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"DETECTION AND TREATMENT OF CLOSTRIDIOIDES DIFFICILE INFECTION IN A PRIVATE HOSPITAL COMPARED TO A PUBLIC HOSPITAL IN MEXICO CITY"

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INDICE

ABSTRACT

Introduction: Clostridioides Difficile infection prevalence has been uprising in the last few years. Because of de-novo infections recently identified, risk factors must be determined in order to prevent this infection outside and inside of the hospital.

Objective: To report the diagnosis and treatment of C. difficile and infections, as well as recurrence in two different hospitals in Mexico City.

Material and methods: an observational, retrospective longitudinal study, within the clinical practice of Hospital Angeles Lomas and the National Institute of Respiratory Diseases (INER) of Mexico City from June 2017 until March 2022.

Results: the proportion of women affected was higher in the private hospital compared to the public hospital. Treatment was very similar in both groups, with great results in general. Identifiable risk factors include age between 30-60, previous hospitalizations, prior wide spectrum antibiotic use, among others.

Conclusion: Clostridioides Difficile infection detection is feasible in a normal setting. We do not require PCR test unless we have discordant results. Treatment must be individualized to obtain better results.

RESUMEN

Introducción: La prevalencia de la infección por Clostridioides Difficile ha ido en aumento en los últimos años. Debido a la identificación de casos de novo, es de suma importancia determinar los factores de riesgo para esta infección tanto fuera como dentro del hospital para saber prevenirlo.

Objetivos: Reportar el diagnostico, tratamiento y recurrencia de la infección por Clostridioides Difficile en dos hospitales de la Ciudad de México.

Material y métodos: realizamos un estudio observacional, retrospectivo, longitudinal, en la practica clínica del Hospital Ángeles Lomas y el Instituto Nacional de Enfermedades respiratorias (INER) de la Ciudad de México desde abril 2019 hasta marzo 2022.

Resultados: la proporción de mujeres afectadas fue mayor en el hospital privado en comparación con el hospital publico. El tratamiento fue muy similar en ambos grupos obteniendo resultados igualmente favorables. Los factores de riesgo identificables son edad 30-60 años, hospitalizaciones previas, uso de antibióticos de amplio espectro, entre otros.

Conclusiones: la detección de infección por clostridioides difficile es sencilla en un contexto común. No se requiere la utilización de PCR a menos que se tenga la duda por resultados discordantes. El tratamiento debe ser dirigido e individualizado para mejores resultados.

INTRODUCTION

Gastrointestinal infection by Clostridioides difficile (CD), formerly known as Clostridium difficile, is associated with the disruption of the intestinal microbiota and has shown a significant increase in cases in the last two decades (1,2). According to Global Burden 2019, CD infection represents 0.55-2.3 per 1000 annual admissions within the internal medicine service (3), and 10-20% of all infectious diarrheas (25), with a predominance of females (59%), Caucasians (86%), and patients over 65 years of age (70%) (4). The Centers for Disease Control and Prevention (CDC) reported it as the main etiology of death from gastroenteritis in North America and Europe, with a mortality of 6-17% (5) which represents more than 30,000 annual deaths (6), and a risk of up to 25% of recurrence and serious complications, which entails high costs for the health sector (7).

It was not until the 2000s that a drastic peak in its incidence was reported (8) which led to the implementation of better and faster diagnostic methods, as well as greater clinical suspicion by doctors (9). However, there has recently been an increase specifically in the population traditionally considered low risk, including young people with no history of hospitalizations (1,10), who represent up to 40% of all cases (11), information that makes us wonder about additional risk factors for community infections, in order to improve their prevention and start treatment as early as possible.

-Microbiology:

The infection is caused by a Gram-positive, obligate anaerobic, spore-forming, and toxin-producing bacillus (10). It was first described in 1935 and was initially named Clostridium difficile because of its difficulty in growing in culture and its slow growth in vitro. It was described as the causative agent of acute infectious colitis in 1978 (9), with the beginning of the indiscriminate use of antibiotics (2,12). Prior to this, infections associated with antimicrobial use were related to Staphylococcus aureus (9).

Its transmission is by fecal-oral route or by contact with contaminated areas with ingestion of spores (13). The presence of spores in soil, water, shellfish, vegetables, and meat for human consumption such as pork, horse, and beef has been reported in up to 10% of the samples (10,14).

Toxigenic CD is characterized by the formation of toxins causing colitis and diarrheal stools; toxin A and toxin B, the latter being up to 10 times more potent (2,15). The genes that encode the toxins are found in TcdA and TcdB, respectively, located in the pathogenic locus of 19.6-KB (13). It is not an entero-invasive bacterium, it interrupts intercellular communication and changes the structure of enterocytes by breaking the cytoskeleton of actin, activating the Rho family of guanosine triphosphate, causing direct death of the colonocyte (2,16). Likewise, it activates inflammatory pathways such as

arachidonic acid, substance P, tumor necrosis factor and interleukins, with direct activation of neutrophils, causing loss of the intestinal barrier and neutrophilic colitis (9).

There is a hypervirulent strain called North American pulsed-field type 1 (NAP1/BI/02), which has in vitro resistance to nalidixic acid (9,17). It has a mutation in the tcdC gene that alters the regulation of the TcdC protein, responsible for the production and secretion of toxins A and B, increasing its level by 16-23 times (15,17,18). This strain has severe manifestations in up to 12.5% of patients compared to 5.9% of other strains, with a lower percentage of cure and higher risk of recurrence, which in turn facilitates hospital transmission (19).

