The situation and management of *Clostridium difficile* infection in Spain: an opinion document

Emilio Bouza[1, 2, 3, 4]
Mercedes Marín[1, 3, 4]
Teresa Peláez[1, 2, 3, 4]
Luis Alcalá[1, 3, 4]
On behalf of the Study Group for *Clostridium difficile* Infection of the Spanish Society for Chemotherapy*

[1]Clinical Microbiology and Infectious Diseases Department, Hospital General Universitario Gregorio Marañón, Madrid, Spain.
[2]Department of Medicine. Universidad Complutense, Madrid, Spain.
[3]Red Española de Investigación en Patología Infecciosa (REIPI), Spain.
[4]Centro de Investigación Biomédica en Red Enfermedades Respiratorias (CIBERES), Spain.

INTRODUCTION

Clostridium difficile is the most common etiologically-defined cause of health-care-related diarrhea[1] and it is being progressively recognized as a cause of community-acquired diarrhea[2-5]. It is caused by the toxins of certain strains of *C. difficile* and it represents a growing concern.

In recent years, several guidelines and recommendations have been published by different societies helping to clarify the management of *C. difficile* infection (CDI)[6-10]. However, a high proportion of those recommendations are not based in A-I type of evidence and still leave many questions open for opinion. Furthermore, those guidelines usually reflect the views of a few highly expert opinion leaders but not the opinion of a large group of practicing physicians and microbiologists interested in the field of CDI. A group for the study of CDI of the Spanish Society for Chemotherapy (SEQ) carried out recently a nationwide study of the situation of the diagnosis of *C. difficile* in Spanish hospitals, involving more than 100 different institutions. It came clear that CDI is still a neglected disease, either because of clinical unawareness or because of defective microbiological processing of samples[11]. The professionals involved in that study gather together in a one day meeting to discuss the results of the study and to assemble an opinion document that may be helpful to other colleagues in order to improve the management of CDI. An opinion document never replaces but complements and potentiates the more formal and structured official guidelines.

The meeting was held in Madrid on March the 14th, 2012 and this document reflects the main questions, answers and conclusions of the meeting updated by the literature available up to may 2013.

Correspondence:
Emilio Bouza
Servicio de Microbiología Clínica y Enfermedades Infecciosas.
Hospital General Universitario Gregorio Marañón.
Dr. Esquerdo 46, 28007 Madrid, Spain.
Phone +34-91-5868452 - Fax +34-91-3721721
E-mail: ebouza@microb.net

METHODS

A list of questions about controversial issues in the clinical management of CDI in Microbiology and Clinical Departments was drafted by the principal investigators. The potential responses were subsequently evaluated by infectious disease specialists and clinical microbiologists.

Before the meeting was convened, each convener was assigned different questions and invited to review and summarize the evidence supporting or refuting the issues raised by each one. All the material obtained was edited in a document that was sent to all the members of the CDI study group before publication.

The panel consisted of more than 100 participants, mainly ID physicians and clinical microbiologists belonging to the Study Group for CDI Infections of the Spanish Society for Chemotherapy (SEQ), who chose between different approaches for each question. The casting of opinion was individual and secret.

The following document summarizes the literature review and the participant's opinion.

QUESTION 1.- WHEN SHOULD CDI BE SUSPECTED?

1.- It must be suspected in adult patients with diarrhea with or without common risk factors for this infection.

68% in favor

2.- It must be suspected in adult patients with diarrhoea only when well known risk factors are present.

21% in favor

3.- It must be suspected only in diarrheic episodes that are health-care related, with or without risk factors

11% in favor

4.- The attendee recognizes a not well formed opinion on this point

0%

Summary of the convener

Several studies reported on the importance of CDI as a community-acquired disease[12]. A recent population based study, carried out by Khanna et al.[2] in Olmsted county (USA) showed that community-acquired CDI cases (CA-CDI) accounted for 41% of 385 definite CDI cases. The incidence of CA-CDI increased significantly over the study period. Compared with those with hospital-acquired infection (HA-CDI), patients with CA-CDI were younger (median age 50 years compared with 72 years), more likely to be female (76% vs. 60%), had lower comorbidity scores, and were less likely to have severe infection (20% vs. 31%) or have been exposed to antibiotics (78% vs. 94%). The study did not show differences in the rates of complicated or recurrent infection in patients with CA-CDI compared with HA-CDI. Another population-based study[4] carried out in Iowa from January 2004 to December 2007 showed incidence rates for CA-CDI and HA-CDI of 11.16 and 12.1 cases per 100,000 person-years, respectively. Overall, 27% of CA-CDI cases did not receive antimicrobials in the 180 days before their diagnoses, and 17% did not have any traditional risk factors for CDI.

Several studies estimate the proportion of CDI episodes of community origin in proportions ranging from 15 to 18%[12, 13].

The community-acquired disease is generally more benign that the health-care associated one and rarely the patients with CA require intensive care admission or colectomy[12, 14]. However, in Durham, North Carolina, USA, in 2005[14], 59% of the CA cases required hospitalization, and 15% reported an emergency department visit. None of the patients required admission to intensive care units or surgical interventions, such as colectomy. Another study in Connecticut, USA, in 2006[15] showed that 111 (46%) of 241 CA-CDI case-patients required hospitalization, 29 (12%) required admission to intensive care units, 5 (2%) had toxic megacolon or colectomy, and 5 (2%) died of CDI, however in that study the search for the more severe cases may have bias the data.

A case control study of CA-CDI[13] showed that community-associated case patients were not receiving prior antibiotics in 40% of the episodes and had more frequently than controls malignancy, exposure to high-risk persons, and remote health care exposure. Stomach-acid suppressants were not associated with community-associated infection, and 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors appeared protective.

Molecularly it is difficult or impossible to differentiate HA-CDI strains and community acquired strains of *C. difficile*. Toxin A-negative/toxin B-positive isolates were highly associated with hospital-acquired CDI in a recent study[16].

In summary, data in the literature and the majority of the experts attending the meeting support that CDI should be suspected in diarrhoeic episodes acquired in or outside the hospital and in patients with or without traditional risk factors for this entity.

QUESTION 2.- HOW MANY STOOL SAMPLES SHOULD BE SENT TO THE MICROBIOLOGY LABORATORY FOR THE DIAGNOSIS OF CDI?

1.- The best cost-effective option is to send one stool specimen and, in some circumstances, two stool specimens.

82% in favor

2.- The best cost-effective option is to send two stool specimens and, in some circumstances, three stool specimens.

12% in favor

3.- The best cost-effective option is to send three stool specimens.

4% in favor

4.- The attendee recognizes a not well formed opinion on this point.

2% in favor

Summary of the convener

The need to test successive stool specimens in order to increase the diagnostic yield of CDI is still under discussion. In an attempt to clarify this issue, Aichinger and colleagues[17] compared the impact of repeating testing in the diagnosis of CDI. They analyzed any patient who had two or more tests performed in 7 days, including all patients with exactly two tests and patients with three or more tests during this period. Of the 1,321 patients who had an initial negative EIA test, 25 (1.9%) were positive on the second test (95% CI, 1.2% to 2.7%), compared to 1.7% (95% CI, 0.7% to 3.5%) becoming positive for the 401 patients having an initial negative PCR test (P =1.0). They concluded that the diagnostic gains of repeat testing are equally low for PCR and EIA and that repeat testing for CDI should not be routine. In a similar study van den Berg et al.[18] found that out of 78 patients, only one became positive upon repeat EIA testing for toxin A/B within a 7-day period. They concluded that if the initial test is negative, repeat testing is unnecessary. In another interesting study[19], all CDI tests (Wampole *C. difficile* Tox A/B II enzyme immunoassay kit) performed during 2006 were retrospectively analyzed. Out of a total of 8,256 tests from 3,112 patients; 49% of tests were repeated. Of the 3,749 initially negative patient tests, 96 were positive (2.5%) upon repeat testing within 10 days of the first test. Thirty-eight patients had a positive test within 48 h of an initial negative test, and based on chart review, 18 patients were treated empirically while only 16 were treated following the new result. None had evidence of medical complications. Of initially positive patients, 91% were positive upon repeat testing on day 0, 75% on day 1, and 58% on day 2, to a low of 14% on days 7 to 10. Depending on the clinical setting, these data support not repeating *C. difficile* tests within 2 days of a negative result.

International guidelines of the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA)[8] recommend only one stool specimen for the diagnosis of CDI. Moreover, the American Society for

Microbiology (ASM) (http://www.asm.org/images/pdf/ Clinical/clostridiumdifficile9-21.pdf) does not recommend repeating testing following a negative test if one of the suggested algorithms is used because nearly all positive patients will be detected in the first test. According to the ASM, testing a second specimen from a negative patient is more likely to be a false-positive. Recent guidelines from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID)[20] do not recommend repeated sample submission during the same episode in a endemic situation (level 2) although may be useful in a epidemic situation (level 3 of scientific evidence).

In conclusion, data in the literature and the majority of the experts attending the meeting support that the best cost-effective number of stool specimens needed for the diagnosis of CDI is one stool specimen and only exceptionally two stool specimens.

QUESTION 3.- REGARDING THE TRANSPORTATION MEDIA TO SEND SAMPLES TO THE LABORATORY. WHAT OF THE FOLLOWING SENTENCES IS MORE CONCORDANT WITH YOUR OPINION?

1.- Recipients both with and without transport medium for enteropathogens are suitable for the diagnosis of CDI.

64% in favor

2.- Only recipients without any transport medium are suitable for the diagnosis of CDI.

28% in favor

3.- Only recipients with transport medium for enteropathogens are suitable for the diagnosis of CDI.

1% in favor

4.- The attendee recognizes a not well formed opinion on this point.

7% in favor

Summary of the convener

As C. *difficile* is a spore-forming anaerobe it is not necessary to use anaerobic media to transport specimens for the diagnosis of CDI[21-25]. International guidelines recommend transporting stool specimens in a clean, watertight container[8]. However, it is not infrequent that laboratories receive diarrheal stools preserved in transport media as Cary-Blair medium from patients suffering for acute gastroenteritis because clinicians suspect of aerobic enteropathogens like *Salmonella* spp., *Shigella* spp., or *Campylobacter* spp., especially when patients are not hospitalized[26]. Besides, strict application of international guidelines in these specimens would prevent diagnosis of CDI. Cary-Blair medium is a non-nutritive transport medium that prevents overgrowth of most *Enterobacteriaceae* and is effective in the preservation of common enteropathogens as *Salmonella, Shigella* and *Vibrio* for long periods[27]. In a non-comparative study, Brown and col. found that four different diagnostic methods (glutamate dehydrogenase and toxin A and B enzyme immunoassays, cell culture cytotoxicity assay, and real-time PCR targeting the toxin B gene) performed well with stools preserved in Cary-Blair medium[28]. In another study, designed for the validation of a liquid Cary-Blair faecal swab, authors showed that there was a 100% correlation of results obtained from stools transported in this media versus those obtained from stools without transport media[29]. In our institution we checked the viability of *C. difficile* spores and toxins inoculated in transport vials with and without Cary-Blair transport media. Results were equivalent and in our opinion fecal samples submitted in transport medium should not be systematically rejected in Microbiology laboratories [Alcalá et al. Unpublished information]

In summary, data in the literature and the majority of the experts attending the meeting support that both recipients without any transport medium and recipients with transport medium for aerobic enteropathogens as Cary-Blair are suitable for the diagnosis of CDI.

