	18 (16.4)	12 (17.4)	6 (14.6)	0.79
- Chronic immunosuppression	42 (38.2)	31 (44.9)	11 (26.8)	0.06
No. of comorbidities per patient (mean±SD, min–max)	3.6±1.5 (0–8)	3.5±1.5 (0–7)	3.8±1.4 (1–8)	0.21
Charlson Comorbidity Score (mean±SD, min–max)	6.5±2.1 (1–10)	6.4±2.0 (1–10)	6.8±2.2 (2–10)	0.3
Risk factors for CDI (n, %):				
- Antibiotic use within 3 months	95 (86.4)	60 (87.0)	35 (85.4)	1.0
- Hospitalization for ≥3 days within 6 months	101 (91.8)	65 (94.2)	36 (87.8)	0.29
- Long-term care facility resident	21 (19.1)	15 (21.7)	6 (14.6)	0.45
Recurrent CDI episode (n, %)	43 (39.1)	26 (37.7)	17 (41.5)	0.84
No. of previous CDI episodes (median±IQR, min–max)	0±1 (0–5)	0±1 (0–4)	0±1 (0–5)	0.61
Treatment for previous CDI episode (n, %):				
- Metronidazole	35 (31.8)	22 (31.9)	13 (31.7)	1.0
- Vancomycin	31 (28.2)	17 (24.6)	14 (34.1)	0.38
- Tigecycline	2 (1.8)	1 (1.4)	1 (2.4)	1.0
CDI onset (n, %):				
- Hospital	64 (58.2)	40 (62.3)	24 (58.5)	1.0
- Community	14 (12.7)	10 (14.5)	4 (9.8)	0.66
- Long-term care facility	32 (29.1)	19 (27.5)	13 (31.7)	0.56
Symptom duration before admission (days, median±IQR, min–max)	10.0±11.0 (1–60)	12.0±14.0 (2–60)	7.0±9.8 (1–60)	**0.01**
No. of stools per day (median±IQR, min–max)	7.0±4.5 (1–20)	7.0±4.5 (1–20)	6.0±4.0 (2–17)	0.41
Bristol stool score (median±IQR, min–max)	6.0±1.0 (6–7)	7.0±1.0 (6–7)	6.0±1.0 (6–7)	0.43
Physical signs of CDI (n, %):				
- Fever (≥38.5 °C)	38 (34.5)	21 (30.4)	17 (41.5)	0.31
- Chills	18 (16.4)	11 (15.9)	7 (17.1)	1.0
- Abdominal pain	75 (68.2)	48 (69.6)	27 (65.9)	0.86
- Meteorism (tympanites)	83 (75.5)	52 (75.4)	31 (75.6)	1.0
- Respiratory failure	16 (14.5)	6 (8.7)	10 (24.4)	0.05
- Haemodynamic instability	18 (16.4)	12 (17.4)	6 (14.6)	0.79
- Peritonitis	11 (10.0)	2 (2.9)	9 (22.0)	**<0.01**
Laboratory signs of CDI (median±IQR, min–max)				
- White blood cell count ($x10^9$/L)	21.5±15.3 (2.1–68.0)	21.5±16.7 (2.1–68.0)	21.4±12.9 (3.2–47.7)	0.83
- Band neutrophil percentage (%)	86.5±9.1 (28.7–98.5)	87.0±8.4 (28.7–98.4)	85.5±9.2 (30.6–98.5)	0.84
- Serum creatinine (μmol/L)	133.0±170.4 (37.0–785.1)	121.0±156.0 (40.0–653.0)	161.1±202.0 (37.0–785.1)	0.84
- Serum lactate (mmol/L)	4.2±2.1 (1.8–16.9)	4.3±2.4 (1.8–16.9)	4.1±1.6 (2.3–13.4)	0.16
- Serum albumin (g/L)	26.5±6.8 (13–42)	27.0±6.0 (19–39)	26.0±6.0 (13–42)	0.68
- Serum CRP (mg/dL)	172.0±132.8 (12–520)	157.0±107.0 (12–479)	191.0±135.0 (19–520)	0.03
Imaging signs of CDI (n, %):				
- Dystension of colon (>6 cm)	55 (50.0)	30 (43.5)	25 (61.0)	0.11
- Mural thickening of colon	76 (69.1)	49 (71.0)	27 (65.9)	0.68
- Ascites	69 (62.7)	42 (60.9)	27 (65.9)	0.67
Patients with performed endoscopy (n, %)	63 (57.3)	42 (60.9)	21 (51.2)	0.42
No. of pseudomembranous colitis (n, %)	54 (49.1)	38 (55.1)	16 (39.0)	0.11
Patients with performed blood cultures (n, %)	79 (71.8)	52 (75.4)	27 (65.9)	0.38
Patients with detected bloodstream-infections (n, %)	17 (15.5)	9 (13.0)	8 (19.5)	0.41
ICU admission (n, %)	21 (19.1)	7 (10.1)	14 (34.1)	**0.01**
LOS at ICU (days, median±IQR, min–max)	5.0±13.0 (1–53)	9.0±12.0 (1–25)	5.0±10.3 (1–53)	0.45
LOS at ward (days, median±IQR, min–max)	22.0±17.0 (1–173)	24.5±16.5 (9–173)	20.0±21.0 (1–124)	**0.01**
ATLAS score (mean±SD, min–max)	6.9±1.4 (5–10)	6.8±1.3 (5–10)	7.2±1.4 (5–10)	0.16

Among patients with subsequent treatment failure, symptom duration before admission was shorter (12.0±14.0 vs. 7.0±9.8 days, p=0.01), peritonitis was more

frequent (2/69, 2.9% vs. 9/41, 22.0%, p<0.01), and median CRP values (157.0±107.0 mg/dL vs. 191.0±135.0 mg/dL, p=0.03) were higher. Other physical and laboratory findings of severe CDI were statistically similar between groups. The rate of bloodstream-infections was higher (9/52,17.3% vs. 8/27, 29.6%, p=0.25), whereas pseudomembranes were detected less frequently (38/42, 90.5% vs. 16/21, 76.2%, p=0.14) in the failure group. Patients with clinical failure had higher ICU admittance rates (7/69, 10.1% vs. 14/41, 34.1%, p=0.01, and lower LOS (24.5±16.5 days vs. 20.0±21.0 days, p=0.01). Bacterial and fungal pathogens of detected bloodstream-infections are shown in *Table 12.* Most breakthrough bloodstream-infections were caused by *Escherichia coli*, and interestingly, there was a trend for higher incidence among patients with treatment success (5.8% vs. 2.4%). Among Gram positive pathogens, infections caused by *Enterococcus faecalis* and *Enterococcus faecium* were prevalent. Only one case of bloodstream-infection was caused by an acquired multidrug-resistant organism, namely *Acinetobacter baumannii*, in a patient treated at the ICU.