On the other hand, CD can be found in the intestinal microbiota of healthy patients as a toxigenic strain without expression or non-toxigenic (23), known as asymptomatic carriers, representing 10% of the general population, 20-30% of the hospitalized population and up to 50% of elderly people in nursing homes (20). Its presence is associated with previous CD infection, previous hospitalizations in the last 3 months, and previous use of antibiotics, specifically fluoroquinolones (16). Its clinical relevance lies in the potential to contaminate skin and surfaces, with transmission of spores and infection to more susceptible populations, especially in hospital settings (16,21).

-Risk factors:

There are 3 main risk factors associated with CD infection: antimicrobial exposure, older age, and previous hospitalization (9). The use of antibiotics is the most related factor, causing loss of colonic microbiota non-pathogenic, thus allowing the propagation of toxigenic CD strains (11,15). In 1970 the CD-associated antibiotics were mainly clindamycin and penicillins, but in 1980 secondary to antimicrobial resistance, they began to be used indiscriminately cephalosporins and fluoroquinolones (8,9), with an increase in the hypervirulent strain NAP1/B1/027 (8,15,22). Today, any antimicrobial family predisposes to infection for CD, including vancomycin and metronidazole (9,13). The dose and exposure time necessary to cause intestinal microbiota depletion are not exactly known, but there is a directly proportional relationship between dose, number of antibiotics and days used. The use of two antibiotics increases the risk 2.5 times compared to patients with antimicrobial monotherapy, the use of 3-4 antibiotics increases the risk 5 times and the use of more than 5 antibiotics increase the risk 9.6 times (12). It has been documented that more than 14 defined daily doses in the previous 3 months have a greater association with CD (5), although in some studies the use of cefoxitin even in single doses as preoperative prophylaxis increased risk of infection (19). During the first month after exposure, the risk of infection is 6 times higher than patients without antimicrobial use, with extension up to 3 months later (5, 16, 25). Advanced age is another important risk factor, with a 10-20% higher risk

compared to those under 20 years of age (9). This is secondary to a state of immunosenescence, greater exposure to antibiotic therapy, use of predisposing medications, presence of comorbidities and higher hospitalizations (8). Globally, it has been seen that, for each additional year over 18 years, the risk of nosocomial CD increases by 2% (8,15). Similarly, mortality increases proportionally with age, with an average 30-day mortality of 13% in those over 80 years of age (3). Patients with previous hospitalizations (last 3 months) have a 63% probability of presenting CD colonization compared to non-hospitalized patients, with a 6-fold greater risk of manifesting toxigenic strains in case of disruption of the normal intestinal flora (23).

Up to 20-40% of hospitalized patients present colonization by CD, even after outpatient hospitalizations or exposure to transient settings such as doctors' offices, emergency departments, gastrointestinal surgery or dialysis units. Of these patients, 8% are asymptomatic carriers, compared to 2-3% of the healthy non-hospitalized population, reflecting a high rate of nosocomial contamination (9,14).
Hydrochloric acid inhibitors, including proton pump inhibitors (PPIs) and H2 antihistamines, have been associated with an increased risk of infection. They inhibit normal defense against ingested pathogens and promote colonization of the upper gastrointestinal system, which under normal conditions should be sterile, with a significant increase in bacterial germination in bile acid (1). Increased gastric pH has also been associated with alterations in leukocyte function, generating a higher incidence of enteric infections including traveler's diarrhea, salmonellosis and cholera (1). In a study of 1672 patients with CD, 74% were community infections associated with chronic use of PPIs, with a relative risk of 2.9 (1). The time and dose required to increase the risk of infection, as well as the time needed to return to baseline risk after discontinuation of drugs, are unknown (24).

Other preexisting factors related to the presence of CD infection are described in table 1, including stay in nursing homes, enteral feeding, chronic kidney disease, obesity and post-hematopoietic transplant. Environmental contamination and poor handwashing by health services promote the nosocomial spread of the infection (25).

Table 1. Risk factors for Clostridioides difficile infection (15,23).

External factors	Internal factors	Nosocomial contamination
Stay in nursing homes	Chronic kidney disease	Systemic chemotherapy
Use of systemic steroids 90 days prior	Obesity	Recent hospitalization
Enteral feeding	Post hematopoietic transplant	Use of hydrochloric acid inhibitors
Recent GI surgery	Neoplasms	Presence of antibodies against Toxin B
Non-surgical GI procedures	Inflammatory bowel disease	Poor handwashing by health services
Stay in intensive care units	Chronic liver disease	

GI: gastrointestinal.

Recurrence and reinfection by CD are one of the most serious problems within this illness. 13-47% of patients have a first recurrence and 38-45% have risk of a second recurrence, with a risk of subsequent recurrence of 30-65% (26,27). It is believed to be secondary to the persistence of spores resistant to the implemented treatment, with a predisposition in elderly patients, concomitant use of antibiotics, comorbidities, a history of severe CD, infection by the B1/NAP1/027 strain, and suboptimal levels of antibodies against Toxin A and toxin B (11,15,26,27). Up to 50% of patients with recurrences have a history of using hydrochloric acid inhibitors, increasing the absolute risk of recurrence by 1.5% (11).

Community infections represent up to 25% of cases and are defined as the presence of CD without a history of hospitalization in the last 3 months. The most associated risk factor is the use of previous antibiotics in up to 64% of cases, followed by proton pump inhibitors in 27.7% and immunosuppressants in 9.2% (14).