QUESTION 4.- SHOULD FECAL SAMPLES WITHOUT A *C. difficile* REQUEST BE PROCESSED FOR *C. difficile*?

1.- Only stool samples received in the laboratory that have a request of CDI diagnosis must be processed for *C. difficile*

35% in favor

2.- All stool samples received in the laboratory must be processed for *C. difficile*, independently of the request.

1% in favor

3.- All unformed stool samples, in patients older than 2 years, received in the laboratory must be processed for diagnosis of CDI, independently of the request.

61% in favor

4.- The attendee recognizes a not well formed opinion on this point.

3% in favor

Summary of the convener

Question number 1 has emphasized the need of clinicians to suspect CDI in all patients with diarrhea acquired in or outside the hospital and in patients with or without traditional risk factors for this illness. It would be naïve to accept that clinicians always know the risk factors for CDI of the patients or always remember to include the request for CDI in those circumstances. Wanahita et al.[30] showed that unrecognized CDI in inpatients was responsible for 58% of episodes of unexplained leukocytosis in a tertiary hospital. Vaessen et al.[31] performed a 5-month study in which they compared the frequency of toxigenic *C. difficile* in stool specimens from patients hospitalized for more than 3 days with and without a physician's request for detection of CDI and found similar values in both groups (8.5 and 8.0%, respectively). A one-day point study of CDI performed in 118 different institutions in Spain showed that only 55.2% of unformed stool specimens

received in the laboratories from patients hospitalized ≥3 days had been processed for CDI diagnosis. This led to the missing of 25% of nosocomial CDI episodes due to the not processing of specimens for CDI[11].

At this point, it seems clear that microbiologists in the laboratory can have an important role for the improvement of the CDI diagnosis. Even in the best scenario for CDI recognition, as is the case of nosocomial diahrroea, the clinical suspicion of CDI is far from optimal.

The majority of the experts attending the meeting support that all unformed stool samples, in patients older than 2 years, received in the laboratory should be processed for an optimal diagnosis of CDI, independently of the request by the clinicians.

QUESTION 5.- SHOULD SAMPLES OTHER THAT DIARRHEIC STOOLS BE PROCESSED FOR CDI ?

1.- Rectal specimens are not recommended for the diagnosis of CDI.

28% in favor

2.- Rectal specimens are recommended for the diagnosis of CDI in patients suffering ileus.

37% in favor

3.- Colon biopsies are more effective than stool specimens in unspecific colitis for the diagnosis of CDI.

8% in favor

4.- The attendee recognizes a not well formed opinion on this point.

27% in favor

Summary of the convener

International guidelines agree that, in diarrheic patients with suspicion of CDI, only watery or loose stools should be collected and tested to establish the diagnosis of CDI[8,20] (http://www.asm.org/images/pdf/Clinical/clostridiumdifficile[9-21].pdf). In situations in which patients suffer for ileus, toxic megacolon or abdominal distension without diarrhoea, it usually cannot be possible to obtain an unformed stool specimen for CDI diagnosis. In this situation, diagnostic procedures recommended by international guidelines are very unlike. English guidelines recommend using diagnostic procedures such as colonoscopy, white cell count, serum creatinine or abdominal computerized tomography scanning (http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_132927). SHEA and IDSA clinical guidelines recommend rectal specimens obtained by means of cotton swabs for the etiologic diagnosis of CDI[8,20]. On the contrary, ASM guidelines do not recommend rectal specimens in this situations and only suggest using formed stool specimens, when present, and after consensus with clinicians (http://www.asm.org/images/pdf/Clinical/ clostridiumdifficile[9-21].pdf). One study that could clarify this issue is that performed by McFarland et al.[32] in which the authors compared the diagnostic yield of rectal swabs and stool specimen cultures from hospitalized patients with CDI. The work showed that rectal specimens were as sensitive as stool cultures for the diagnosis of CDI. In a recent publication, Kundrapu et al.[33] compared the value of testing for CDI by polymerase chain reaction, perirectal swabs or stools. The sensitivity, specificity, positive predictive value, and negative predictive value of testing perirectal swabs vs stool specimens were 95.7%, 100%, 100%, and 99.1%, respectively.

The value of determination of the presence of toxigenic *C. difficile* in colonic mucosal biopsies in patients with colitis is another issue for debate[26]. Although culture of colon biopsies obtained by colonoscopy traditionally has been an acceptable procedure for diagnosis of CDI, stool cultures, which are less invasive and cheaper, could be a better option for CDI diagnosis. In a retrospective study performed in the Clinical Microbiology and Infectious Diseases Department of the Hospital General Universitario Gregorio Marañón (Madrid, Spain) the diagnostic yield of both colon biopsies and stool specimens from patients with both types of samples and suspected of having CDI were compared (1997-2011). From 500 specimens pairs analyzed, a total of 75 patients (15.0%) were diagnosed of CDI from, at least, one of the analyzed specimens. Sensitivity of stool specimens (94.7%) was significantly higher than that of colon biopsies (21.3%) ($p<0.001$). Results of this study clearly show that stool specimens are a more recommendable specimen for CDI diagnosis [Alcalá L. et al. Unpublished information].

Limited data from the literature and the opinion of the majority of those attending the meeting support that rectal or perirectal specimens are suitable for CDI diagnosis in patients whose stool specimens cannot be obtained and that stool specimens are more sensitive than colonic biopsies for the diagnosis of CDI in patient suffering colitis.

QUESTION 6.- ARE ALL RAPID TESTS BORN EQUAL. HOW TO SELECT ONE?

1.- Enzyme immunoassays (EIA) that detect both toxins A and B are good tests for diagnosis of CDI.

19% in favor

2.- A screening using an EIA that detects glutamate dehydrogenase, followed by one or more confirmatory techniques is the most cost-effectiveness technique for the rapid diagnosis of CDI.

72% in favor

3.- Commercial genetic detection of toxins A or B is a cost-effective and accessible technique for the laboratories for the rapid diagnosis of CDI.

6% in favor

4.- The attendee recognizes a not well formed opinion on this point.

3% in favor

Summary of the convener

The optimal rapid diagnosis of CDI in the laboratory remains an area of controversy. The availability of multiple tests with different *C. difficile* targets both reflects and contributes to this uncertainly[34-37]. Commercial enzyme immunoassays (EIAs) tests that either detect toxin A only or detect both toxins A and B have been the most used techniques in the laboratories all over the world. Until a few years ago, most laboratories performing EIAs tests used those detecting only toxin A, however, with the knowledge of the existence of toxigenic strains toxin A-/toxin B+ EIAs that detect both toxins were developed[38-41]. In spite of the extensive use of EIAs in the diagnostic laboratories, these tests have showed to have poor sensitivity and specificity when compared with toxigenic culture[42-48]. EIA sensitivities may be as low as 40% and are rarely above 60%. Besides, at low prevalence, the positive predictive values of these tests may be as low as 50%. For these reasons, the IDSA and the SHEA consider these tests as a suboptimal alternative approach for diagnosis of CDI (strength of recommendation: BII)[8]. Similarly, the ASM considers these tests as insensitive and strongly recommends that these tests do not be used as a stand-alone test (http://www.asm.org/images/pdf/Clinical/clostridiumdifficile9-21.pdf). After a review of 13 commercial EIAs detecting toxins A and/or B an ESCMID report concluded recommending to use another tests that is more sensitive and with a greater positive predictive value for rapid CDI diagnosis[20].

Another rapid test that allows the detection of *C. difficile* is the detection of glutamate dehydrogenase (GDH), an antigen specific of both toxigenic and non-toxigenic *C. difficile*. The initial test was a latex agglutinin assay that had a sensitivity of only 58-68% and a specificity of 94-98%[49,50]. However, GDH detection was later adapted using EIA methodology and showed an increased sensitivity of 85%–95% although maintained an specificity of 89%–99%[51,52] that led that this test has not been recommended as a single test for rapid diagnosis of CDI by international societies[8].

In recent years, the CDI diagnostic conundrum has been dramatically transformed by the development of commercial molecular assays for toxigenic *C. difficile* that utilize real-time PCR or loop-mediated isothermal amplification to directly detect the *tcdA* or *tcdB* genes encoding toxin A or B, respectively, from fecal specimens[53-63]. Some of these tests has been approved by FDA as are the BD MAX system (Becton Dickinson), Xpert® *C. difficile* (Cepheid), prodesse® proGastro™ CD (Gen-Probe) and Illumigene® *C. difficile* (Meridian) platforms. Sensitivity of most of these techniques is very high with values of 90-100% when are compared with toxigenic culture, as well as being highly specific. However, the high cost that have commercial molecular test precluded them as an alternative for diagnosed CDI in most laboratories[35].

Given the limitations of rapid tests, various multistep algorithms have been devised in which initial screening are performed using GDH EIA test due to its high sensitivity to detect CDI[64-67]. As most specimens are negative, the GDH screening step substantially reduces the number of specimens that require evaluation with more specific methods. Since both toxigenic and nontoxigenic *C. difficile* strains express GDH, a positive GDH EIA requires confirmation testing with a toxin EIA and/or a sensitive assay for toxin A or B (i.e., a molecular assay). Overall performance including turnaround time of a GDH-based algorithm depends on the secondary tests used to follow up a positive GDH result. In the update of CDI guidelines published in 2010 by IDSA and SHEA there is a 2-step method recommendation (strength of recommendation: B-II) that uses GDH detection by EIA as initial screening and a cell cytotoxicity assay or toxigenic culture as the only confirmatory test for GDH-positive stool specimens[8,20]. However, this recommendation implicates delayed information for positive results of 1-3 days. More recently, the ASM has published a guidance document in which the proposed algorithms enable a rapid diagnosis of CDI avoiding the overall use of molecular tests (http://www.asm.org/images/pdf/Clinical/clostridiumdifficile9-21.pdf). The two combinations proposed use GDH antigen followed by a molecular test, alone or combined with a toxin A/B EIA, when specimens are GDH-positive. These procedures have been evaluated by several authors and have a sensitivity of 85-90% and specificity greater than 99%[43,55,68-72].