Table 12. Isolated bacterial and fungal pathogens of detected bloodstream-infections in adult patients with severe *Clostridium difficile* infection included in the second phase, subgrouped by response to tigecycline treatment

Isolated pathogens* (n, %)	Total (n=110)	Treatment success (n=69)	Treatment failure (n=41)	*p* value
Escherichia coli	5 (4.6)	4 (5.8)	1 (2.4)	0.69
Enterococcus faecalis	3 (2.7)	0	3	0.05
Enterococcus faecium	3 (2.7)	2 (2.9)	1 (2.4)	1.0
Klebsiella pneumoniae	2 (1.8)	1 (1.5)	1 (2.4)	1.0
Proteus mirabilis	2 (1.8)	2 (2.9)	0	0.52
Pseudomonas aeruginosa	2 (1.8)	1 (1.5)	1 (2.4)	1.0
Staphylococcus aureus	2 (1.8)	1 (1.5)	1 (2.4)	1.0
Acinetobacter baumannii	1 (0.9)	1 (1.5)	0	1.0
Enterococcus gallinarum	1 (0.9)	1 (1.5)	0	1.0
Enterobacter aerogenes	1 (0.9)	1 (1.5)	0	1.0
Klebsiella oxytoca	1 (0.9)	0	1 (2.4)	0.37
Staphylococcus epidermidis	1 (0.9)	1 (1.5)	0	1.0
Staphylococcus lugdunensis	1 (0.9)	0	1 (2.4)	0.37
Candida inconspicua	1 (0.9)	1 (1.5)	0	1.0
Candida albicans	1 (0.9)	1 (1.5)	1 (2.4)	0.37

* Six patients had mixed bloodstream-infections, the isolates are reported individually.

2.2. Clinical outcomes and therapeutic measures

Clinical outcomes and therapeutic measures are shown in *Table 13*, and *Figures 5-6.* In-hospital all-cause mortality was lower in the treatment success group (7.2% vs. 75.6%, p<0.01), in-hospital relapse (4.3% vs. 4.9%, p=1.0) and sepsis (13.0% vs. 26.8%, p=0.07) rates were similar. Among patients with treatment failure, CDI specific mortality was 34.1%, ileus (7.2% vs. 26.8%, p=0.01) and toxic megacolon (1.4% vs. 24.4%, p<0.01) were prevalent, colectomy was occasionally needed (0 vs. 12.2%, p<0.01). There was no statistically significant difference regarding the rate of patients receiving standard therapy, or the duration of standard therapy before tigecycline (*Figure 5.*). Tigecycline was started earlier in patients with subsequent failure (6.0±7.0 days vs. 2.5±4.0 days, p=0.19), treatment duration was shorter (10.0±2.0 days vs. 8.5±5.0 days, p=0.01), possibly explained by more patients dying during the first few days of therapy. In addition, total parenteral nutrition (20.3% vs. 46.3%, p=0.01) and vasopressor support (15.9% vs. 36.6%, p=0.02) were more commonly administered among them (*Figure 6.*).

Table 13. Clinical outcomes and therapeutic measures of adult patients with severe *Clostridium difficile* infection included in the second phase, subgrouped by response to tigecycline treatment

Parameter	Total (n=110)	Treatment success (n=69)	Treatment failure (n=41)	*p* value
In-hospital mortality, all-cause* (n, %)	36 (32.7)	5 (7.2)	31 (75.6)	**<0.01**
In-hospital mortality, CDI specific (n, %)	14 (13.6)	0	14 (34.1)	**<0.01**
In-hospital relapse (n, %)	5 (4.5)	3 (4.3)	2 (4.9)	1.0
Colectomy rate (n, %)	5 (4.5)	0	5 (12.2)	**<0.01**
Complicated disease course (n, %):				
- Any manifestation	38 (34.6)	16 (23.2)	22 (53.7)	**<0.01**
- Sepsis	20 (18.2)	9 (13.0)	11 (26.8)	0.07
- Ileus	16 (14.5)	5 (7.2)	11 (26.8)	**0.01**
- Toxic megacolon	11 (10.0)	1 (1.4)	10 (24.4)	**<0.01**
Duration of standard therapy before tigecycline** (days, median±IQR, min–max):				
- Metronidazole	4.0±6.8 (1–18)	4.0±6.0 (1–18)	3.0±3.0 (1–13)	0.24
- Vancomycin	7.0±6.0 (0–38)	8.0±5.3 (0–38)	6.0±7.5 (1–21)	0.06
Tigecycline therapy characteristics (days, median±IQR, min–max):				
- Starting day from diagnosis	4.0±6.0 (0–19)	6.0±7.0 (1–19)	2.5±4.0 (0–14)	0.19
- Duration of therapy	10.0±3.0 (2–22)	10.0±2.0 (4–22)	8.5±5.0 (2–21)	**0.01**

* Including CDI specific deaths.