Risk factors associated with higher mortality and complications are shown in table 2. They include older age >80 years, comorbidities such as heart, lung or kidney failure, obesity, leukocytosis or leukopenia, hypoalbuminemia <2.5mg/dl, elevated markers of inflammation with CRP >150mg/ l, recent elective surgery, tachycardia and tachypnea, concomitant intestinal infection, as well as fever or hypothermia (13,28,29).

Table 2: Risk factors for higher mortality and complications due to Clostridioides difficile infection (13,28,29).

Risk factor for higher mortality and complications
Older age >80 years
Heart failure
Kidney failure
Obesity
Leukocytosis or leukopenia
Hypoalbuminemia <2.5 gr/dl
CRP > 150 mg/L
Recent elective surgery
Tachycardia or tachypnea
Concomitant intestinal infection
Fever or hypothermia

CRP: C reactive protein.

-Clinical manifestations:

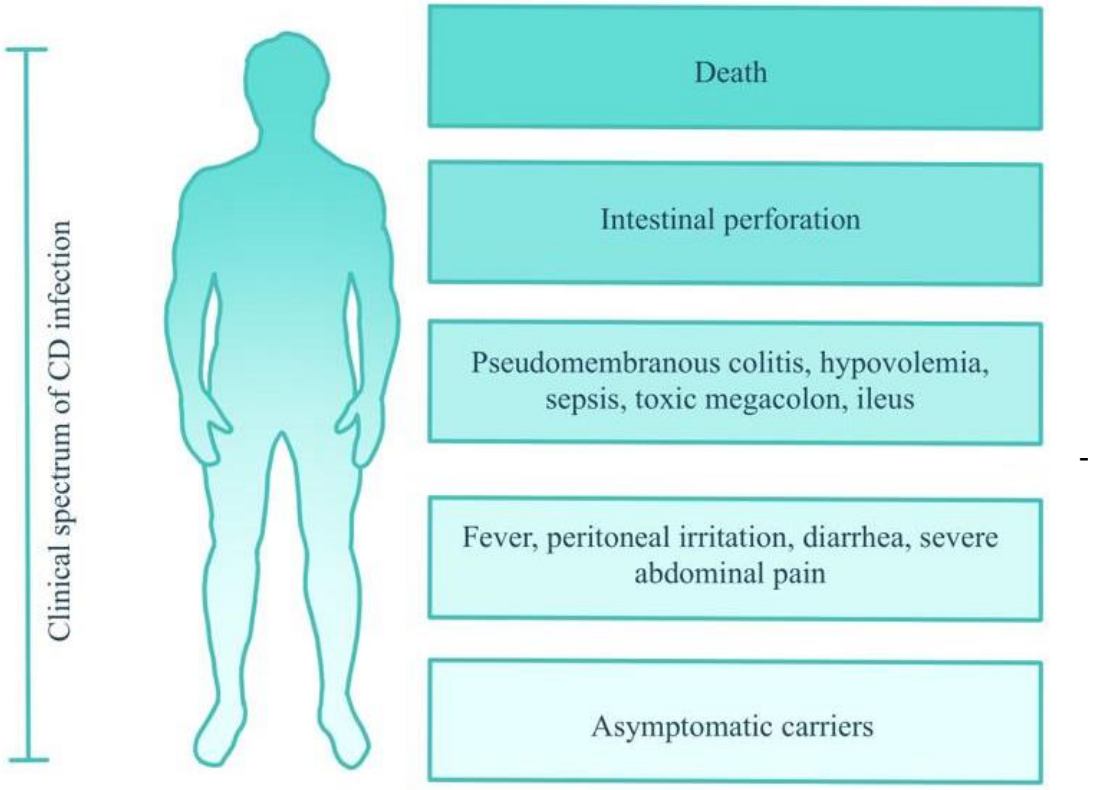
The clinical spectrum of CD infections is variable **(Figure 1),** expressing as asymptomatic carriers to toxic megacolon, ileus, intestinal perforation, sepsis, and death (8,15,21).

The presence of watery diarrhea is the cardinal manifestation and is defined as the presence of more than 3 diarrheal stools in 24 hours (15). It is generally accompanied by intermittent colicky abdominal pain, fecal leukocytosis, nausea without vomiting, anorexia, and signs of inflammation in colon biopsies (9). Up to 10-15% of patients have low-grade fever as a sign of severity (30).

Severe patients present with fulminant colitis and toxic megacolon in 11%, with mortality of up to 13% (28). Clinical manifestations include explosive diarrhea, bloating, and severe abdominal pain with data of peritoneal irritation or ileus (2.4%), hypovolemia with lactic acidosis, hypoalbuminemia, anemia, elevated creatinine (15.8%), marked leukocytosis, and toxicity data severe systemic or organ failure (2.9%) (8,9), although only 0.7% need colectomy and 0.2% present intestinal perforation (8). Up to 50% of hospitalized patients will present a co-infection of which stands out urinary tract infection in 21.1%, pneumonia in 13.6%, and sepsis in 13.2% (8). Another serious manifestation is pseudomembranous colitis, considered pathognomonic of this infectious agent. An atypical manifestation is protein-losing

enteropathy, characterized by hypoalbuminemia secondary to colonic barrier injury and loss of albumin through the intestinal lumen, causing loss of oncotic pressure with edema and ascites (9).

Figure 1: Clinical spectrum of CD infection



DIAGNOSIS:

Early diagnosis of a CD infection is of great importance for early and targeted initiation of antibiotic therapy and preventing nosocomial transmission. Clinical suspicion should be present in any patient with diarrhea defined as >3 watery stools in 24 hours, with or without the presence of known risk factors (13). The definitive diagnosis is made through two main diagnostic branches, where the presence of toxigenic CD in feces or the presence of toxins is sought (21).