Data in the literature and the majority of the experts attending the meeting support that detection of GDH by EIA as screening test, followed by a rapid confirmatory technique, as a molecular test alone or together with a toxin A/B EIA, is the most cost-effectiveness procedure for the rapid diagnosis of CDI. In laboratories without severe economic restrictions, optimal rapid diagnosis of CDI can be achieved using a molecular technique as a standalone test.

QUESTION 7.- WHAT COMBINATION OF LABORATORY TESTS IS NOW RECOMMENDED FOR AN OPTIMAL CONFIRMATION OF CDI?

1.- Detection of GDH by EIA followed by a rapid confirmatory test.

37% in favor

2.- Detection of GDH by EIA followed by a rapid confirmatory test and a cytotoxicity assay from stool specimens.

14% in favor

3.- Detection of GDH by EIA followed by a rapid confirmatory test and a toxigenic culture.

46% in favor

4.- The attendee recognizes a non well formed opinion on this point.

3% in favor

Summary of the convener

Multistage algorithms based in an initial screening with a GDH EIA are excellent cost-effectiveness procedures for the rapid diagnosis of CDI. Although the high specificity of these algorithms

warrants a high positive predictive value even in low prevalence situations, their relatively limited sensitivity causes that about 5-15% of stool specimens containing toxigenic *C. difficile* not be detected by these procedures[43,55,68-72]. Therefore, optimal diagnosis of CDI requires an additional procedure with a high sensitivity but also very specific to recover those cases missed by these algorithms. Traditionally, the gold standard for CDI diagnosis has been considered the cytotoxin assay, which uses tissue culture to detect a cytotoxic strain directly from diluted stool specimens. Antibody neutralization makes this assay highly specific. However, numerous studies have shown that the cytotoxin neutralization assay is only 65 to 80% sensitive to detect toxigenic *C. difficile* isolates[73-75] in comparison to toxigenic culture, which is performed by isolating *C. difficile* on selective media and demonstrating cytotoxin production by the cultured organism. These reasons have induced that IDSA and SHEA societies excluded in their guidelines the cytotoxin assay as the gold standard in favor of toxigenic culture (strength of recommendation: BIII)[8]**.** Similarly, the ASM recommends using toxigenic culture (or nucleic acid amplification test) as a confirmatory test of the proposed algorithms (http://www.asm.org/images/pdf/Clinical/clostridiumdifficile9-21.pdf). However, the ESCMID consider both cytotoxicity assay and toxigenic culture as the gold standards for the diagnosis of CDI, although considers the last as a more sensitive method[20].

In conclusion, data in the literature and the majority of the meeting attendees support that detection of GDH by EIA as screening test following by a rapid confirmatory technique as a molecular test alone or together with a toxin A/B EIA and the use of toxigenic culture in order to recover up to 5-15% of CDI episodes lost by the rapid procedure is the optimal combination of laboratory tests to confirm CDI.

QUESTION 8.- WHEN AND HOW TO PERFORM ANTIMICROBIAL SUSCEPTIBILITY TESTS TO *C. difficile* ISOLATES

1.- The increase of resistance to metronidazole and vancomycin requires a systematic sensitivity testing.

5% in favor

2.- Sensitivity testing should be done periodically to monitor the emergence of resistance or in specific situations and in reference centers.

57% in favor

3.- Sensitivity testing should be done periodically in all Microbiology laboratories.

31% in favor

4.- The attendee recognizes a not well formed opinion on this point.

7% in favor

Summary of the convener

Metronidazole and vancomycin are the drugs of choice for the treatment of CDI. Fidaxomicin, is a recently FDA-approved drug entering the treatment options at present.

Until recently, the activity of traditional antimicrobials metronidazole and vancomycin for the treatment of CDI was not argued and susceptibility testing was not even recommended. However, several reports of toxigenic isolates of *C. difficile* resistant to metronidazole have been communicated during the last 15 years. Barbut et al.[76] found one resistant toxigenic strain (MIC: 16 mg/L, agar dilution method) isolated during 1997. Peláez et al.[77] detected a 6.3% of resistance to metronidazole (26/415 isolates, MICs: ≥32 mg/L, agar dilution method) in toxigenic strains isolated from 1993 to 2000 in a hospital in Spain. A posterior analysis of the resistant strains showed that resistance to metronidazole is heterogeneous and that it can be lost in strains after prolonged periods of storage due to freezing and thawing[78]. In a study performed in a medical center in Israel[79], the authors described a 2% of resistance to metronidazole (1/49 isolates, MIC: ≥32 mg/L, E-test method), and a similar resistance rate was find in toxigenic strains isolated during 2004 to 2006 in Ontario, Canada (19/1,080 isolates, MICs: ≥32 mg/L, E-test method)[80]. Recently, Huang et al.[81] reported a 23.1% of resistance to metronidazole in primary fresh toxigenic *C. difficile* strains isolated from 2008 to 2009 in China (18/78 isolates, MICs ≥32 mg/L, E-test method). As in the Spanish study, authors from both the Canadian and Chinese studies identified a heterogeneous resistance in their resistant isolates in such a way that most of resistant isolates were converted in sensitive to metronidazole after serial passages[78,80].

Although not so frequently, isolates of *C. difficile* have been reported to have intermediate resistance to vancomycin with MICs above 2 mg/L[77,82-85]. On the other hand, fidaxomicin has shown a strong activity against *C. difficile* with most isolates having MICs lower than 1 mg/L being the highest MIC ever reported, to our knowledge, of 2 mg/L[86-93].

Faecal metronidazole and hydroxymetronidazole concentrations are considered bactericidal in patients with acute disease receiving oral or intravenous metronidazole but as the diarrhoea improves neither substance is detectable in the faeces of diarrhoea caused by *C difficile* (mean concentration of 9.3 µg/g in watery stools and of 1.2 µg/g in formed stools)[94]. This finding has led to the EUCAST committee to decrease the metronidazole breakpoint from 16 mg/L to only 2 mg/L (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_2.0_120221.pdf).

Conversely, fecal levels of vancomycin and fidaxomicin in the colon lumen are greater than metronidazole with concentrations of 64-760 µg/g on day 2 and 152-880 µg/g on day 3 post-treatment for vancomycin[95] and as high as 3,000 mg/L for fidaxomicin[96].

Although Clinical and Laboratory Standards Institute (CLSI) guidelines do not recommend to routinely do susceptibility testing of *C. difficile* isolates because correlation of MICs with clinical failures has not been established they advocate performing a surveillance testing annually to detect emerging resistance. The surveillance should be done by the hospital laboratory if expertise is available or, if not, by a re-

ference laboratory. If possible, this institute recommend to test isolated collected over several months and stored until a total of 50-100 strains for later batch testing using preferably agar dilution method[97].

In summary, data in the literature and the majority of the experts attending the meeting suggest that sensitivity testing should be done periodically to monitor the emergence of resistance or in specific situations and in reference laboratories.

QUESTION 9.- ARE FOLLOW-UP LABORATORY TESTS REQUIRED IN PACIENTES WITH CDI?

1.- Toxin B detection is recommended to follow-up the evolution of patients with CDI.

13% in favor

2.- Toxin B detection is not recommended to follow-up the evolution of patients with CDI.

45% in favor

3.- Toxin B detection is not recommended to diagnosed recurrences as patients could be colonized.

18% in favor

4.- The attendee recognizes a not well formed opinion on this point.

24% in favor

Summary of the convener

Treatment of non-severe CDI episodes with metronidazole or vancomycin at standard doses and duration have showed similar efficacy with response rates near 90-97%[98-100]. In cases with severe disease, however, a recent study performed by Zar et al.[101] showed that metronidazole was inferior to vancomycin. The therapeutic response usually involves the resolution of fever on the first day and of diarrhea before fourth or fifth day[102]. In spite of the clinical resolution of CDI episode, *C. difficile* can survive in the lumen of cured patients during several weeks or months[103,104]. In a study performed in cured patients with previous recurrent CDI, McFarland et al.[105] found that persistence of *C. difficile* spores by the end of antibiotic therapy was of 56% and 43% for those treated with metronidazole and vancomycin, respectively. Similarly, an observational study involving patients receiving therapy for CDI showed that near 20% of successfully treated patients had detectable spores in stools at the time of resolution of diarrhea and increased to 56% 1-4 weeks later[106]. This lack of correlation showed by these studies between clearance of colonic *C. difficile* and resolution of CDI had led to the main international guidelines to recommend not to use culture or toxin detection to follow-up the evolution of patients with CDI (level of evidence of SHEA-IDSA: B-III)[8,20].

Evidence in the literature and most of the experts attending the meeting suggest that *C. difficile* detection is not a good method to follow-up the evolution of patients with CDI.

QUESTION 10.- WHEN AND HOW TO REPORT CLINICIANS THE RESULTS OF LABORATORY TESTS FOR CDI?

1.- Prompt written information obtained from the rapid diagnostic test is a necessary and sufficient condition for a optimal control and treatment of CDI.

32% in favor

2.- Phone information of positive results obtained from the rapid diagnostic test clearly improves the control and treatment of CDI.

66% in favor

3.- Rapid information of negative results obtained from the rapid diagnostic test barely has effect in the control and treatment of CDI.

0%

4.- The attendee recognizes a not well formed opinion on this point.

2% in favor

Summary of the convener

Early recognition of CDI is a critical step to control the transmission of *C. difficile* to other patients and to optimize the treatment of this illness, and must be based in a right suspicion of the illness by clinicians, an accurately laboratory diagnosis of CDI and a rapid and effective transmission of information of these results to the attending physician, infection preventionist, and nursing staff[8,20,107]. The CDC recommend to work with microbiology laboratories to ensure rapid reporting of test results for CDI, including weekends and holidays, and to ensure that there is a process for providing results to the patient care area so isolation precautions can be initiated promptly (Center for Diseases Control and Prevention, Guidelines for preventing transmission of MDROs, 2006, http://www.cdc.gov).

C. difficile is a highly transmissible organism able to produce spores that can persist in the environment for many months and are highly resistant to cleaning and disinfection measures[103,104]. Transmission of *C. difficile* to the patient via transient hand carriage on healthcare workers´hands is thought to be the most likely mode of transmission[108]. In order to avoid transmission, prestigious authors and international Scientific Committees as the Healthcare Infection Control Practices Advisory Committee, the SHEA, the Association for Professionals in Infection Control and Epidemiology, the CDC and the Infectious Diseases Society of America[109-115] recommended several points to the health care facilities as are to quarantine CDI patients, to use antiseptic procedures as the utilization of disposable gloves, mask and gown and the handwashing with soap and water, to clean patient-care equipment (such as thermometers, stethoscopes, etc) before it is used for another patient, to enhance environmental cleaning with dilute bleach from all patient contact surface areas, to restrict the use of antimicrobials implicated as risk factors for CDI, to provide an easy laboratory access for prompt and active sur-

veillance toxin B detecting at the earliest indication of a case of CDI and to use rapid and accuracy tests to diagnosis CDI in the laboratory.