** If combination was used, durations were counted separately for each drug.

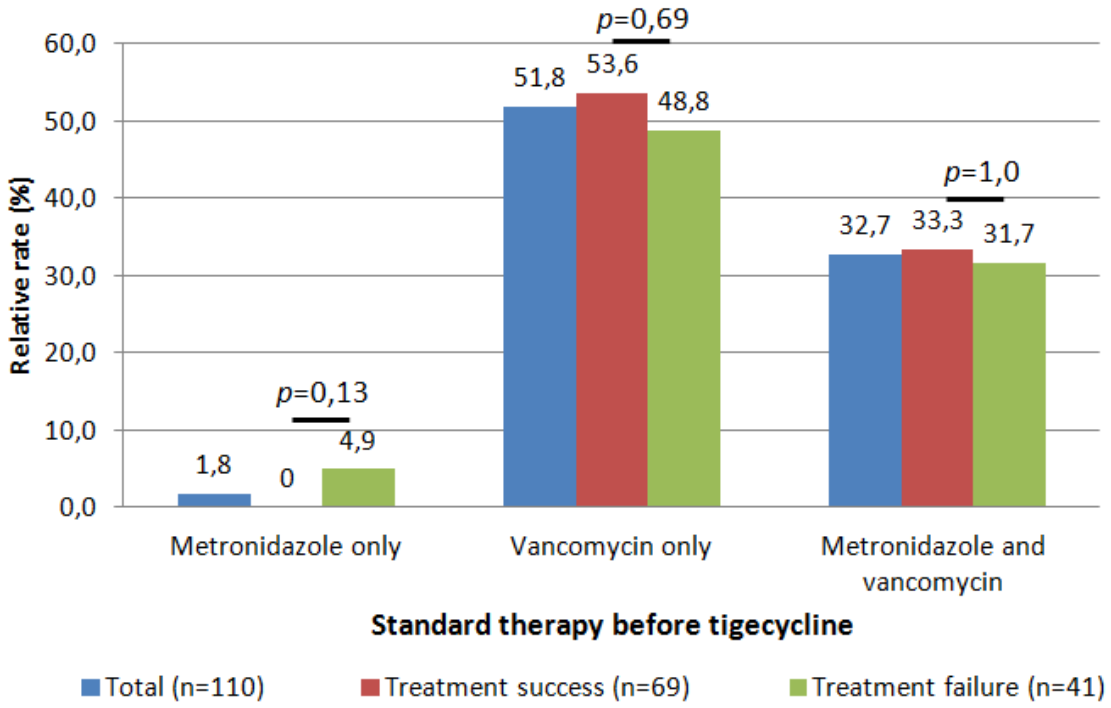

Figure 5. Standard therapy administered before tigecycline among adult patients with severe *Clostridium difficile* infection included in the second phase, subgrouped by response to tigecycline treatment

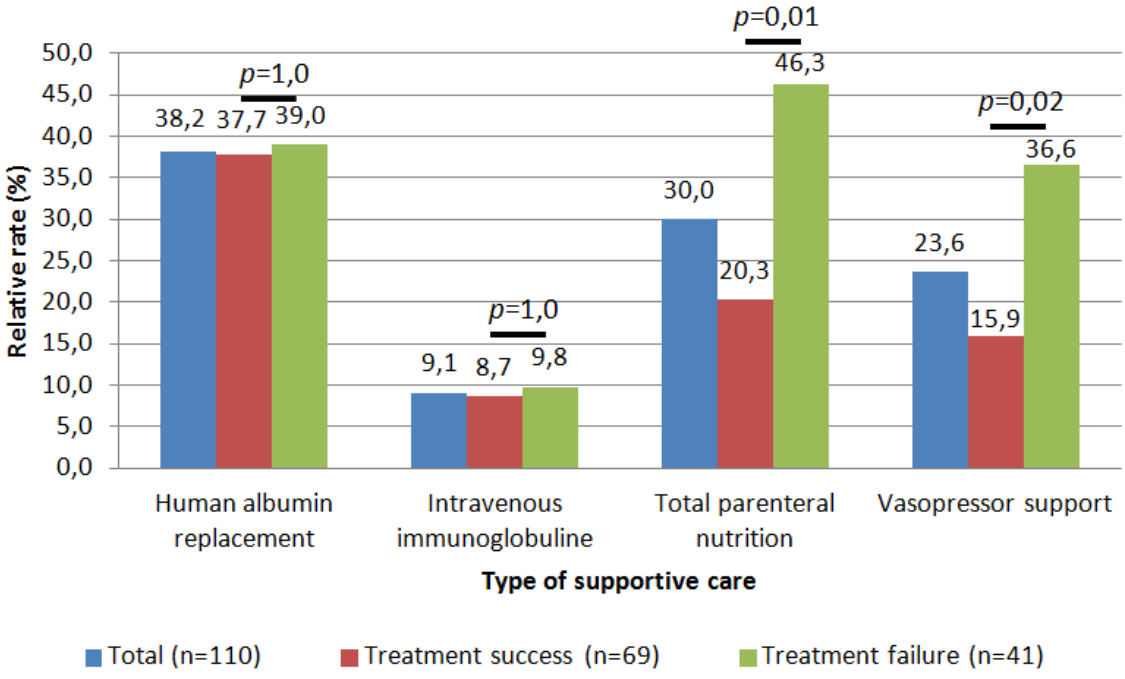

Figure 6. Supportive care among adult patients with severe *Clostridium difficile* infection included in the second phase, subgrouped by response to tigecycline treatment

2.3. Predictors of tigecycline treatment failure

Predictors for tigecycline treatment failure in uni- and multivariate logistic regression analysis are shown in *Table 14.* In univariate analysis, 11 possible covariates were selected, and in the final multivariate model, chronic pulmonary disease (OR 3.48, 95%CI 1.06–11.49, p=0.04), development of ileus (OR 2.38, 95%CI 0.53–10.75, p=0.01), need for total parenteral nutrition (OR 7.04, 95%CI 2.02–24.56, p<0.01) and duration of therapy (OR 0.81, 95%CI 0.69–0.94, p<0.01) were retained as independent predictors of treatment failure.