The gold standard is the detection of CD in feces by means of a cytotoxicity assay in cell culture (12), with a high sensitivity (67-100%) and specificity, but with an important limitation in that it requires 48-72 hours to its processing and high costs (9,31,32), making it less valuable. Culture is a slow and complicated process, and it does not differentiate toxigenic from non-toxigenic strains, which is why it is losing its clinical relevance. Available tests for CD infection detection shown in table 3.

Table 3: summary of available tests for Clostridioides difficile infection, in decreasing order of sensitivity (IDSA 2021) *(12)*

Test	Sensitivity	Specificity	Substance detected
Toxigenic culture	High	Low*	Clostridium difficile vegetative cell spores
Nucleic acid amplification tests	High	Low-moderate	C. Difficile nucleic acid (toxic genes)
Glutamate dehydrogenase (GDH)	High	Low*	C. Difficile common antigen
Cell culture cytotoxicity neutralization assay	High	High	Free toxins
Toxin A and B enzyme immunoassays	Low	Moderate	Free toxins

Diagnosis by immunoassay for toxin A (TcdA) commercially available since 1980, or toxin B (TcdB) has a low sensitivity of 48%-75%, easy to process, low cost and fast results (9,31), but it is considered a suboptimal study individually (33,34). The test requires 100-1000pg of toxin to be positive, compared to <10pg required for the cytotoxicity assay (32), so the risk of false negatives is high and there may be discordance between diagnostic tests. The American College of Gastroenterology suggests that a negative immunoassay test should be repeated after 24 hours or complemented by another diagnostic study (34). The stool sample used must have a consistency on the Bristol scale of 5-7 (32,33) (figure

2), this being a visual table that classifies the shape of the stool into seven groups. The use of laxatives should be avoided approximately 48 hours beforehand and it should be processed in less than 2 hours, avoiding the degradation of toxins at room temperature (12). If timely processing is not achieved, the sample must be stored at a temperature of 4 Cº for a maximum of 72 hours. Patients with clinical resolution persist with positive toxins in up to 7% of cases, with more than 50% of patients excreting spores and 56% with positive cultures for 1-4 weeks after treatment, which is why it is not recommended to take serial samples (20,34).

Figure 2: Bristol stool chart (35).

Type*		Form of human faeces
1		Separate hard lumps, like nuts (hard to pass)
2		Sausage-shaped but lumpy
3		Like a sausage but with cracks on its surface
4		Like a sausage or snake, smooth and soft
5		Soft blobs with clear-cut edges
6		Fluffy pieces with ragged edges, a mushy stool
7		Watery, no solid pieces, entirely liquid

*Formed feces were defined as type 1–4.

Diagnosis by means of nucleic acid amplification by real-time polymerase chain reaction (rT-PCR) of toxin B is highly recommended due to its high sensitivity (87.1%-95%) and specificity (95%-99.4%) as well as quick results (25, 33, 37). This study became commercial in 2008 (36) and is considered the test of choice (30). Its main disadvantage is cost, the need for highly specialized equipment, and the inability to differentiate between infected patients and asymptomatic carriers (37). Up to 30% of hospitalized patients are colonized, so it is important to only test patients with clinical gastrointestinal suspicion (9,20). Another study showed that RT-qPCR for TcdC has a sensitivity of 86% and a specificity of 97% compared to the cellular cytotoxicity assay (34). The sample must have a high number of pathogens, so it must not be a diluted sample, with a minimum detection limit of 1x 105 per gram of stool (36,37), again without recommendations for serial testing (34).

For patients with ileus who cannot provide a fecal sample, rectal swab sampling for rT-PCR is recommended as an acceptable alternative diagnosis (16).