On the other hand, rapid and accurate laboratory recognition of a CDI episode is a key step to optimize the treatment of patients suffering for CDI. Rapid report of a positive result can facilitate a prompt treatment that avoids the risk that an initial mild CDI episode may progress to severe colitis and toxic megacolon[116]. Besides, delayed diagnosis can increase the time of patient exposition to inappropriate drugs as antiperistaltics or narcotics that can complicate CDI[117]. Similarly, fast information of a negative result advance the withdrawal of antimicrobial in patients with empiric treatment for CDI and permit to find the true cause of the symptoms that suffer the patient[19].

In summary, data from literature and most of the experts attending the meeting suggest that immediate laboratory recognition of CDI is a crucial step for the control and management of this illness. Preliminary phone information of results obtained from the rapid diagnostic tests to the appropriate health care workers is recommended.

QUESTION 11.- WHAT IS YOUR OPINION REGARDING THE INCIDENCE OF CDI IN SPAIN?

1.- Between 1-5 episodes/10,000 days of hospital stay.

36% in favor

2.- Between 5-10 episodes/10,000 days of hospital stay.

35% in favor

3.- The attendee recognizes not to have a figure in mind not even approximative.

29% in favor

Summary of the convener:

The report of the incidence of CDI is still a subject of considerable variability[118,119] because different nominators and denominators have been used for the reports. Episodes may be collected from microbiological data or from chart reports with significant differences[120-122]. At the same time, failure to include post-discharge CDI cases can lead to further underreporting of CDI and inaccurate incidence rates. Data from a retrospective cohort study carried out in the USA show that when post-discharge CDI events were included, incidence figures raised from 29 per 10,000 admissions to 52 per 10,000 admissions[16].

Furthermore, the number of episodes is frequently referred to figures for 1000 or 10000 days of admissions or days of hospital stay, adding again to confusion in this area. Population based studies provide a broader spectrum of the situation of CDI but are more difficult to convey and may be less representative for the assessment of the situation in a particular hospital or institution.

Accurate diagnosis of CDI is also a pre-requisite for obtaining reliable epidemiological data on incidence and prevalence rates and yet, as a comprehensive survey of diagnostic protocols across Europe suggests, testing for CDI is suboptimal in many countries[123]. A significant percentage of CDI cases are missed today because clinicians often fail to request tests for *C. difficile* toxins in cases of unexplained diarrhoea and widely used diagnostic tests have low sensitivity or are not applied appropriately in microbiology laboratories[11].

According to the SHEA-IDSA guidelines[8], the minimum surveillance that should be performed by all healthcare facilities is to report on new cases with onset at least 48 hours after inpatient admission (nosocomial cases) per 10,000 patient days. In addition, measures should be considered for tracking severe outcomes and comparison of incidence rates between hospitals could be more meaningful if rates are age-standardized or are limited to specific age groups.

Community-associated CDI requires excluding an overnight stay in an inpatient healthcare facility in at least the 12 weeks prior to symptom onset. A reasonable denominator for community-associated CDI is the number of person-years for the population at risk.

With these limitations in mind, evidence for a change in the epidemiology of CDI first emerged in the USA and Canada, where rates of CDI were seen to increase markedly between 2000 and 2006[124,125]. Data on discharge diagnosis rates in US hospitals showed that rates of CDI more than doubled from fewer than 150,000 cases in 2001 to more than 300,000 in 2005 (http://www.hcup-us.ahrq.gov/reports/statbriefs/sb50.pdf). This change in incidence marked the start of what has become a continuous rise in rates of CDI not only in North America but also in Europe[126-128]. The incidence of CDI in a USA population-based study estimated 13.5 CDI cases per 10,000 person-years. This could represent figures of about 220,000 cases of CDI occurring among persons older than 20 years in 2007 in the USA. Overall 55% of the episodes occurred out of the hospital setting[129].

Historically, rates of CDI in Europe have been broadly similar to those reported in the USA, although surveillance for CDI has been more variable reflecting differences in reporting regulations across Europe. To address deficiencies in CDI reporting across Europe, a pan-European hospital-based survey of CDI was carried out in November 2008 by Bauer et al.[130]. Information was based on data provided by a network of 106 laboratories in 34 European countries and collected in 2008. The incidence of CDI was, overall, of 4.1 per 10,000 patient-days with a very broad range (0.0-36.3). The figure collected from the Spanish hospitals involved in that survey was a mean of 4.3 episodes/10,000 admissions, again with a variability ranging from 0 to 16.7 episodes/10,000 admissions. The survey showed that CDI remains a predominantly nosocomial infection in Europe, with 80% of cases acquired in hospitalised patients compared with 14% in the community and 6% of indeterminate origin[12].

Some data suggest however, very recent and important

decreases in the incidence of CDI in several countries attributed to different intervention programs and also a recent significant reduction in the participation of the 027 epidemic strain[131].

CDI is not yet a reportable disease in Spain but indirect data obtained through the hospital records suggest an increase in the episodes of CDI in recent years with estimates of 41.2 cases per 100,000 discharges[132]. Data obtained through a review of point prevalence study series of nosocomial infections showed prevalence rates of CDI increasing from 3.9 to 12.2 cases per 10,000 hospitalized patients from 1999 to 2007[133].

A recently reported prospective nationwide diagnostic study, in which confirmatory cultures were performed to all diarrheic stools arriving into 118 Spanish microbiology laboratories[11] estimated a nosocomial incidence of 3.8 cases of CDI per 10,000 patient days in Spain.

The incidence of CDI infections is probably underestimated not only in Spain but also in other European countries. A large proportion of health-care workers with interest in the field of CDI recognized to ignore the national incidence or missed the published figures.

QUESTION 12.- THE ESTIMATION OF THE EXTRA-COST OF AN EPISODE OF CDI BY THE ATTENDEES WAS AS FOLLOWS:

1.- The extra-cost is estimated between 2,000 and 5,000 US dollars ($2008) per episode:

47% in favor

2.- The extra-cost is estimated between 5,001 and 10,000 US dollars ($2008) per episode:

20% in favor

3.- The extra-cost is estimated in more than 10,000 US dollars ($2008) per episode:

8% in favor

4.- The attendee recognizes not to have a figure in mind not even approximative

25% in favor

Summary of the convener:

The costs associated with each hospitalised case of CDI are by no means trivial. Patients who develop CDI require isolation, supportive therapy for underlying diseases, as well as specific antibiotic therapy to treat *C. difficile*. In the small percentage of patients who develop serious complications, significant additional costs arise from the need for surgery and post-operative care.

On average, patients with CDI spend an extra 1–3 weeks longer in hospital compared with non-infected patients. Increased duration of hospitalisation is a major, if not the major, contributor to increased costs[134,135]. Additional costs accrue from the need for rigorous hygiene in patient care, environmental decontamination and, when outbreaks occur, cohort isolation and ward closure[126].

A matched case-control study was carried out to determine hospital-wide excess costs due to CDI in a tertiary care university hospital in 2006. The difference in the length of stay showed that CDI cases stayed significantly longer (median 7 days) than their matched controls. The average cost per CDI patient was €33,840. The difference in the cost per patient showed that the cost for CDI patients was significantly more than for their matched controls (median € 7,147; 95% confidence interval: 4,067-9,276)[136].

In a recent systematic review carried out by Ghantoji et al.[137] from 1980 to 2010, the authors included 13 studies and estimated CDI extra costs in 2008 US dollars in between $2,871 to $4,846 per case for primary CDI and from $13,655 to $18,067 per case for recurrent CDI. In special populations (subjects with irritable bowel disease, surgical inpatients, and patients treated in the intensive care unit) they showed an incremental cost range from $6,242 to $90,664. Non-US-based studies showed an estimated incremental cost of $5,243 to $8,570 per case for primary CDI and $13,655 per case for recurrent CDI.

McGlone et al.[138] estimated the economic burden of CDI for hospitals, third-party payers and society using an economic computer simulation model. The median cost of a case ranged from $9,179 to $11,456 from the hospital perspective, $8,932 to $11,679 from the third-party payor perspective, and $13,310 to $16,464 from the societal perspective. Most of the costs incurred were accrued during a patient's primary CDI episode. They estimated that the annual US economic burden of CDI would be ≥$496 million (hospital perspective), ≥$547 million (third-party payer perspective) and ≥$796 million (societal perspective).

Separately, the high rates of recurrent CDI associated with currently available antibiotics not only increase morbidity, with some patients experiencing repeated recurrences over months and years leading to exhaustion and protein-losing enteropathy[139], but also add to the burden of costs of care.

In Europe, estimates suggest that the potential costs associated with the management of CDI are in the region of €3,000 millions. This figure is likely to rise in line with an ageing population. By 2050, more than 134 million Europeans will be aged 65 years or older[126].

A recent estimation of the burden of CDI in Spain[140] shows a calculated burden of 7,601 episodes per year with expenses for the National Health Service of 32,157,093 € . Mean estimated extra-cost per episode is 3,901 € in initial episodes, 4,875 € for first recurrences and 5,916 € for subsequent episodes in Spain.

Overall, 72% of physicians and microbiologist with interest in the field of CDI, either widely infra-estimate the cost of the disease or recognize to ignore data on the issue. Costs ranging, at least, from 3,900 to 5,900 € per episode in Spain can be provisionally considered.

QUESTION 13.- REGARDING THE INCLUSION OF NOSOCOMIAL CDI AMONG THE QUALITY ASSURANCE MARKERS OF ANTIBIOTIC STEWARDSHIP IN HOSPITALS, THE OPINION OF THE ATTENDEES WAS AS FOLLOWS:

1.- Believe that nosocomial CDI rates are good indicators of antibiotic stewardship in an institution and should be included as benchmarking values

15% in favor

2.- There is no enough evidence to use incidence of nosocomial CDI as a marker of quality and proper antibiotic stewardship.

79% in favor

3.- Participant recognize not to have an opinion on this issue

6% in favor

Summary of the convener:

Reported data suggests that good antimicrobial stewardship can lead, overall, to less antimicrobial use and to less inappropriate antimicrobial use, lower drug-related costs, reductions in CDI, and, in some studies, less emergence of antimicrobial resistance[141-147]. However, the value of nosocomial CDI incidence as an index of antibiotic misuse is unknown[148,149].

Revised guidelines of antimicrobial use, recommending the avoidance of broad-spectrum antibiotics, was associated with a significant reduction in the use of fluoroquinolones and cephalosporins and to a significant decrease in CDI in a retrospective quasi-experimental study[150].