Table 14. Predictors for tigecycline treatment failure among adult patients with severe *Clostridium difficile* infection included in the second phase, subgrouped by uni- and multivariate logistic regression analysis

Parameter	Univariate analysis		Multivariate analysis	
	OR (95%Cl)	*p value*	*OR (95%Cl)*	*p value*
Age at diagnosis	1.0 (0.96–1.04)	0.86		
Male gender	0.64 (0.29–1.41)	0.25		
Chronic heart disease	5.0 (1.33–20.0)	**0.01**	–	–
Chronic pulmonary disease	3.13 (1.29–7.69)	**0.01**	3.48 (1.06–11.49)	**0.04**
No. of comorbidities per patient	1.19 (0.91–1.56)	0.21		
Charlson index	1.10 (0.91–1.33)	0.29		
ATLAS score at diagnosis	1.25 (0.94–1.67)	0.12		
Peritonitis at diagnosis	10.0 (1.92–50.0)	**0.01**	–	–
Appearance of CDI associated sepsis	2.43 (0.92–6.67)	0.07	–	–
Appearance of ileus	4.76 (1.52–14.28)	**0.01**	2.38 (0.53–10.75)	**0.01**
Appearance of toxic megacolon	25.0 (2.71–100.0)	**0.01**	–	–
Presence of bloodstream-infection	1.61 (0.57–4.55)	0.37		
Recurrent CDI episode	0.85 (0.38–1.88)	0.69		
ICU admission	4.55 (1.67–12.5)	**0.01**	–	–
LOS at ward (not ICU)	0.99 (0.97–1.01)	0.47		
Symptom duration before admission*	0.95 (0.91–1.0)	0.05	n.a.	
White blood cell count at diagnosis	0.99 (0.96–1.02)	0.66		
Serum albumin at diagnosis	0.96 (0.91–1.02)	0.17		
Serum CRP at diagnosis	0.99 (0.98–1.0)	**0.02**	–	–
Starting day of tigecycline therapy from diagnosis	1.09 (1.02–1.19)	**0.02**	–	–
Duration of tigecycline therapy	0.85 (0.76–0.96)	**0.01**	0.81 (0.69–0.94)	**<0.01**
Need for total parenteral nutrition	3.39 (1.45–7.93)	**0.01**	7.04 (2.02–24.56)	**<0.01**
Need for vasopressor support	3.04 (1.23–7.52)	**0.01**	–	–

* The parameter was not included in the final model as co-linearity was not proven by the *Box-Tidwell* test (p < 0.05)
n.a. Not applicable

VI. DISCUSSION

1. Key findings of the present study

In the first phase of the study, patients who were administered tigecycline had significantly better outcomes with regard to higher rate of overall clinical cure (75.6%) and lower occurence of complicated disease course (28.9%) and CDI sepsis (15.6%), compared to patients receiving standard therapy alone. Upon tigecycline initiation, all patients had unresolved disease symptoms corresponding to CDI, so most of them (84.4%) received tigecycline as last resort therapy after failure of standard antibiotics. Initial vancomycin and metronidazole treatment intervals did not differ between groups, and tigecycline was not administered simultaneously with standard treatment. Although not statistically significant, 90-day mortality was found to be lower among recipients of tigecycline. Rates of ileus and toxic megacolon, in-hospital mortality and relapse were similar between groups. These findings may imply that positive differences observed among outcome measures were attributable to tigecycline therapy alone. In general, patients who responded poorly to tigecycline were those with chronic illnesses and were diagnosed early with severe forms of complication. Thus, it is assumed that when tigecyclin failed to resolve signs and symptoms of clinically severe CDI, the drug was initiated too late and the disease had already progressed beyond the point where any additional effect would appear.

In the second phase of the study, 37.3% of patients treated with intravenous tigecycline had subsequent treatment failure. These patients had a relevant comorbidity burden and shorter pre-diagnosis symptom duration, possibly due to faster progression of disease, prompting for earlier hospitalization and change to tigecycline. Frequency and duration of initial standard therapy did not differ between treatment groups. A relevant percentage (34.1%) of failure may be assigned to CDI specific death during treatment. Although more than half (53.7%) of failures had complications, 42.1% of complicated cases could be treated successfully. This might suggest that earlier stages of ileus and sepsis, could still be attenuated by tigecycline administration. In logistic regression, independent predictors of tigecycline treatment failure were chronic pulmonary disease, development of ileus and the need for total parenteral nutrition, whereas the duration of therapy had a protective effect. It might be possible that ileus or

the need for total parenteral nutrition may be considered as an overall indicator for serious gastrointestinal disease with dysmotility, malabsorption and inflammation associating with severe CDI. Based on our data, a minimal therapy duration of 10 days might be required for success.

2. Key findings of previous *in vitro / in vivo* and clinical studies

Tigecycline was approved in the European Union and the USA for treatment of complicated intra-abdominal infections in 2006. Since then, its significant *in vitro* activity against *C. difficile* was proven by experimental studies demonstrating low minimum inhibitory concentrations never exceeding 2 mg/L, even for multidrug-resistant human isolates (79-81). Furthermore, using *in vivo* mice and three-stage chemostat human gut models, it was proven that tigecycline does not provoke intraluminal *C. difficile* proliferation, or toxin production, whereas sporulation might also be prevented (82, 83). Additionally, tigecycline is excreted into the bile in high concentrations, and only minimally and transiently disrupts the normal intestinal microbiome (84).

Following the promises of *in vitro* microbiological data, the first report on succesful usage of intravenous tigecycline in the treatment of refractory CDI was published by Herpers *et al.* in 2009, which was followed by other small case reports describing clinical cure after tigecycline initiation either as monotherapy or in combination (85-89). In contrast, Kopterides *et al.* documented the first case of an elderly patient hospitalized at ICU receiving tigecycline, oral vancomycin, metronidazole and human immunoglobuline, who later succumbed to *Proteus mirabilis* breakthrough sepsis (90). One of the first retrospective case–control studies, which included 18 patients with severe CDI did not find any significant difference in outcome measures between patients who were administered tigecycline in combination with vancomycin+metronidazole or fidaxomicin, and those who only received standard treatment (91). However, the relatively low case count and the lack of matching cases and controls may account for this finding. A narrative literature review published by Di Bella *et al.* found 11 articles as of 2015 describing patients with refractory CDI who were treated with tigecycline. From a total number of 47 subject cases included, 44 (94%) received tigecycline together with other anti-CDI drugs. Overall clinical cure was

74%, while 15% of the patients died and 4% experienced recurrence (92). It should be noted that the patient group pooled during review was probably heterogeneous, and the various follow-up times of original studies may have contributed to the low recurrence and mortality rates. In contrast, a recent systematic review and meta-analysis of 8 studies published between 2015 and 2018, gave a pooled clinical cure rate of 79% (95%CI 73.0–84.5%) among 186 patients treated with tigecycline, without statistically significant heterogeneity between included studies (p=0.84, I^2=0.0%) (93). Obviously, prior evidence from the literature appears to be ambiguous, as the majority of earlier studies are lacking adequate comparisons and high case numbers to determine the role of tigecycline monotherapy in the cure of severe or refractory CDI.