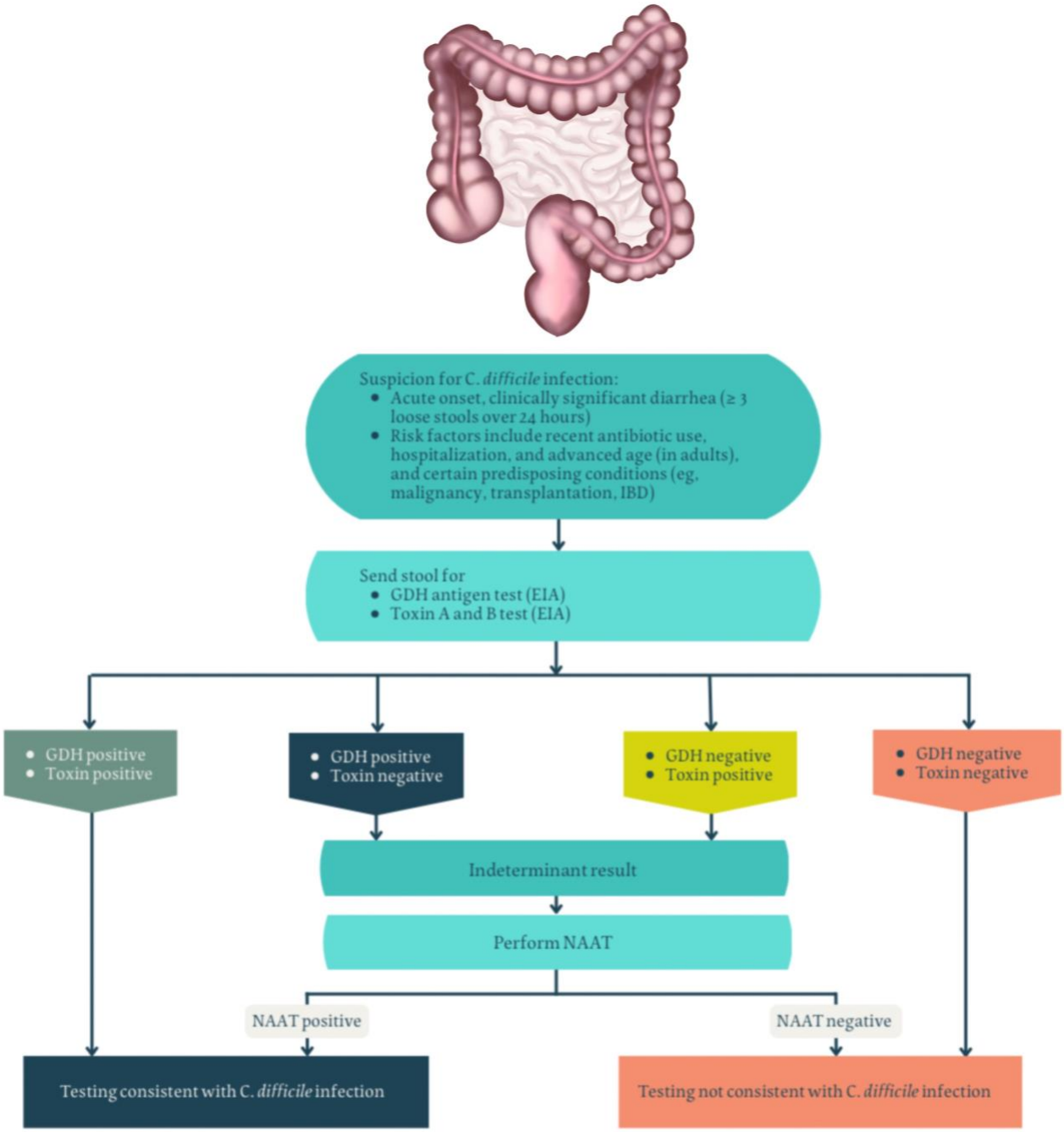
The immunoassay for glutamine dehydrate antigen (GDH), a enzyme that is produced constitutively by all strains of CD, is another method diagnosis, however, its presence does not differentiate between toxigenic and non-toxigenic strains (34), so there is the possibility of a cross-reaction against GDH from other anaerobes including Clostridium sporogenes, Peptostreptococcus anaerobius, and Clostridium botulinum (37). This test has a high sensitivity (96%) with a positive predictive value of 93.4% (32), up to 100% negative, and your result can also be delivered in less than an hour (31,37).

Some adjunctive diagnostic studies include abdominal radiography, abdominal computed tomography, and colonoscopy to visualize pseudomembranes, toxic megacolon, perforation, or ileus. Diagnosis is made with an abdominal x-ray when visualizing intestinal dilation >7cm in the colon or >12cm in the cecum, ruling out signs of intestinal perforation.

Screening studies are not recommended for asymptomatic carrier patients, as there is no evidence on the effects of the eradication of non-toxigenic strains or the risk of progression to manifestations of cytotoxicity (36). However, asymptomatic patients at high risk of colonization should be considered, such as patients over 65 years of age, with a history of CD infection and previous use of antibiotic therapy, to avoid continuous nosocomial transmission as much as possible (19,20,38), and taking into account that the concentrations of toxins in feces of symptomatic patients and asymptomatic carriers are similar (21,38).

Diagnostic algorithm:

Diagnostic algorithms seek to optimize resources and time, using two low-cost and fast methods with high sensitivity, followed by a confirmatory test with high specificity. **(Figure 3)**



IBD: Inflammatory bowel disease; GDH: Glutamate dehydrogenase; EIA: Enzyme immunoassay; NAAT: Nucleic acid amplification testing

Different diagnostic algorithms have been proposed, the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) recommends the two-step method, initially with a high-sensitivity test (nucleic acid amplification or GDH), then the diagnosis is confirmed with a technique more specific as the immunoassay test for toxins. GDH in combination with PCR has a sensitivity of 86.1% and a negative predictive value of 97.8% compared to PCR alone (31).

On the other hand, the American Society of Infectious Diseases (12) proposed an algorithm multistep diagnosis that is based on concomitantly ordering an immunoassay to toxins and GDH, if both tests are positive, you have a diagnosis definitive, and in case of discrepancy, a confirmatory test with rT-PCR is requested (12,21). This algorithm, compared to the gold standard, has a sensitivity and specificity of 55.6% and 98.3% respectively, with a positive predictive value of 87% and a negative predictive value of 91.7% (31).

TREATMENT:

1. Prevention:

The use of non-essential and prophylactic antibiotics should be minimized, as well as the time of exposure to them should be reduced to avoid as much as possible the depletion of the non-pathogenic intestinal microbiota and the development of CD (12,19,22). The implementation of hospital antimicrobial control programs has reduced the presence of CD by up to 60% in some hospitals (8,14). It is also recommended to suspend and limit the non-indicated use of proton pump inhibitors, educating the population about the adverse effects related to their use, which can reduce CD infection by 11.2% (1,14,16). Hospital-acquired infections should be managed optimally to avoid nosocomial transmission (1,8,39), reporting up to 29% of cases with a history of exposure to asymptomatic carriers and 30% to patients with active infection (12,20).

2. Medical treatment:

Medical treatment should be implemented immediately empirically in all patients with compatible symptoms, even before confirmatory diagnostic tests.