Few studies have examined the risk of CDI associated with total dose, duration, or number of antibiotics while taking into account the complex changes in exposures over time. In a study including 10,154 hospitalizations, the authors observed dose-dependent increases in the risk of CDI associated with increasing cumulative dose, number of antibiotics, and days of antibiotic exposure. Compared to patients who received only 1 antibiotic, the adjusted hazard ratios (HRs) for those who received 2, 3 or 4, or 5 or more antibiotics were 2.5 (95% confidence interval [CI]: 1.6-4.0), 3.3 (CI: 2.2-5.2), and 9.6 (CI: 6.1-15.1), respectively. The receipt of fluoroquinolones was associated with an increased risk of CDI, while metronidazole was associated with reduced risk. Antimicrobial stewardship programs that focus on the overall reduction of total dose as well as number and days of antibiotic exposure and the substitution of high-risk antibiotic classes for lower-risk alternatives may reduce the incidence of hospital-acquired CDI[151].

The overall majority of the attendees think that the information available at present time do not permit to include the incidence of hospital acquired episodes of CDI as an overall indicator of antimicrobial stewardship quality.

QUESTION 14.- WHAT IS THE DEFINITION AND THE CONCEPT OF "SEVERE CDI" RECOMMENDED BY THE ATTENDEES?

1.- This concept is unclear and not unquestionably defined and needs further precision

16% in favor

2.- There are many systems to classify a case as severe, all are reliable and easy to use

13% in favor

3.- Due to the many different and variable criteria it is better to adopt those recommended by large scientific societies and particularly those provided by SHEA/IDSA

71% in favor

Summary of the convener:

A case of CDI was considered severe by the Centers for Disease Control (CDC) when the case requires admission in an intensive care unit due to *C. difficile* infection, has colonic perforation, has toxic megacolon or requires colonic surgery, requires more than 10 days of extra hospital admission or ends in death[152,153]. Clinicians, however, need to assess severity much earlier in the clinical process in order to take the proper therapeutic solutions and with that intention several severity scores have been proposed, however none with the proper validation. Fujitani et al.[154], compared 8 severity score indices for CDI in a prospective observational study, carried out in 3 university hospitals. Sensitivities of the 8 severity score indexes ranged from 63.2% to 84.2%, and specificities ranged from 59.4% to 93.9%. The Hines VA index had the highest kappa score. The Hines VA CDI severity score index included the following: fever≥ 38ºC (1 point), ileus diagnosed by radiologic or clinical findings (1 point), systolic blood pressure < 100 mmHg (1 point), elevated WBC (< 15,000=0 points, 15,000 to 30,000= 1 point, > 30,000= 2 points) and CT scan findings (No findings=0 points, 1 finding=1 point, 2 or more findings=2 points). A score of 3 or more points indicates severe CDI[155]. The Hines score however includes CT findings that are not obtained routinely in the management of CDI infections and does not include laboratory data such as the albumin level or the creatinine level.

The Society for Healthcare Epidemiology of America (SHEA)[8] made recommendations to consider a CDI episode as severe or severe and complicated. They are based in opinion of experts and include as severe all cases with either leukocytosis (> 15,000 wbc/uL) or elevation of more than 1.5 times over basal levels of creatinine serum level. A CDI is considered severe and complicated if one or more of the following is present: low blood pressure, sepsis, ileus, toxic megacolon, perforation, need to ICU admission, need of surgery due to CDI complications and death.

In a nationwide survey, acute kidney injury is considered as an independent marker of severity in *Clostridium difficile* Infection[156].

The majority of the attendees accepted the SHEA/ID-

SA score for severity definition as the most convenient for clinical practice.

A CDI episode is considered as severe if one or more of the following criteria are fulfilled:

White blood cell count ≥ 15.000 uL

Increase in Creatinine> 50% over basal levels

An episode is considered severe and complicated if any of the following criteria is fulfilled

Low blood pressure or sepsis

Paralytic ileus

Toxic megacolon

Bowel perforation

ICU admission required

Need for surgery due to CDI complications

Death

QUESTION 15.- WHAT IS THE DRUG OF CHOICE FOR THE TREATMENT OF SEVERE CDI IN YOUR INSTITUTION AT THE PRESENT TIME?

1.- Oral vancomycin at regular doses

16% in favor

2.- Oral vancomycin at higher doses and/or longer duration

28% in favor

3.- Oral Vancomycin plus IV Metronidazole

25% in favor

4.- Oral metronidazole

31% in favor

Summary of the convener:

The first step in the treatment of patients with CDI is to withdraw antimicrobials, whenever possible. Up to 25% of CDI episodes may resolve with this simple measure[98,157,158]. However, frequently it is not feasible to predict which subset of patients will respond to the withdrawal of antibiotics only. On the other hand, in severe hospitalized patients it is hardly possible to stop antimicrobial therapy altogether in many cases.

The administration of either metronidazole or vancomycin is the mainstay for the treatment of CDI. The normal duration of therapy is 10-14 days, although there are no well-performed studies that have established the possible advantage of shortening or lengthening this course. Some authors advocate longer therapy (14 days) to avoid recurrence. All antimicrobials should be administered orally as *C. difficile* is in the lumen of the colon. If the intravenous route is required, only metronidazole is effective, as intravenous vancomycin only achieves very low concentrations in the colon lumen[159]. The therapeutic response usually involves the resolution of fever and of diarrhea within the next five days[159].

The guidelines jointly published in 2010 by the SHEA and IDSA committees[8] recommend using metronidazole (500 mg, orally, 4 times per day for 10-14 days) for the initial mild to moderate CDI episode while vancomycin is reserved for severe (125 mg, orally, 4 times per day for 10-14 days) and severe-complicated cases (500 mg, orally, 4 times per day; with or without metronidazole, 500 mg iv, 3 times per day) (BII). This guidelines recommend treating with the same regimen the first recurrences and always with vancomycin the second and later recurrences (BIII). The European treatment guidance document[20] advocates to treat initial episodes and first recurrences with metronidazole in non-severe cases and vancomycin in severe cases or second or subsequent recurrences. When oral therapy is not possible, the guidelines recommend using a combination of intravenous metronidazole plus intracolonic vancomycin (500 mg, every 4-12 hours) and/or vancomycin administered by a nasogastric tube (500 mg, 4 times per day) (C-III). In second and later recurrences, vancomycin alone (oral therapy possible) (B-II) or in combination with metronidazole (oral therapy is not possible) (C-III) is the recommendation of the European guidelines.

In a prospective, randomized study reported by Zar et al.[101] the authors compared the efficacy of metronidazole and vancomycin stratifying patients according to disease severity. Among the patients with mild CDI, treatment with metronidazole or vancomycin resulted in clinical cure in 90% and 98% of the patients but among the patients with severe CDI, treatment with metronidazole or vancomycin resulted in clinical cure in 76% and 97% of the patients, respectively (P=.02). Clinical symptoms recurred in 15% of the patients treated with metronidazole and 14% of those treated with vancomycin.

Regarding other potential drugs, bacitracin was used in the treatment of CDI in the eighties but when compared to vancomycin persistence of toxins in the stools is higher in patients treated with bacitracin. Nevertheless, the rate of recurrence in patients treated with bacitracin was not higher than that in patients on vancomycin[160-162].

Teicoplanin is an alternative to vancomycin though with no clear benefit and with the disadvantage of not being available at present in all countries[99,163,164]. Fusidic acid is associated with more recurrences and it is worse tolerated by patients when compared to vancomycin[99] and shows similar results when compared to metronidazole[165].

Nitazoxanide is an antihelminthic and antiprotozoal agent with activity against a broad range of parasites that also shows "in vitro" activity against *C. difficile*[166-169]. After its oral administration it reaches high concentrations in the lumen of the colon. It has achieved cure rates of 75% in patients who failed metronidazole treatment but relapse occurs in one out of every three patients[100,170,171].

Rifaximin is a synthetic antibiotic derived from rifamycin in order to achieve low gastrointestinal absorption while retaining good antibacterial activity. It has a broad spectrum of antibacterial action including aerobic and anaerobic Gram-positive and Gram-negative microorganisms. It has not been finally approved for the treatment of CDI[172-175].

Fidaxomicin ia a new macrocyclic, RNA polymerase-inhibiting antibiotic for the treatment of *C. difficile* infections[176] that is now approved for the treatment of the severe or multi-recurrent episodes of CDI infections. This new drug has demonstrated non-inferiority in cure rates in patients with first and subsequent episodes of CDI and a lower incidence of recurrent episodes[177]. Occurrence of treatment-emergent adverse events did not differ between groups in the pivotal clinical trials[178,179]. Fidaxomicin (former OPT-80) has a narrow spectrum of activity against *C. difficile* isolates and causes less alteration to the bowel microbiota of *C. difficile*-infected patients than does vancomycin[87,180]. Fidaxomicin, has a significantly higher cost that the other available agents and this precludes the drug to be elected as the drug of choice in most episodes of CDI by many physicians.

The majority of the attendees accept widely established guidelines favoring the use of vancomycin in CDI episodes qualified as severe, in those that are the second or subsequent recurrences or in patients intolerant to metronidazole.

Fidaxomicin is non-inferior to vancomycin and associated with lower rates of recurrences but its order of election in many episodes of CDI is limited by its present price of acquisition.

QUESTION 16.- WHAT IS YOUR OPINION REGARDING THE RISK OF RECURRENCES AFTER TREATMENT OF CDI EPISODES WITH APROVED DRUGS?

1.- Patients treated with either vancomycin, metronidazole or fidaxomicin have similar risk of recurrences

18% in favor

2.- Patients treated with vancomycin recur less frequently than those treated with metronidazole

29% in favor

3.- Patients treated with metronidazole recur less frequently than those treated with vancomycin

4% in favor

4.- Fidaxomicin is associated with lower recurrence rates than vancomycin

49% in favor

Summary of the convener:

As already mentioned, one of the main complications of CDI is recurrence which is described in 8-50% of the cases[98, 161, 163, 181-191]. Recurrences are multiple in a significant percentage of the patients. Risk factors for recurrence are: advanced age, remaining on antimicrobial therapy after a first CDI episode, low serum albumin levels, a long hospital stay, admittance to an intensive care unit and a severe underlying disease[186,192-195]. It is essential to know whether there is a relapse with a reactivation of the disease by the previous clone or if it is due to the acquisition of a new clone. Different typing techniques have shown that 10-50% of recurrences are caused by a new clone ("re-infections")[196-199]. In a series of HIV patients with CDI a third part of recurrences were in fact re-infections[183].

The risk of recurrence is similar both in patients on metronidazole or on vancomycin[182,200]. Recurrence appears 3-21 days (mean 6 days) after completion of therapy. Most patients with a recurrence respond to another 10-day course of therapy with the same antimicrobial agent but 3-5% of patients may have up to 5 subsequent recurrences[201].