Previous studies did not explicitly deal with characteristics associating with treatment failure during tigecycline therapy. In most studies, patients were administered concurrent non-CDI antibiotics, or received tigecycline only as an adjunctive to standard treatment, which calls for a cautious overall interpretation of data (94-98, 91). Despite this, some factors of tigecycline treatment failure could be outlined from literature. In a retrospective cohort, *Mirea et al.* reported that tigecycline is likely to be beneficial if initiated early in severe CDI course parallel to standard therapy, as survival rose from 12.1% to 80% (98). This observation may be mirrored by a study of *Bishop et al*., in which clinical cure was documented in 77.0% of patients to whom adjunctive tigecycline was administered earlier during the clinical course of infection (94). In our study, we also found that starting day of therapy might have some effect on outcomes. Another potential factor of failure was highlighted by *Brinda et al*., in a study conducted among oncology patients with severe CDI receiving tigecycline: 18.2% of patients had breakthrough infections (severe or invasive co-infections documented during tigecycline treatment), which might have shifted clinical courses to a worse outcome (95). In our study, we also documented cases of bloodstream-infections, but regression analysis did not seem to validate this as a predictor of clinical failure.

In summary, recent expert statements and guidelines suggest that tigecycline might be considered as a potential adjunctive or salvage agent in severe and refractory CDI or among severely ill patients, especially when immediate surgery could not be

performed, but more studies are needed to enhance our understanding of possible treatment failure in everyday practice (99-103, 93, 104-106, 57, 107)**.**

3. Limitations and future directions

Our study has several limitations. Firstly, during planning, the study was designed as retrospective in nature, and as in all retrospective cohort designs, a prospective controlled trial is needed to determine the definite role of tigecycline among anti-CDI strategies before stronger recommendations could be proposed. The study had a single-centre design, thus it only reflects one approach to treatment. During data collection, recall bias might have influenced the quality of non-objective data. Although a standardized approach to CDI was in place at our centre, some observations might have been affected by prescription bias. Isolated *C. difficile* strains are not routinely tested for *in vitro* antibiotic susceptibility, and ribotyping is not available, so these potentially relevant data were not obtainable during the study period. At the analysis stage, despite multiple models of regression was executed to control for confounding during the study phases, some additional influence of unmeasured confounders or residual confounding cannot be excluded. Despite these limitations, we feel that our study is one of the first ones reporting details on efficacy and characteristics of treatment failure with intravenous tigecycline monotherapy among a relatively homogeneous adult cohort hospitalized with severe CDI, and might aid future refinements to therapeutic algorithms.

VII. CONCLUSIONS

In the first phase of our study, we described the clinical cure of severe CDI among hospitalized adult patients treated with intravenous tigecycline monotherapy. Favourable outcomes suggest that intravenous tigecycline might be a reasonable therapeutic choice for cases of severe CDI refractory to standard therapy. Further research, particularly a prospective, randomized controlled trial is warranted for *proof-of-concept* validation and additional evidence.

In the second phase of our study, we described charateristics and predictors of treatment failure with intravenous tigecycline monotherapy administered among hospitalized adult patients with severe CDI. Data suggests that a higher probability for clinical failure might be identified by some independent predictors, such as chronic pulmonary disease, development of ileus and need for total parenteral nutrition, while longer duration of therapy might be a protective factor against treatment failure.

VIII. SUMMARY / ÖSSZEFOGLALÁS

In this dissertation, we reviewed the current *state-of-the-art* knowledge on the microbiological and clinical aspects of human *C. difficile* infections in adults, and presented original research, analysing the efficacy and treatment failure with intravenous tigecycline monotherapy among adult patients hospitalized with severe CDI.

C. difficile is a Gram positive obligate anaerobic rod, transmitted mainly by the faecal-oral route between humans, animals and the environment. Sporulation, toxin and biofilm formation are the main virulence factors of *C. difficile*. Diagnosis of *C. difficle* infection relies on organism detection by EIA, NAAT or toxigenic culture from a stool sample during a clinically compatible case presentation, such as diarrhea, toxic megacolon or paralytic ileus. After considering clinical signs, endoscopic, laboratory and imaging findings, an appropriate anti-CDI treatment could be decided after risk stratification. *C. difficile* infections possess an overall high morbidity burden, and in severe and fulminant clinical forms of CDI, the prognosis is unfavourable.

To assess the role of intravenous tigecycline monotherapy among adult patients hospitalized with severe CDI, we designed a two-phase retrospective observational cohort study at our tertiary referral centre, collecting anonymous data beween 2014 and 2018. In the first phase, patients who were administered tigecycline had better outcomes considering the higher rate of clinical cure and lower propensity for complications and CDI sepsis, compared to patients receiving oral vancomycin and intravenous metronidazole. Favourable clinical outcomes might suggest that tigecycline can be a reasonable choice for severe CDI cases refractory to standard therapy. In the second phase, we described charateristics and predictors of treatment failure with tigecycline monotherapy. Patients with subsequent failure had a relevant comorbidity burden and shorter pre-diagnosis symptom duration, possibly due to faster progression of disease, prompting for earlier hospitalization. A relevant percentage of failure was attributable to CDI specific death during treatment. Independent predictors of tigecycline treatment failure were chronic pulmonary disease, development of ileus and the need for total parenteral nutrition, whereas the duration of therapy had a protective effect.

A disszertációban áttekintettük a felnőttkori *C. difficile* fertőzések mikrobiológiai és klinikai vonatkozásaival kapcsolatos korszerű ismereteket, és bemutattuk a súlyos CDI-vel kórházba került felnőtt betegek körében alkalmazott intravénás tigecyclin monoterápia hatékonyságát, és a kezelés sikerességét elemző kutatásainkat.