As mentioned above, strict contact isolation measures should be immediately implemented up to 48 hours after clinical resolution (19), surface washing with a 1:10 dilution of chlorine solution (9), avoiding

rectal thermometers, and washing should be encouraged. Continuous washing of hands with soap and water for both patients and healthcare personnel, to avoid nosocomial transmission, and use disposable material as much as possible (12,19).

The use of non-essential antibiotics should be discontinued early (9,40) since the concomitant use of other antibiotics is associated with prolonged diarrhea, treatment failure and early recurrences (12).

Formerly, the treatment of choice was metronidazole for non-severe cases, with an approximate clinical response of 87-90%, being a cheap drug and with minimal association with microorganisms resistant to vancomycin (18,29,40). However, in the last few years, studies have shown that 25-30% of patients presented recurrence and it is associated with potentially irreversible neurotoxicity (12,16,18,40,41). In the 2021 IDSA guidelines, treatment for an initial episode od CD infection has shown better results with oral fidaxomicin 200 mg given twice daily for 10 days. This bactericidal macrolide is specific against CD, avoids the destruction of commensal intestinal flora and has been associated with a lower number of recurrences in non-NAP 1 strains (41). Oral vancomycin is considered a good alternative at 125 mg four times daily for 10 days, reaching levels in the colon lumen up to 100 times higher than the minimum inhibitory concentration necessary for the destruction of the infectious agent possessing no systemic absorption abilities (42) nevertheless, it has devastating effects on the composition of the microbiota (41). Clinical resolution is similar for fidaxomicin and vancomycin, at 88% and 86%, respectively (12), but fidaxomicin is associated with fewer short-term recurrences and less risk of overgrowth of vancomycin-resistant enterococcus. The higher sustained clinical response associated with fidaxomicin may be especially beneficial in patients at greater risk for recurrence of CDI. No statistical differences have been reported between high-dose and low-dose oral vancomycin in non-severe CD (42). If the aforementioned medications are not available, metronidazole can be used at a dose of 500mg orally every 8 hours only in non-severe CD (9). If they have a concomitant infection, treatment should be extended one week after the end of concomitant antibiotic therapy (12). The clinical response to management varies, but in non-severe cases it is 4-6 days (9), with a faster response to vancomycin compared to metronidazole of 1.6 days (43).

On the other hand, the use of imidazoles, bacitracin, rifaximin and fusidic acid for the treatment of CD has been studied, without good results (4). However, the use of nitazoxanide, being an antibiotic that intervenes in the metabolism of anaerobic bacteria, has noninferiority results against vancomycin in the treatment of severe CD (44).

In patients with severe or fulminant disease (defined by shock, hypotension, megacolon or ileous), immediate management should be started with oral vancomycin at high doses of 500 mg orally or by nasogastric tube every 6 hours together with intravenous metronidazole 500mg every 8 hours,

particularly if ileus is present. In these patients, clinical cure with vancomycin has been reported in up to 97% and 76% with metronidazole (12). If they have ileus, transrectal vancomycin can be administered at a dose of 500 mg in 100ml of saline solution every 4-6hrs as a retention enema (12). The use of fidaxomicin in severely ill patients is not recommended due to lack of evidence and clinical experience (12).

General surgical evaluation should be done in patients with severe CD with evidence of hypotension, fever, ileus or significant abdominal distention, peritoneal irritation, altered alertness, leukocytosis, serum lactate >2.2mmol/L, multiple organ failure or lack of clinical improvement in 3-5 days (16,45).

Age <65 years is the only independent factor that predicts a good clinical response, while age >80 years, leukocytosis >15x109 leukocytes/liter and renal failure with creatinine >1.5mg/dl are risk factors associated with mortality and failure to treatment (45,46). The ATLAS scale for CD is a useful clinical tool that uses five clinical and laboratory values, with a scoring system capable of predicting response to optimal treatment and mortality, allowing a comparison between groups of patients (47).

3. Recurring disease:

Recurrence has an increase in mortality at 180 days of 33%, compared to patients without recurrences (12). Recurrences have been reported in 25% of patients treated with vancomycin and 30% of patients treated with metronidazole. In case of a first recurrence, the indicated treatment is fidaxomicin 200 mg given twice daily for 10 days although the IDSA 2021 guidelines also propose vancomycin as a good alternative regimen, given 125 mg 4 times daily by mouth for 10 days. This implementation depends upon available resources within every region. In case of a second recurrence, the appropriate treatments are: fidaxomicin 200 mg given twice daily for 10 days, OR twice daily for 5 days followed by once every other day for 20 days; vancomycin 125 mg 4 times daily by mouth for 10 days followed by rifaximin 400 mg 3 times daily for 20 days and/or fecal microbiota transplantation (12). As an adjunctive treatment, it is recommended: Bezlotoxumab 10 mg/kg given intravenously once during administration of standard of care antibiotics. There is no benefit to extending treatment time for CD >14 days (40).

4. Alternative treatment:

-Faecal microbiota transplantation has been used in patients with fulminant colitis or patients with multiple recurrences only in regions with high capacity and experience, however, it is not a routine treatment for early and/or uncomplicated treatment, with therapeutic efficacy reported 77-94% (12).

- There are no recommendations at this time for the use of probiotics (12), even with systematic reviews that support the use of probiotics as a preventive measure for CD specifically Lactobacillus acidophilus and Lactobacillus casei administered within 2 days of starting antibiotic therapy (7).

- In patients at high risk for recurrence, the use of prophylactic vancomycin at doses of 125-250mg orally every 12 hours has been recommended, without clinical relevance in patients with >2 previous recurrences (6,12).