In a recent meta-analysis, Vardakas et al.[202] evaluated the frequency of treatment failure and recurrence of CDI following treatment with vancomycin or metronidazole in recently performed studies (last 10 years). In a total, of 39 articles (7005 patients) suitable for study, the reported recurrence of CDI occurred in 27.1% of patients following metronidazole treatment (18 studies) and 24.0% of patients following vancomycin treatment (8 studies). The reported outcomes depended on the study design (higher in prospective and retrospective cohort studies than in randomized controlled trials), geographic location of the study (higher in North America than in Europe and Asia), funding (higher in studies funded by non-profit organizations than pharmaceutical companies), mean age of the studied population (higher in older patients) and duration of follow-up (higher in studies with follow-up >1 month).

In patients with a poor response or with a second recurrence both the patient and his family requires a therapeutic alternative[203]. An option is to keep on using the same agent, though on a different dosage or with a longer duration. There are protocols which recommend a double dose of vancomycin for 10 days; others which prolong the administration of vancomycin for 3 weeks and others which follow a decreasing dosage scheme on vancomycin, 500 mg daily during the first week, 250 mg daily during the second week, 125 mg daily during the third week, followed by 125 mg every 3 days for 21 days[203]. There are no reports on prolonged or intermittent use of metronidazole.

As we already mentioned, Fidaxomicin, formerly known as PAR-101 or OPT-80 (Difimicin®; Dificlir®), is a new macrocyclic antibiotic approved in the USA and in Europe for the treatment of CDI. The drug showed in clinical trials non-inferiority than oral vancomycin in the rate of cures but at the same time a significant reduction in the rates of recurrences with an increase in the rate of sustained responses[204,205]. Plasma concentrations for fidaxomicin are very low and fecal levels are >1000 microg/g for fidaxomicin and >800 microg/g for the metabolite OP-1118. Fidaxomicin mean fecal levels were >5000 times the minimum inhibitory concentration for *C. difficile* of 0.25 mg/L.

A phase 3 clinical trial carried out in adults, randomly assigned patients to receive fidaxomicin (200 mg twice daily) or vancomycin (125 mg four times daily) orally for 10 days, enrolled 629 patients. The rates of clinical cure with fidaxomicin were no inferior to those with vancomycin in both the modified intention-to-treat analysis (88.2% with fidaxomicin and 85.8% with vancomycin) and the per-protocol analysis (92.1% and 89.8%, respectively). Significantly

fewer patients in the fidaxomicin group than in the vancomycin group had a recurrence of the infection, in both the modified intention-to-treat analysis (15.4% vs. 25.3%, P=0.005) and the per-protocol analysis (13.3% vs. 24.0%, P=0.004). The lower rate of recurrence was seen in patients with non-North American Pulsed Field type 1 strains[179]. A second randomized trial of a similar design carried out partially in Europe confirmed the former data showing that fidaxomicin could be an alternative treatment for CDI[178]. In phase 3 clinical trials, fidaxomicin was well tolerated, with a safety profile comparable with oral vancomycin. There were no differences in the incidence of death or serious adverse events between the 2 drugs[206].

Due to the higher cost of fidaxomicin than either vancomycin or metronidazole, the proper position of this new drug in the therapeutic armamentarium is still under debate. A cost-effectiveness analysis reported by Bartsch et al.[207] suggest that at present cost the drug is not cost-effective as a first line treatment for CDI.

Fidaxomicin is being promoted for severe CDI episodes and secondary episodes with high rates of expected recurrences.

Only half of the attendees were aware of the data showing that fidaxomicin was associated with significant fewer recurrences than vancomycin in randomized phase 3 clinical trials.

QUESTION 17.- WHAT IS YOUR OPINION REGARDING THE USE OF PROBIOTICS FOR THE TREATMENT OR PROPHYLAXIS OF CDI AT THE PRESENT TIME?

1.- You support the use of probiotics as coadyuvant agents in the treatment of CDI.

14% in favor

2.- You support the use of probiotics as agents for the prevention of CDI.

15% in favor

3.- In your opinion probiotics have not yet demonstrate utility neither in the treatment nor in the prevention of CDI

39% in favor

4.- Participant recognize not to have an opinion on this issue

32% in favor

Summary of the convener:

The IDSA/SHEA Guidelines state that "Administration of currently available probiotics is not recommended to prevent primary CDI, as there are limited data to support this approach and there is a potential risk of bloodstream infection (C-III)"[8].

In a recent meta-analysis[208], Videlock et al., however, suggested that probiotic administration reduces the incidence of antibiotic-associated diarrhoea (AAD) but this cannot be extrapolated to CDI episodes.

Published data analyzed in a systematic review by Avadhani et al.[209] in both AAD and CDI showed that administration of probiotics led to a statistically significant relative risk reduction of 44% for AAD and 71% for CDI.

In the case of *Saccharomyces boulardii*, of 31 randomized trials (encompassing 5029 study patients), this probiotic was found efficacious and safe also in the prevention of AAD but there is not evidence of efficacy in the prevention of CDI[210]. In two randomized studies in patients with CDI recurrences, intestinal recolonization with *Saccharomyces boulardii* has been evaluated[200,211]. In one of them *S. boulardii* was administered for 4 weeks after treatment with vancomycin (2 g daily) for 10 days. Recurrences decreased but only when vancomycin was administered at such a high dose[211]. The efficacy of *S. boulardii* to decrease recurrences has been shown in several studies[200,212]. The use of *S. boulardii* has been associated with the belief that it has no risks and, since it is not expensive, it has been widely prescribed. Our group has published a study on one of its complications, fungemia by *Saccharomyces*, that may present as small epidemic outbreaks particularly in ICU patients with intravascular catheters[213]. Furthermore, the PROPATRIA study, related to the use of probiotics in patients with severe pancreatitis showed a worse evolution in patients on probiotics, rising concerns on the future design of the studies with probiotics[214].

Some authors have reported small series of patients[215,216] in which the administration of *Lactobacillus rhamnosus* or *Lactobacillus plantarum* stopped recurrences, yet in two prospective and comparative studies with this probiotic and placebo there was no decrease in recurrences[217,218].

The higher proportion of the attendees believes that probiotics have not proved efficacy neither in prevention nor in coadyuvant treatment of CDI in agreement with the present guidelines. However, the opinion is broadly distributed and the majority of the attendees either recognized not to be sufficiently acquainted with the issue or believe that probiotics may be useful in prevention or treatment of CDI.

QUESTION 18.- WHAT IS YOUR OPINION REGARDING THE USE OF IMMUNOTHERAPY FOR THE TREATMENT OF CDI?

1.- You believe than monoclonal antibodies have proven useful but immunoglobulins and vaccines have not yet demonstrated efficacy.

9% in favor

2.- You believe than immunotherapy is promising but has not yet demonstrated efficacy in any of the different approaches.

32% in favor

3.- You believe that the use of IV immunoglobulins is highly recommended in patients with multiple recurrences

10% in favor

4.- Participant recognize not to have an strong opinion on this issue

49% in favor

Summary of the convener:

Humoral immunity is essential in resistance to both CDI infection and recurrence. Antibodies against toxins A and B of *C. difficile* are readily detectable in both the general population and in patients with CDI[219-221]. The level of anti-toxin antibody response, against toxin A, correlates with resistance to symptomatic infection and high levels protect against recurrence[158,192,222]. These are the rational basis for using intravenous immune globulins (IVIG) and the development of antitoxin monoclonal antibodies as adjunctive therapy in severe, refractory or recurrent CDI[223-225].

Intravenous immunoglobulins have been used in a few patients with severe disease or multiple recurrences but there is not any prospective and comparative study to establish their definite role in the treatment of this disease[223-225]. O'Horo and Safdar[226] undertook a systematic review to examine the published literature pertaining to the use of immunoglobulin for *C. difficile* infection. Four retrospective studies and five case reports were identified. Although the overall impression is that using IVIG in recurrent severe disease may be helpful, the small sample sizes and lack of control groups in three of the four studies do not allow recommendations to be made regarding the use of IVIG in CID. The doses elected are usually the administration of 200-500 mg/kg.

Muñoz et al.[227] in a group of patients with solid-organ transplantation (SOT) and a high incidence of CDI demonstrated the remarkable decrease in the incidence in the incidence of CDI after hypogammaglobulinemia (HGG) was systematically corrected in those patients. Incidence was 20.6% in the pretreatment period and 6.4% in the post-intervention period. Severe HGG was found to be the only independent risk factor for CDI in heart transplant patients. The feasibility of oral immune whey protein concentrate (40%; immune WPC-40) to aid the prevention of relapse of *C. difficile* diarrhea has also been evaluated. Immune WPC-40 was made from milk after immunization of Holstein-Frisian cows with *C. difficile*-inactivated toxins and killed whole-cell *C. difficile*. Immune WPC-40 contained a high concentration of specific IgA antibodies and was effective in neutralizing the cytotoxic effect of *C. difficile* toxins in cell assays in vitro[228-230]. WPC-40 was administered to 11 patients who failed treatment or had a history of relapsing *C. difficile* after a 14-day treatment course. All patients were cured and none of them suffered another episode of diarrhea.

The potential use of monoclonal antibodies has also been evaluated[231]. Lowy et al. reported in 2010[232] a randomized, double-blind, placebo-controlled study of two neutralizing, fully human monoclonal antibodies against *C. difficile* toxins A and B. The antibodies were administered together as a single infusion, each at a dose of 10 mg per kilogram of body weight, in patients with symptomatic *C. difficile* infection who were receiving either metronidazole or vancomycin. Among the 200 patients who were enrolled the recurrence rate of CDI was lower among patients treated with monoclonal antibodies (7% vs. 25%; 95% confidence interval, 7 to 29; P<0.001), including cases with the BI/NAP1/027 strain. A new multicentric study with monoclonal antibodies is ongoing.

The search for CDI vaccines in the last 2 decades[233] included formalin-inactivated *C. difficile* cultures[234] and toxoid vaccines[235,236] that protected hamsters. In humans, the use of vaccines has been limited to a few isolated patients with very promising results[237]. Currently, a parenteral toxoid vaccine directed against toxin A and toxin B is undergoing clinical trials[238,239] and it seems to be safe and associated with high serum antibody responses. Another vaccine under phase I trials is a subunit recombinant protein vaccine consisting of two truncated toxins A and B from *C. difficile* I phase I safety and immunogenicity testing in volunteer subjects (Intercell, 2011).

Sanofi Pasteur's *C. difficile* candidate vaccine is being developed for the prevention of primary disease. The target population is adults at risk of CDI, those with planned hospitalization, long-term care/nursing home residents, and adults with co-morbidities requiring frequent/prolonged antibiotic use[240].

Overall the majority of the attendees recognized their limited knowledge or skepticism in the present role of immunotherapy in the coadyuvant treatment of CDI.