A *C. difficile* Gram-pozitív obligát anaerob pálca, mely főként fekális-orális úton terjed az emberek, állatok és a környezet között. A sporuláció, a toxin- és a biofilmképzés a *C. difficile* főbb virulenciafaktorai. A *C. difficile* fertőzés diagnosztikája a toxintermelésre képes baktérium székletbeli jelenlétének EIA, NAAT vagy toxogenikus tenyésztésen alapuló igazolásával történik a fertőzés tünettanával kompatibilis klinikai esetek során (pl. hasmenés, toxikus megacolon vagy paralitikus ileus). A tünetek, endoszkópos, laboratóriumi és képalkotó leletek rizikóstratifikálása után történhet a megfelelő anti-CDI kezelés kiválasztására. A *C. difficile* fertőzés magas morbiditási terhet jelent, a súlyos és fulmináns klinikai formákban a prognózis kedvezőtlen.

Az intravénás tigecyclin monoterápia szerepének felmérésére kétfázisú retrospektív, obszervációs kohorszvizsgálatot terveztünk, mely során anonimizált adatokat gyűjtöttünk a 2014 és 2018 között súlyos CDI-vel saját centrumunkban ellátott felnőtt betegek körében. Az első fázis tapasztalatai alapján a tigecyclinkezelésben részesülő betegek az orális vancomycint és intravénás metronidazolt kapó betegekhez viszonyítva magasabb klinikai gyógyulási arányt, valamint a szövődményekre és a CDI szepszisre való kisebb hajlamot mutattak. A kedvezőbb klinikai eredmények azt sugallhatják, hogy a tigecyclin reális választás lehet standard terápiára nem reagáló súlyos CDI során. A második fázisban ismertettük a tigecyclinkezelés sikertelenségének jellemzőit és független prediktorait. A kezelési kudarcot mutató betegek jelentős komorbiditási teherrel rendelkeztek, és a diagnózisuk előtti tünetes periódus rövidebb volt, ami valószínűleg a betegség gyorsabb progressziója miatt korábban megkezdett kórházi kezeléshez vezetett. A sikertelenség releváns aránya a kezelés során bekövetkezett CDI-specifikus halálozásnak tulajdonítható. A sikertelenség független prediktora a krónikus tüdőbetegség, az ileus, és a teljes parenterális táplálás szükségessége volt, míg a terápia időtartama protektív hatásúnak bizonyult.

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X. PUBLICATIONS LIST

I. Publications in connection to the dissertation / Az értekezés témájában megjelent eredeti közlemények:

Szabo Balint Gergely, Duma Lilla, Lenart Katalin Szidonia, Kiss Rebeka, Vad Eszter, Petrik Borisz Raban, Ostorhazi Eszter, Kadar Bela

Characteristics and predictors of treatment failure with intravenous tigecycline monotherapy among adult patients with severe Clostridioides (Clostridium) difficile infection: a single-centre observational cohort study

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Scopus - Medicine (miscellaneous) SJR indikátor: Q1

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Scopus - Microbiology (medical) SJR indikátor: Q2

IF: 2,803**

Szabo B Gergely, Kadar B, Szidonia Lenart K, DezsenyiB, Kunovszki P, Fried K, Kamotsay K, Nikolova R, Prinz G

Use of intravenous tigecycline in patients with severe Clostridium difficile infection: a retrospective observational cohort study

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Folyóiratcikk/Szakcikk (Folyóiratcikk)/Tudományos

Scopus - Infectious Diseases SJR indikátor: D1

Scopus - Medicine (miscellaneous) SJRindikátor: D1

Scopus - Microbiology (medical) SJR indikátor: D1

IF: 5,292

II. Publications not in connection to the dissertation / Egyéb – nem az értekezés témájában megjelent – eredeti közlemények:

Bartoletti Michele, Azap Ozlem, Barac Aleksandra, Bussini Linda, Ergonul Onder, Krause Robert, Paño-Pardo José Ramón, Power Nicholas R, Sibani Marcella, **Szabo**

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ESCMID COVID-19 Living guidelines: drug treatment and clinical management

CLINICAL MICROBIOLOGY AND INFECTION 2021. Paper: DOI: 10.1016/j.cmi.2021.11.007, 18 p. (2021)

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El-Sokkary Rehab, UysalSerhat, Erdem Hakan, Kullar Ravina, Pekok Abdullah Umut, Amer Fatma, Grgic Svjetlana, Carevic Biljana, El-Kholy Amani, Liskova Anna, Mehmet Özdemir, Ejaz Ahmed Khan, Yesim Uygun-Kizmaz, Nenad Pandak, Nirav Pandya, Jurica Arapović, Rıdvan Karaali, Nefise Oztoprak, Michael M Petrov, Rami Alabadla, Handan Alay, Jehan Ali El Kholy, Caroline Landelle, Reham Khedr, Dhruv Mamtora, Gorana Dragovac, Ricardo Fernandez, Emine Unal Evren, Lul Raka, Antonio Cascio, Nicolas Dauby, Ahsen Oncul, Safak Ozer Balin, Yasemin Cag, Natalia Dirani, Mustafa Dogan, Irina Magdalena Dumitru, Maha Ali Gad, Ilad Alavi Darazam, Behrouz Naghili, Rosa Fontana Del Vecchio, Monica Licker, Andrea Marino, Nasim Akhtar, Mostafa Kamal, Goffredo Angioni, Deana Medić, Aliye Esmaoğlu, **Szabo Balint Gergely**, AndréSilva-Pinto, Lurdes Santos, Ionela Larisa Miftode, Recep Tekin, Phunsup Wongsurakiat, Mumtaz Ali Khan, Yesim Kurekci, Hema Prakash Pilli, Krsto Grozdanovski, Egidia Miftode, Rusmir Baljic, Haluk Vahabolgu, Jordi Rello

Profiles of multidrug-resistant organisms among patients with bacteremia in intensive care units: an international ID-IRI survey

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Folyóiratcikk/Sokszerzős vagy csoportos szerzőségű szakcikk (Folyóiratcikk)/Tudományos

IF: 3,267**

Lakatos Botond[1], **Szabo Balint Gergely**[1], Bobek Ilona, Gopcsa Laszlo, Bekő Gabriella, Kiss-Dala Noémi, Petrik Borisz, Gáspár Zsófia, Farkas Balazs Ferenc, Sinko Janos, Reményi Péter, Szlávik János, Vályi-Nagy István