-Since 2016, the use of bezlotoxumab has been implemented, a monoclonal antibody that binds directly to CD toxin B and inhibits its action, for secondary prevention of recurrences (27). The monoclonal antibody Actoxumab binds to toxin A and neutralizes it, but has not had good clinical results (16).

-The use of antimotility drugs for the treatment of enterocolitis associated with invasive bacteria is not recommended since intestinal motility promotes a decrease in the enteropathogens (48), at the moment there is insufficient evidence to discontinue or recommend their use in CD (12,39,43).

OBJECTIVES:

• Primary objective:

-To report the diagnosis and treatment of C. difficile and infections, as well as prognosis in two different hospitals in Mexico City.

• Secondary objectives:

- To describe the socio-demographic and clinical characteristics of patients with infection by C. difficile.
- Quantify the incidence of recurrent infections by C. difficile.
- Report the prevalence of other concomitant gastrointestinal infections
- Describe the use of antibiotics prior to infection and the medical treatment implemented in patients with primary and recurrent infection by C. difficile.
- Describe the risk factors associated with recurrent C. difficile infection.

MATERIAL AND METHODS:

Methology

An observational, retrospective longitudinal study, within the clinical practice of Hospital Ángeles Lomas and the National Institute of Respiratory Diseases (INER) of Mexico City was performed. We obtained data from June 2017 to March 2022

Study population:

• Inclusion criteria:

Patients over 18 years of age, hospitalized at Hospital Angeles Lomas and National of respiratory illnesses (INER) with a diagnosis of Clostridioides difficile infection, established either from a positive nucleic acid amplification test by polymerase chain reaction or positive toxin B and GDH.

• Exclusion criteria:

1.- Patients under 18 years of age

2.- Outpatient treatment patients

3.- Incomplete electronic file

• Clinical and sociodemographic characteristics:

The clinical records (electronic) of 111 patients diagnosed with C. difficile infection by rT-PCR, who were hospitalized at Hospital Ángeles de las Lomas in the period November 2017-March 2022, were reviewed. Demographic, serological and clinical state within a database created for the study.

• Diagnosis of C. difficile infection:

-DNA extraction was performed using QIAgen stool and Estratec stool, following the manufacturing instructions, looking for the amplification of the gene for Toxin B (tcdB). Isolation and rT-PCR was performed with the Seegene Allplex gastrointestinal panel, using Allplex GI-Bacteria (I), Allplex GI- virus assay, Allplex Roti molecular. Briefly, the technique consists of taking a fecal sample for extraction and amplification of the toxin B gene (tcdB), initially it must be centrifuged, and the specific fluorescent reagents are added, later it is introduced to the previously mentioned interpretation system. The results are reported as positive or negative, in an average of 2 hours.

-Detection of toxins A-B and/or GDH from stool samples using by immunoassays (IA) techniques, such as lateral flow-based immunochromatographic techniques or enzyme-linked immunosorbent assay techniques based on a final reading by spectrophotometry or chemiluminescence.

METHODOLOGY:

An observational, retrospective, case series study was conducted in patients who had Clostridium difficile infection from June 2017 to March 2022 at Hospital Ángeles Lomas (HAL) and the National Institute of Respiratory Diseases (INER); hospitals in Mexico City. Information was collected from the electronic files, recording the following variables: age, sex, type of hospitalization, concomitant diseases, diagnostic tests, concomitant microorganisms detected, previous treatments, previous C. difficile infections, and treatment received. Any patient diagnosed with CD by PCR or toxin B or GDH positive were included. Patients under 18 years of age who received treatment outside the hospital and with incomplete medical records were excluded.

For statistical analysis, the IBM SPSS program, version 21, was used. All categorical variables are expressed as frequencies and percentages. $\chi 2$ was used for difference of proportions and Student's t for difference between two means and ANOVA for difference between three or more means. An alpha value less than 0.05 was considered significant.

RESULTS

A total of 189 patients were analyzed, 89 from the HAL hospital and 100 from the INER hospital. Table 4 shows the diagnostic tests used with their percentage of positivity for each hospital, where we can see that the PCR had a similar performance between both hospitals, with a positivity of 85% for HAL and 89% for INER. In Table 5 we observe the associated microorganisms that were identified by the PCR test, observing that in the HAL hospital there were more pathogens identified, compared to the INER group, however, it is interesting that in 15% of the cases of the INER hospital they were found cryptosporidium, perhaps because of high incidence of people being treated there for human immunodeficiency virus (HIV) infection. Sixty-three percent of the subjects in the private hospital were women while fifty one were female in the public hospital group (Table 4) lacking statistical significance thus we can expect a more homogeneous incidence in the present. Our data showed that the mean prevalence of infections occurs around 30 to 60 years-old ($p = 0.7$). Diagnostic methods are similar between both groups (PCR and/or GDH and toxins) as well as treatment options that resulted in favorable outcomes. Most physicians prefer using vancomycin as a first line treatment given the fissionability of medications in Mexico. One relevant difference is that in INER, all the antibiotics are given by an infectiology specialist, not like in HAL where a variety of physicians can provide antibiotic

treatment (internal medicine, gastroenterology and infectiology). The doctors that did not use vancomycin as a first line treatment did use nitazoxanide instead with similar results. The prevalence of certain comorbidities (as diabetes mellitus) are more common in the INER given that is a reference center and many diabetic patients were already being treated there. More doctors in HAL provided treatment with both vancomycin and metronidazole compared to INER doctors (40% vs 17%). Recurrent infection was reported in similar percentages in both groups and deaths by any cause showed equivalent data.