Only low proportions of the participans accept a role for the treatment with IV immunoglobulins in patients with severe, multiple recurrent cases or value as highly promising the use of monoclonal antibodies or vaccines in a near future.

QUESTION 19.- WHAT ARE YOUR EXPECTATIONS OF FECAL MICROBIOTA TRANSPLANTATION (FMT) AS A FUTURE THERAPY FOR MULTIPLY RELAPSING EPISODES OF CDI?

1.- It may be very effective and feasible in episodes refractory to conventional treatment

5% in favor

2.- It may be very effective but unfeasible in most institutions in episodes refractory to conventional treatment.

7% in favor

3.- Fecal transplantation is not effective nor feasible at the present time

30% in favor

4.- Participant recognize not to have an strong opinion on this issue

58% in favor

Summary of the convener:

Local bacteriotherapy, stool transplantation or fecal microbiota transplantation (FMT) is the name for the lavage of the lumen of the colon and the administration of enemas prepared with fresh feces from healthy volunteers[241-244]. Reports are almost always of isolated cases or short series[245-249].

In a recent series of 26 patients, Kelly et al.[250] provide a simple treatment protocol and review their results. Twenty-six patients with relapsing CDI underwent FMT over a 28-month period by colonoscopy. The mean duration of CDI was 12.6

months (range, 4 to 84 mo) before FMT. Twenty-four patients have remained free of significant diarrhea or CDI. The authors qualify their experience as simple, safe, and 92% effective in preventing further CDI relapse.

A group from Minnesota has recently report on the results of FMT after simplification of the procedure[251], overcoming barriers for feasibility. They report clinical experience with 43 consecutive patients. This group moved from patient-identified individual donors to standard volunteer donors. They also shifted preparation from the endoscopy suite to a standardized process in the laboratory, and ultimately to banking frozen processed fecal material that is ready to use when needed. This was performed without loss of apparent efficacy in clearing recurrent CDI. Approximately 30% of their patients had underlying inflammatory bowel disease, and FMT was equally effective in this group.

Several systematic reviews[252-254] failed to found controlled trials but concur in recognize a high success rate in most cases and small series reported. In 317 patients treated across 27 case series and reports Gough et al.[254], FMT was highly effective, showing disease resolution in 92% of cases. Effectiveness varied by route of instillation (better if directly intracolonic), relationship to stool donor (better form related donors), volume of FMT given (better with higher volumes), and treatment before infusion. Death and adverse events were uncommon.

Postigo et al.[255] compared the best route of administration with FMT. They presented a pooled analysis of the reported cases of CDI treated with FMT via colonoscopy or nasogastric tube (NGT). They collected a total of 182 patients from 12 published studies; 148 patients received FMT via colonoscopy (colonoscopy group) and 34 patients received by NGT (NGT group). There were differences regarding pre-treatment for CDI and other variables but with those limitations the treatment efficacy did not differ significantly; 93.2 % (138/148) success for the colonoscopy group as compared to 85.3 % (29/34) success for the NGT group.

Van Nood et al.[256] reported recently the results of a randomized clinical trial comparing three therapies: an initial vancomycin regimen followed by bowel lavage and subsequent infusion of donor feces; a standard vancomycin regimen or a standard vancomycin regimen with bowel lavage. Of 16 patients in the infusion group, 13 (81%) had resolution of *C. difficile*-associated diarrhea after the first infusion. The 3 remaining patients responded to a second infusion. Resolution of *C. difficile* infection occurred in 4 of 13 patients (31%) receiving vancomycin alone and in 3 of 13 patients (23%) receiving vancomycin with bowel lavage. The infusion of donor feces was significantly more effective for the treatment of recurrent *C. difficile* infection than the use of vancomycin.

Fecal microbiota therapy (fecal transplantation) was only considered effective and feasible for a minority of the participants in the meeting. However, data accumulated before and after the meeting showed it to be one of the more effective and feasible techniques to control recurrent CDI.

QUESTION 20.- REGARDING THE MORE IMPORTANT MEASURES FOR PREVENTION OF CDI IN INSTITUTIONS?

1.- You subscribe the recommendations of the SHEA

98% in favor

2.- You believe that particular recommendations should be issued for Spain

0%

3.- You don't have a particularly strong opinion on this issue

2% in favor

Summary of the convener:

Guidelines and recommendations for preventing CDI have evolved during the last decades as new information and knowledge on the epidemiology of health care–associated CDI was being acquired. Starting in 1994, many reports and updates have been produced[113,257-259]. Most recent recommendations provided in 2008 by the Association for Professionals in Infection Control and Epidemiology (APIC) have been published as an executive summary in 2011[7]. The main recommendations include: CDI surveillance, contact precautions, adherence to hand hygiene, antimicrobial stewardship, education of patients and staff and administrative support.

Surveillance for CDI requires clear, well stated definitions and a clear separation between community-associated and health-care associated episodes of CDI, as well as between community-onset and health-care facility onset cases. In the case of hospitals, surveillance should include, at least health care facility onset and health care–associated cases of CDI. Episodes that develop within 4 weeks of discharge from a health care facility should be considered as nosocomially-acquired.

The SHEA Guidelines[8] include measures for healthcare workers, patients, and visitors requiring the use gloves por "measures for healthcare workers, patients, and visitors requiring the use of gloves (A-I) and gowns (B-III) to entry a room of a patient with CDI. They emphasize compliance with the practice of hand hygiene (A-II), including the use of soap (or antimicrobial soap) and water and to accommodate patients with CDI in a private room with contact precautions (B-III). If single rooms are not available, cohort patients, providing a dedicated commode for each patient (C-III). Those measures should be maintained, at least for the duration of diarrhea (C-III).

Routine identification of asymptomatic carriers for infection control purposes is not recommended (A-III) as treatment of such identified patients is not effective (B-I).

Regarding environmental cleaning and disinfection, it is recommended to identify and remove environmental sources of *C. difficile*. An example may be to replace electronic rectal thermometers with disposables and use chlorine-containing cleaning agents or other sporicidal agents to address environmental contamination in areas associated with increased rates of CDI (B-II).

Routine environmental screening for *C. difficile* is not recommended (C-III).

Regarding restrictions of antimicrobial use, the guide recommends to minimize the frequency and duration of antimicrobial therapy and the number of antimicrobial agents prescribed, to reduce CDI risk (A-II) and the implementation of an antimicrobial stewardship program.

The participants were clearly in favor o implementing recommendations already issue by international agencies or countries with issued documents.

Appendix I

The members of the Study Group for *Clostridium difficile* Infection of the Spanish Society for Chemotherapy are as follows.

F. E. Calvo Muro and M. J. Lezaun Burgui, Hospital Unificado de Álava-Sede Txagorritxu, Vitoria-Gasteiz, Álava; M. Martínez Serrano and M. D. Crespo Sánchez, Complejo Universitario Hospitalario de Albacete, Albacete; M. Ruiz García, L. Álvarez Paredes and G. Royo García, Hospital General Universitario de Elche, Elche, Alicante; M. Elía López and J. M. Andrés, Hospital General Universitario de Elda, Elda, Alicante; N. Gonzalo Jiménez, Hospital del Servicio Valenciano de la Vega Baja, Orihuela, Alicante; W. Sánchez-Yebra Romera, M. Morales Torres and A. Sicilia Enríquez de Salamanca, Complejo Hospitalario Torrecárdenas, Almería; B. Iglesias Rodríguez, G. Sierra, P. de la Iglesia, and H. Villar, Hospital San Agustín, Avilés, Asturias; L. Barreiro Hurlé, Hospital Carmen y Severo Ochoa, Cangas del Narcea, Asturias; M. D. Miguel Martínez and L. Otero Guerra, Hospital de Cabueñes, Gijón, Asturias; P. Suárez Leiva and M. J. Santos Rionda, Hospital Universitario Central de Asturias, Oviedo, Asturias; F. Vázquez Valdés, Hospital Monte Naranco, Oviedo, Asturias; M. I. Blanco Costa, Hospital Comarcal de Jarrio-Coaña, Jarrio, Asturias; R. Sánchez Arroyo and A. Gómez del Campo, Hospital Nuestra Señora de Sonsoles (Complejo Hospitalario de Ávila), Ávila; M. Fajardo Olivares, Hospital Universitario Infanta Cristina, Badajoz; M. Álvarez and J. Vila Estape, Hospital Clinic de Barcelona, Barcelona; I. Sanfeliú Sala and D. Fontanals Aymerich, Corporació Sanitària Parc Taulí, Sabadell, Barcelona; J. López Madueño and M. Horta, Fundación Althaia-Hospital San Joan de Deu, Manresa, Barcelona; A. Gené Giralt and C. Latorre Otín, Hospital Sant Joan de Déu, Esplugues de Llobregat, Barcelona; J. Niubó Bosh and M. A. Domínguez Luzón, Ciutat Sanitària i Universitaria de Bellvitge-Hospital Princeps d´Espanya, L´Hospitalet de Llobregat, Barcelona; V. Plasencia Miguel and M. Salvadó Costa, Laboratorio de Referencia de Cataluña, El Prat de Llobregat, Barcelona; M. Micó García, B. Mirelis Otero and P. Coll Figa, Hospital de la Santa Creu i Sant Pau, Barcelona; C. San José Alemany and A. Vilamala Bastarras, Hospital Comarcal de L´Alt Penedès, Vilafranca del Penedès, Barcelona; A. Vilamala Bastarras and J. Prat Quinzaños, Consorci Hospitalari de Vic, Vic, Barcelona; F. Álvarez Lerma, Hospital del Mar, Barcelona; J. Pérez Jove and M. Xercavins Valls, Hospital Mútua de Terrassa, Terrasa, Barcelona; M. Simó Sanahuja, Catlab Centre Analitiques Terrassa Aie, Viladecavalls, Barcelona; V. Rodríguez Garrido, Hospital Vall d´Hebrón, Barcelona; J. Sánchez Lafuente, J. L. Díaz de Tuesta del Arco and R. Cisterna Cáncer, Hospital de Basurto, Bilbao, Bizkaia; L. Elorduy Otazúa, P. Irargui and I. Marzana, Hospital San Eloy, Barakaldo, Bizkaia; A. P. Martínez de la Fuente and M. J. López de Goikoetxea, Hospital de Galdakao-Usansolo, Galdakao, Bizkaia; I. Perales Palacios, M. Montejo Baranda and M. Sota Busselo, Hospital Universitario de Cruces, Barakaldo, Bizkaia; G. Megías Lobón and E. Ojeda Fernández, Hospital Universitario de Burgos, Burgos; C. Gimeno Crespo, Hospital Comarcal Santiago Apostol, Miranda de Ebro, Burgos; R. Íñiguez Ovando, M. R. Sánchez Benito and P. Teno Sánchez, Complejo Hospitalario de Cáceres-Hospital San Pedro de Alcántara, Cáceres; F. Galán Sánchez and M. A. Rodríguez Iglesias, Hospital Universitario Puerta del Mar, Cádiz; A. Sánchez Porto, Hospital del S.A.S. de La Línea de la Concepción, La Línea de la Concepción, Cádiz; M. D. López Prieto, Hospital del S.A.S. de Jerez de la Frontera, Jerez de la Frontera, Cádiz; M. P. Roiz Mesones and L. Martínez Martínez, Hospital Universitario Marqués de Valdecilla, Santander, Cantabria; S. Sabater Vidal and R. Moreno Muñoz, Hospital General de Castellón, Castellón; A. López Llopis and S. Pesudo Calatayud, Hospital de La Plana, Villareal, Castellón; J. López Barba, Hospital Universitario de Ceuta, Ceuta; I. Barba Ferreras, J. Martínez Alarcón, M. Torres Narbona and M. D. Romero Aguilera, Hospital General Universitario de Ciudad Real, Ciudad Real; E. Manrique González and R. Carranza González, Hospital General La Mancha Centro, Alcázar de San Juan, Ciudad Real; M. Causse del Río and M. Casal Román, Hospital Universitario Reina Sofía, Córdoba; M. J. Rodríguez Escudero, Hospital General Virgen de la Luz, Cuenca; A. Peña Monje and F. García García, Hospital Clínico Universitario San Cecilio, Granada; J. Rodríguez Granger, C. Gómez Camarasa and J. M. Navarro Mari, Hospital General Universitario Virgen de las Nieves, Granada; C. Gimeno Fernández and J. Bisquert Santiago, Hospital General Universitario de Guadalajara, Guadalajara; M. Gomáriz Díaz and E. Pérez Trallero, San Sebastián, Gipuzkoa; J. A. Jiménez Alfaro, Policlínica de San Sebastián, San Sebastián, Gipuzkoa; J. M. Saavedra Martín and M. de la Iglesia Salgado, Hospital General Juan Ramón Jiménez, Huelva; A. Milagro Beamonte and M. Ferrero Cáncer, Hospital General San Jorge, Huesca; A. Mena Ribas and J. L. Pérez Sáenz, Hospital Son Espases, Palma de Mallorca, Islas Baleares; A. Hurtado Fernández and J. Sánchez Gómez, Hospital Can Misses, Ibiza, Islas Baleares; P. Ordóñez Barrosa and J. A. Agulla Budiño, Hospital Arquitecto Marcide-Novoa Santos, El Ferrol, La Coruña; A. Álvarez Alba and P. Castro Romero, Hospital General Juan Cardona, El Ferrol, La Coruña; M. D. Rodríguez Mayo and G. Bou Arévalo, Complejo Hospitalario Universitario A Coruña, La Coruña; J. Llovo Taboada and J. L. Barrios Andrés, Hospital Clínico de Santiago de Compostela, Santiago de Compostela, La Coruña; M. M. Ojeda Vargas and A. M. Martín Sánchez, Hospital Universitario Insular de Gran Canaria, Las Palmas de Gran Canaria, Las Palmas; R. I. Elcuaz Romano, Hospital Universitario de Gran Canaria Doctor Negrín, Las Palmas de Gran Canaria, Las Palmas; R. López Medrano and C. Fuster Foz, Hospital del Bierzo, Ponferrada, León; T. Marrodán Giordia, T. Nebreda Mayoral and F. Cachón García, Hospital de León, León;