Laboratory parameters predicting mortality of adult in-patients with COVID-19 associated cytokine release syndrome treated with high-dose tocilizumab

ACTA MICROBIOLOGICA ET IMMUNOLOGICA HUNGARICA 68: 3 pp. 145-152. (2021)

Folyóiratcikk/Szakcikk (Folyóiratcikk)/Tudományos

[1] Megosztott első szerzők

IF: 2,048**

Szabo Balint Gergely[2], Lenart Katalin Szidonia[2], Petrik Borisz, Gaspar Zsofia, Kiss-Dala Noemi, Szlavik Janos, Valyi-Nagy Istvan, Lakatos Botond, Saint Ladislaus COVID-19 Collaborative (Kollaborációs szervezet)

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GEROSCIENCE: OFFICIAL JOURNAL OF THE AMERICAN AGING ASSOCIATION (AGE) 43: 5 pp. 2205-2213. (2021)

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[2] Megosztott első szerzők

IF: 7,713**

Szabo Balint Gergely, Lakatos Botond, Bobek Ilona, Szabo Edina, Szlavik Janos, Vályi-Nagy István

Invasive fungal infections among critically ill adult COVID-19 patients: First experiences from the national centre in Hungary

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Folyóiratcikk/Szakcikk (Folyóiratcikk)/Tudományos

IF: 2,391**

Babarczy Balázs, Bertókné Tamás Renáta, Biró Krisztina, Bobek Ilona, Bognár Zsófia, Bogos Krisztina, Dánielisz Ágnes, Deutschman-Horváth Zsuzsanna, Elek Jenő, Farkas Ferenc Balázs, Gercsák Márta, Gopcsa László, Gődény Mária, Grmela Gábor, Hajdu

Ágnes, Kerpel-Fronius Anna, Kurcz Andrea, Lakatos Botond, Madurka Ildikó Eszter, Markóczy Zsolt, Molnár Zsuzsanna, Müller Cecília, Pápai-Székely Zsolt, Reményi Péter, Sárosi Veronika, Sebestyén Beáta, Sinkó János, Surján Orsolya, **Szabó Bálint**, Széll Enikő Ágnes, Szlávik János, Temesi Gabriella, Vályi-Nagy István
A 2020. évben azonosított új koronavírus (SARS-CoV-2) okozta fertőzések (COVID-19) megelőzésének és terápiájának kézikönyve
Budapest, Magyarország: Emberi Erőforrások Minisztériuma(2020)
Könyv/Kézikönyv (Könyv)/Tudományos

Hajdú Edit, Horváth István, Kardos Gábor, Kristóf Katalin, Matuz Mária, Nagy Kamilla, Pataki Margit, Sümegi Viktória, **Szabó Bálint Gergely**, Szabó Éva, Szabó Judit, Vitális Eszter
Az akut nem komplikált cystitis antimikrobiális kezelése a járóbeteg ellátás keretében
In: Belicza Éva, Lám Judit, PölöskeiPetra (szerk.) Aktívan a betegbiztonságért!: Az Egészségügyi ellátórendszer szakmai módszertani fejlesztése című, EFOP 1.8.0 - VEKOP 17 jelű pályázati konstrukció betegbiztonsági alprojekt szakmai koncepciója és eredményei
Budapest, Magyarország: Semmelweis Egyetem Egészségügyi Menedzserképző Központ (2021) pp. 4-16.
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Diktas Husrev, Uysal Serhat, Erdem Hakan, Cag Yasemin, Miftode Egidia, Durmus Gul, Ulu-Kilic Aysegul, Alabay Selma, **Szabo Balint Gergely**, Lakatos Botond, Ricardo Fernandez, Pinar Korkmaz, Michael Cruz Caliz, Xavier Argemi, Sholpan Kulzhanova, Fatime Kormaz, Fatma Yilmaz-Karadag, Pinar Ergen, Aynur Atilla, Edmond Puca, Mustafa Dogan, Francesca Mangani, Suzan Sahin, Svjetlana Grgić, Krsto Grozdanovski, Gul Ruhsar Yilmaz, Rosa Fontana Del-Vecchio, Aslihan Demirel, Fatma Sirmatel, Alper Şener, Suzan Sacar, Emsal Aydin, Ayşe Batirel, Gorana Dragovac, Rehab El-Sokkary, Crişan Alexandru, Selcan Arslan-Ozel, Sibel Bolukcu, H Deniz Ozkaya, Saygin Nayman-Alpat, Asuman Inan, Fahad Al-majid, Berna Kaya-Ugur, Jordi Rello

A novel id-iri score: development and internal validation of the multivariable community acquired sepsis clinical risk prediction model

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Folyóiratcikk/Sokszerzős vagy csoportos szerzőségű szakcikk (Folyóiratcikk)/Tudományos

IF: 3,267

Laky Boglárka, **Szabó Bálint Gergely**

A 2018/2019. évi légúti szezonban influenzaszerű betegséggel kórházban ellátott felnőtt betegek klinikai és mikrobiológiai jellemzése [Differences in characteristics of adult patients hospitalized with influenza-like illness during the 2018/2019 flu season]

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Folyóiratcikk/Szakcikk (Folyóiratcikk)/Tudományos

IF: 0,540

Szabó Bálint Gergely

Egy új világjárvány közepén – amit eddig a COVID-19-ről tudni vélünk

ORVOSTOVÁBBKÉPZŐ SZEMLE 2020. március 1-12. (2020)

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Szabó Bálint Gergely, Bobek Ilona, Réti Marienn, Gopcsa László, Mathiász Dóra, Lakatos Botond, Bekő Gabriella, Pető Mónika, Sinkó János, Mikala Gábor, Kis Zoltán, Szlávik János, Reményi Péter, Vályi-Nagy István

Az új típusú koronavírus okozta megbetegedés (COVID–19): összefoglaló hematológusoknak II. – a diagnosztika, terápia és prevenció lehetőségei

HEMATOLÓGIA-TRANSZFUZIOLÓGIA 53: 2 pp. 81-95. (2020)