Table 4: Demographic and clinical characteristics of both study groups

Variable	HAL N: 89	INER N: 100	*P*
Age – media ±SD	51.4 ±19.3	50.5 ±15.1	0.70
Age stratification (years) – N. (%)			
• <30	14 (15.7)	4 (4)	
• 31-40	19 (21.3)	23 (23)	
• 41-50	11 (12.4)	25 (25)	
• 51-60	12 (13.5)	22 (22)	
• 61-70	16 (18)	15 (15)	
• >70	17 (19.1	11 (11)	
Sex – N. (%)			0.01
• Woman	63 (70.8)	51 (51)	
• Man	26 (29.2)	49 (49)	
Medical hospitalization – N. (%)			0.00
• Yes	42 (47.2)	84 (84)	
• No	47 (52.8)	16 (16)	
UTI hospitalization – N. (%)			0.60
• Yes	11 (12.4)	10 (10)	
• No	78 (87.6)	90 (90)	
Diabetes mellitus – N. (%)			0.01
• Yes	6 (6.7)	32 (32)	
• No	83 (93.3)	68 (68)	
Other comorbidities – N. (%)			0.02
• Yes	33 (37.1)	100 (100)	
• No	56 (62.9)	0	
Neoplasia/ immunosuppression – N. (%)			0.32
• Yes	32 (36)	43 (43)	
• No	57 (64)	57 (57)	

Table 5: Diagnostic tests

Test	HAL N: 89	INER N: 100	*P*
PCR – N. (%) • Positive • Negative	 76 (85.4) 13 (14.6)	 89 (89) 11 (11)	0.45
C. difficile toxins – N. (%) • Positive • Negative	 38 (42.7) 51 (57.3)	 74 (74) 26 (26)	0.00
GDH – N. (%) • Positive • Negative	 35 (39.3) 54 (60.7)	 91(91) 9 (9)	0.00

HAL: Hospital Angeles Lomas. INER: Instituto Nacional de Enfermedades Respiratorias. PCR: polymerase chain reaction. C. difficile: Clostridioides difficile. GDH: glutamate dehydrogenase.

Table 6: Concomitant microorganisms diagnosed by PCR

Microorganism	HAL	INER	*P*
Rotavirus	2.2%	3%	0.74
Norovirus	5.6%	2%	0.10
Yersinia	1.1%	0%	0.28
Campylobacter	3.4%	2%	0.55
Astrovirus	2.2%	0%	0.13
Cryptosporidium	1.1%	15%	0.12
Giardia	1.1%	7%	0.04
Adenovirus	2.2%	1%	0.13
Shigella	3.4%	1%	0.06
Salmonella	4.5%	2%	0.03
Vibrio	3.4%	1%	0.06

Table 7: Risk factors for C. difficile infection

Variables	HAL N: 89	INER N: 100	*P*
Previous antibiotics – N. (%)			
• Cephalosporin	16 (18)	10 (10)	
• Carbapenems	13 (14.6)	5 (5)	
• Fluoroquinolones	10 (11.2)	6 (6)	
• Macrolides	5 (5.6)	2 (2)	
• Vancomycin	10 (11.2)	0	
• Metronidazole	11 (12.4)	0	
• Another antibiotic	7 (7.9)	0	
Previous PBI treatment – N. (%)			0.68
• Yes	31 (34.8)	32 (32)	
• No	58 (65.2)	68 (68)	
Previous steroids – N. (%)			0.02
• Yes	24 (27)	14 (14)	
• No	65 (73)	86 (86)	
Previous infection – N. (%)			0.00
• Yes	14 (15.7)	3 (3)	
• No	75 (84.3)	97(97)	
Recurrent infection – N. (%)			0.91
• Yes	5 (5.6)	6 (6)	
• No	84 (94.4)	94 (94)	

PBI: proton pump inhibitor. HAL: Hospital Angeles Lomas. INER: Instituto Nacional de Enfermedades Respiratorias.

Table 8: Treatment comparison

Variables	HAL N: 89	INER N: 100	*P*
Vancomycin 500 mg – N. (%)			0.02
• Yes	73(82)	97 (97)	
• No	16 (18)	3	
Metronidazole – N. (%)			0.03
• Yes	67 (75.3)	17 (17)	
• No	22 (24.7)	83 (83)	
Vancomycin + Metronidazole			0.00
• Yes	40 (44.9)	17 (17)	
• No	49 (55.1)	83 (83)	
Another antibiotic			0.00
• Yes	19 (21.3)	1 (1)	
• No	70 (78.7)	99 (99)	
• Treatment duration (days) – media ±SD	10.4 ±2.4	10 ±2	0.05
Death by any cause – N. (%)	2 (2.2)	3 (3)	0.74

CONCLUSIONS

CD infection in Mexico has been treated for decades with the preparation of intravenous (IV) vancomycin given orally because of the lack of oral vancomycin in the country. This context somehow must affect the fissionability of the medication meant to be given IV, nevertheless, over the years and with our previous report, patients have had a generally good outcome with this presentation.

Many physicians are trained to treat CD infection in adults; including internists, gastroenterologists, and infectiology specialists, which should not alter the treatment given the updated guidelines, however, some treatments do differ given the specialty of the named physician giving equivalent results.

Another limitation we have in Mexico is that we do not have fidaxomicin, so we generally use either vancomycin or metronidazole. There were few doctors prescribing nitazoxanide even though it has shown non-inferior results compared with vancomycin.

One limitation of our study was the number of incomplete databases that restricted us to some comorbidities and baseline diseases. We would like to perform a prospective study to have a better comprehension of our work.

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