J. Aramburu Arnuelos and B. Pérez Remón, Hospital Universitari Arnau de Vilanova, Lleida; E. Undabeitia, Complejo Hospitalario San Millán-San Pedro, Logroño, La Rioja; F. García-Garrote, J. M. Pita Carretero and M. P. Alonso García, Hospital Universitario Lucus Augusti (HULA), Lugo; M. V. Portús Marco and R. Cortés Bohórquez, Hospital Central de la Cruz Roja Española San José y Santa Adela, Madrid; A. García Blanco, J. Martiáñez Rodríguez and M. López-Brea, Hospital Universitario de la Princesa, Madrid; L. Alcalá, E. Reigadas, M. Marín, A. Martín, P. Catalán, P. Muñoz, T. Peláez and E. Bouza, Hospital General Universitario Gregorio Marañón, Madrid; M. A. Orellana Miguel, and J. J. Rodríguez Otero, Hospital Universitario 12 de Octubre, Madrid; P. Trevisi, C. Toro Rueda and M. Baquero Mochales, Hospital Universitario Carlos III, Madrid; J. Romero Vivas, M. I. Morosini and R. Cantón Moreno, Hospital Ramón y Cajal, Madrid; I. Sánchez Romero and R. Martínez Ruiz, Hospital Puerta de Hierro, Madrid; P. Merino Amador, F. J. Candel González, E. Baos Muñoz and J. J. Picazo, Hospital Clínico San Carlos-Complejo Hospitalario, Madrid; S. García Bujalance, M. N. Seara Sanz and A. Gutiérrez Altés, Hospital de la Paz, Madrid; J. V. Saz, Hospital Príncipe de Asturias, Alcalá de Henares, Madrid; C. García Esteban, D. Molina Arana, A. Martín Pozo and J. I. Alós, Hospital Universitario de Getafe, Getafe, Madrid; M. C. Gómez Criado, F. J. Merino Fernández and I. Wilhelmi de Cal, Hospital Severo Ochoa, Leganés, Madrid; A. Delgado-Iribarren, and J. F. Valverde, Fundación Hospital Alcorcón, Alcorcón, Madrid; M. T. Pérez Pomata and J. L. Gómez Garcés, Hospital General de Móstoles, Móstoles, Madrid; M. J. González Abad and M. Alonso Sanz, Hospital del Niño Jesús, Madrid; M. A. Izquierdo Ruiz de la Peña, M. F. Ramos Ferriol, F. Hervás Maldonado and J. R. Maestre Vera, Hospital Central de la Defensa Gómez Ulla, Madrid, Madrid; M. C. Mediavilla Gradolph, I. Toro Peinado and B. Palop Borrás, Hospital Regional Universitario Carlos Haya, Málaga; G. Sena Corrales and E. Clavijo Frutos, Hospital Clínico Universitario Virgen de la Victoria, Málaga; F. Díaz Monllor and J. C. Navarro Madrid, Hospital Comarcal de Melilla, Melilla; C. Guerrero Gómez and R. Blázquez Garrido, Hospital José María Morales Meseguer, Murcia; A. Aguinaga, J. R. Azanza and J. Leiva León, Clínica Universitaria de Navarra, Pamplona, Navarra; X. Beristain Rementería, C. Martín Salas and C. Ezpeleta, Complejo Hospitalario de Navarra, Pamplona, Navarra; M. A. Gómez Rial, A. Cid Lama and G. Estebán Meruéndano, Hospital Santa María Nai- Complejo Hospitalario de Ourense, Ourense; M. García Bravo and M. A. García Castro, Hospital General Río Carrión, Palencia; S. Cortizo Vidal, J. Martínez Conde and M. García Campello, Complejo Hospitalario, Pontevedra; L. Martínez Lamas and M. T. González del Blanco, Complejo Hospitalario Xeral-Cies, Vigo, Pontevedra; I. Rodríguez Conde, Policlínico Vigo S. A.-POVISA, Vigo, Pontevedra; M. de Frutos Serna, M. I. García García and J. E. García Sánchez, Hospital Universitario de Salamanca-Hospital Clínico y Hospital Virgen de la Vega, Salamanca; A. Madueño Alonso and M. Lecuona, Hospital Universitario de Canarias, La Laguna, Santa Cruz de Tenerife; J. Alcoba Flores, D. García Martínez de Artola and E. Viejo Rivero, Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife; S. Hernando Real, P. Carrero González and S. García Carbajosa, Complejo Hospitalario de Segovia (SACYL), Segovia; M. Delgado Valverde, E. Muñoz Nuño and A. Pascual Hernández, Hospital Universitario Virgen de la Macarena, Sevilla; M. C. Lozano Domínguez, J. Garnacho and J. Aznar Martín, Hospital Universitario Virgen del Rocío, Sevilla; A. I. Aller García E. Martín Mazuelos, Hospital Universitario Nuestra Señora de Valme, Dos Hermanas, Sevilla; C. Aldea Masilla, S. García de Cruz and A. Campos Bueno, Complejo Hospitalario de Soria (Santa Bárbara-Virgen del Mirón), Soria; J. Tapiol Oliva, G. García Pardo and A. Vilanova Navarro, Hospital de Tarragona Joan XXIII, Tarragona; M. P. Chocarro Escanero and J. Horno, Hospital General de Teruel Obispo Polanco, Teruel; J. A. Rodríguez, P. Zamarrón Fuertes and S. Brea Zubigaray, Hospital Virgen de la Salud, Toledo; J. Colomina Rodríguez, Hospital de la Ribera, Alzira, Valencia; N. Orta Mira and J. Díaz Fernández, Hospital Francesc Borja, Gandía, Valencia; J. M. García Aguayo, Hospital General de Requena, Requena, Valencia; S. Giner Almaraz, Hospital Doctor Moliner, Serra, Valencia; T. García Lozano and J. Maiquez Richart, Fundación Instituto Valenciano de Oncología (IVO), Valencia; A. Valentín Martín and C. Segarra Martínez, Hospital Arnau de Vilanova, Valencia; J. Buesa and J. García de Lomas, Hospital Clínico de Valencia, Valencia; D. González Granda and E. Ochoa Ávila, Hospital Lluis Alcanyis, Xátiva, Valencia; L. Navarro Pérez, M. Jiménez Mayordomo and J. L. Barberá Comes, Hospital de Manises, Manises, Valencia; A. Molina de Diego, J. L. Poveda Andrés and J. L. López Hontangas, Hospital Universitario y Politécnico de La Fé, Valencia; C. Gobernado, M. A. Bratos Pérez and R. Órtiz de Lejarazu, Hospital Clínico Universitario de Valladolid, Valladolid; M. Domínguez-Gil González Eirós, A. Gómez Nieto and J. Eirós Bouza, Hospital del Río Hortega, Valladolid; M. I. Antolín Ayala and M. C. García Loygorri, Hospital Medina del Campo, Medina del Campo, Valladolid; L. López-Urrutia Lorente and M. F. Brezmes Valdivieso, Hospital Virgen de la Concha Área 11, Zamora; C. Miñana Amada, Hospital Real y Provincial Nuestra Señora de Gracia, Zaragoza; C. Seral, F. J. Castillo García and M. C. Rubio Calvo, Hospital Clínico Universitario Lozano Blesa, Zaragoza; and C. Marne and M. J. Revillo Pinilla, Hospital Universitario Miguel Servet, Zaragoza.

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