Folyóiratcikk/Összefoglaló cikk (Folyóiratcikk)/Tudományos

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Dezsényi Balázs, **Szabó Bálint Gergely**, Danka József, Ocskay László, Bor László, Csepregi András, Babarczi Edit, Petrovicz Edina, Budai József
Családi disznótor, avagy a szokások nem változnak: Hagyományőrző infektológiai esetbemutatás [Pig-slaughtering at home. Customs do not change. An infectological case report from a historical point of view]
ORVOSI HETILAP 160: 24 pp. 952-957. (2019)
Folyóiratcikk/Szakcikk (Folyóiratcikk)/Tudományos
IF: 0,497

Maraolo Alberto Enrico, Ong David SY, Cimen Cansu, Howard Philip, Kofteridis Diamantis P, Schouten Jeroen, Mutters Nico T, Pulcini Celine, Harxhi Arjan, **Szabo Balint Gergely** (Kollaborációs közreműködő); ESGAP-EUCIC-TAE Working Group on AMS/IPC mapping in Europe (Kollaborációs szervezet 78 szerző)
Organization and training at national level of antimicrobial stewardship and infection control activities in Europe: an ESCMID cross-sectional survey
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Folyóiratcikk/Sokszerzős vagy csoportos szerzőségű szakcikk (Folyóiratcikk)/Tudományos
IF: 2,837

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IF: 2,688

Szabó Bálint Gergely

Az elmúlt néhány év tapasztalatai az influenzajárványokkal kapcsolatban

HÁZIORVOS TOVÁBBKÉPZŐ SZEMLE 24: 8 pp. 472-477. (2019)

Folyóiratcikk/Összefoglaló cikk (Folyóiratcikk)/Tudományos

Szabó Bálint Gergely, Kiss Rebeka, Lénárt Katalin Szidónia, Radka Nikolova, Kádár Béla

„Tempus fugit, venit mors" – a streptococcalis toxikus sokk szindrómáról egy eset kapcsán [Tempus fugit, venit mors" – about streptococcal toxic shock syndrome: a case report and mini-review]

ORVOSI HETILAP 160: 48 pp. 1887-1893. (2019)

Folyóiratcikk/Összefoglaló cikk (Folyóiratcikk)/Tudományos

IF: 0,497

Beović Bojana, Pulcini Céline, Dumartin Catherine, Béraud Guillaume, Nerat Barbara, Maurel Cristina, Doušak May, Čižman Milan, Allerberger Franz, Benko Ria, Berild Dag, Cunney Robert, Debacker Martine, Deptula Aleksander, Dumpis Uga, Dyar Oliver J, Ergonul Onder, **Szabo Balint Gergely**, Gormley Cairine, Grape Malin, Gudnason Thorolfur, Howard Philip, Huttner Benedikt, Ioannou Petros, Ionescu Ramona, Keuleyan Emma, Knepper Viviane, Kofteridis Diamantis, Kostyanev Tomislav, Krcmery Vladimir, Lakatos Botond, Luzzati Roberto, ten Oever Jaap, Pagani Leonardo, Pardo José Ramón Paño, Popescu Mihaela, Popovici Mihaela, Paul Mical, Bix Hege Salvesen, Schouten Jeroen, Sneddon Jacqueline, Stevanović Goran, Wechsler-Fördös Agnes, de With Katja, Vlahović-Palčevski Vera, Zarb Peter

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IF: 4,615

Szabo BG, Lenart KS, Tirczka T, Ostorhazi E

Clinical and microbiological characteristics of adult invasive Haemophilus influenzae infections: results of a 14-year single-center experience from Hungary

INFECTION 46: 6 pp. 855-860. (2018)

Folyóiratcikk/Szakcikk (Folyóiratcikk)/Tudományos

IF: 2,927

Szabó Bálint Gergely

Atípusos panaszokkal kezdődő invazív pneumococcusinfekció magas rizikójú, oltatlan felnőttben

MEDICA MENTE 3: 2 pp. 2-2. (2015)

Folyóiratcikk/Ismertetés(Folyóiratcikk)/Tudományos

Szabó Bálint Gergely

Szövődménnyel gyógyuló invazív pneumococcusinfekció magas rizikójú, oltatlan felnőttben

MEDICA MENTE 3: 1 pp. 2-2. (2015)

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Szabó Bálint Gergely, Lénárt Katalin Szidónia, Kádár Béla, Gombos Andrea, Dezsényi Balázs, Szanka Judit, Bobek Ilona, Prinz Gyula

A Streptococcus pneumoniae (pneumococcus) -infekciók ezer arca [Thousand faces of Streptococcus pneumoniae (pneumococcus) infections]

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Folyóiratcikk/Összefoglaló cikk (Folyóiratcikk)/Tudományos

IF: 0,291

Szabó Bálint Gergely, Tóvári József, Marton Annamária, Vizler Csaba, Tímár József, Szilák László

Az angiostatin felhasználási lehetőségei a tumorellenes terápiában

In: Csiszár, Imre; Kőmíves, Péter Miklós (szerk.) Tavaszi Szél 2014 Konferencia = Spring Wind 2014: Konferenciakötet V.

Debrecen, Magyarország: Doktoranduszok Országos Szövetsége (DOSZ) (2014) 590 p. pp. 137-147.

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Szabó Bálint Gergely

Dr. Novák Endre egészségügyi és szociális eszményképe a korabeli magyar sajtóforrások tükrében

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Dr. Novák Endre egészségügyi és szociális eszményképe a korabeli magyar sajtóforrások tükrében [Medical and social ideals of dr. Endre Novak in view of the contemporary Hungarian press sources]

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Folyóiratcikk/Ismertetés(Folyóiratcikk)/Tudományos

Szabó Bálint Gergely

Egy szabadon választott növénytársulás megfigyelése

TERMÉSZET VILÁGA (DIÁKMELLÉKLET) 138: 1 pp. X-XIV. (2005)

Folyóiratcikk/Szakcikk (Folyóiratcikk)/Ismeretterjesztő

**** Várható IF érték**

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CONFLICT OF INTEREST

Balint Gergely SZABO and the authors of the publications declare no conflicts of interest regarding this study.