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XII Updating Course of Antimicrobials and Infectious Diseases 2022

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Introduction

Francisco Javier Candel

Introduction to XII Updating Course of Antimicrobials and Infectious Diseases

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Last February, the XII Updating Course of Antimicrobials and Infectious Diseases edition was held at Hospital Clínico San Carlos in Madrid. It was a scientific activity accredited by the Community of Madrid Commission for Continuing Education of Health Professions at the Community of Madrid (file number 07-AFOC-00085.0/2022, 1,3 Credits) and endorsed by Spanish Society of Clinical Microbiology and Infectious Diseases (SEIMC), Spanish Society of Chemotherapy (SEQ) and Madrid Society of Clinical Microbiology (SMMC). This year, the course was online edited and reached peaks over 700 connections with continuous mean over 500. The audience consisted of multidisciplinary professionals of all specialties related to infection, the teachers made an update of the most relevant aspects on bacteriology, mycology, and virology.

Current journal issue includes summaries of the lectures given in the presential course. It also includes the questionnaire with the evaluations made by the students and a sheet of correct answers to being able to contrast the results. The supplement is divided into five headings. First one, entitled "Approach to management of SARS-CoV-2 infection", included topics such as the analysis of most vulnerable groups to infection, as well as the antiviral therapeutic management, the current use of monoclonal antibodies and the integrated management of hyperinflammatory syndrome. Second round table, entitled "Approach to Infection models" dealt with topics such as Integral approach to infection in the diabetic foot, current antimicrobial treatment of nosocomial pneumonia or pharmacodynamic optimization of antimicrobial treatment in sepsis. Also under this heading, topics such as the global strategy for the treatment of HIV infection in 2022 and the transmission of bacterial resistance from the animal and plant world (the one health concept) were also reviewed. Third section entitled "Update on antimicrobial pharmacotherapy against multidrug-resistant Gram-negative bacilli", included controversies in the treatment of infections caused by ESBL-producing enterobacteria in clinical practice and a current approach in the treatment of infections caused by carbapenemase-producing enterobacteria or multidrug-resistant non-fermenting gram-negative bacilli (*Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*). Fourth heading entitled "Approach to infection in immunosuppressed patients" analyzed SARS-CoV-2 infection in donation and transplant, persistent and recurrent infections in primary immunodeficiencies (congenital), the differential diagnosis of pulmonary infiltrates in oncology patients undergoing immunotherapy and current management of cytomegalovirus infection in oncology patients. Last round table, entitled "Current strategies for infectious diseases management", included topics like rapid microbiological techniques for therapeutic optimization (Diagnosis stewardship), clinical applicability of new EUCAST 2019 breakpoint susceptibility classification, new bacteremia prediction models and implications, practical approach to latent tuberculosis infection, real applicability of therapeutic strategies against *Clostridioides difficile* or the duration of antimicrobial treatment were analyzed.

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Pablo Barreiro
Jesús San Román

Vulnerability to SARS-CoV-2 infection and disease: ripping the curl after the storm

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ABSTRACT

SARS-CoV-2 infection now seems to have entered the announced endemic phase. The population's immunity is increasingly more robust, thanks to successive vaccination and booster campaigns, and the almost inevitable exposure and re-exposure to the virus itself, which has truly served as a natural immunizing mechanism. On the other hand, the genetic drift of the virus is leading it to become another catarrhal agent, as are the other endemic human coronaviruses. However, it should not be lost sight of that there are still segments of the population with susceptibility to severe COVID, who will be candidates to continue receiving vaccine boosters or antiviral drugs in the initial stages of infection.

Keywords: COVID, SARS-CoV-2, vulnerability, comorbidity, frailty

INTRODUCTION

The clinical manifestations of the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) infection, named as coronavirus infectious disease (COVID), occur in a wide range, that moves from asymptomatic infection to multi-organ failure and death. The probability of having severe disease has been changing throughout the pandemic, mainly for three reasons: the protective effect of vaccination [1], the selection of less lethal SARS-CoV-2 variants – only demonstrated for lineage B.1.1.529 (Omicron, and subsequent subvariants)— [2] and due to the lower severity of reinfections, particularly in vaccinated individuals. According to descriptive epidemiological studies carried out very early in the pandemic [3,4], symptomatic infection was approximately limited to: i) catarrhal symptoms, including mild pulmonary involvement, in 80% of cases; ii) severe pneumonia with hypoxia in 15% of cases; iii) respiratory distress, shock or multiple organ failure in 5% of cases; and iv) death in 2% of cases. At the end of 2021, among the vaccinated population, the probability of serious illness is estimated at 0.015% and death at 0.003% [5].

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GENETIC FACTORS

Several genetic variants and epigenetic factors have been associated with severe COVID. SARS-CoV-2 uses several receptors (ACE2, TMPRSS2) for entry to cytoplasm of epithelial cells; certain mutations at ACE2 may increase risk of death, while changes at TMPRSS2 reduce susceptibility to infection. Once pathogen-derived molecules are detected by immune cells, interferons (IFN) and other proinflammatory cytokines are released; mutations at the level of these mediators are related with more intense inflammatory response to infection, and therefore with greater incidence of respiratory distress and thrombotic events. At the level of HLA receptors, 3p21.31 and 9q34.2 loci are significantly associated with COVID severity. Epigenetic mechanisms including methylation, histon acethylation, and X chromosome inactivation (XCI) also affect COVID outcomes by regulating IFN signaling and ACE2 expression, and immunity-related genes that particularly escape from XCI [6]

DEMOGRAPHY

Age is the main factor that determines the risk of severe COVID. Infection by SARS-CoV-2 is generally mild or asymptomatic in children, adolescents and young people [7,8]. According to various studies in adults, the age segments of under 50 years of age, from 50 to 64 years of age, from 65 to 74 years of age, from 75 to 84 years of age and over 85 years of age can be established to estimate increasing risks of admission for COVID [9]. Mortality is concentrated in patients older than 65 years [10], and increases especially in those older than 80 years [11]. In vaccinated people, a history of SARS-CoV-2 in-

Table 1 Factors associated with severe COVID

HIGH RISK
Solid organ transplants
Cystic fibrosis, severe asthma, severe COPD
Combined immunodeficiency, sickle cell disease
Pharmacological immunosuppression
Splenectomy
Down's Syndrome
Chronic kidney failure (stage 5)
Pregnancy (heart disease or diabetes)
Chemotherapy or immunotherapy
Radiotherapy for lung cancer
Leukemia, lymphoma, or myeloma
Bone marrow or stem cell transplants
INTERMEDIATE RISK
70 years or older
Under 70 years:
Asthma, COPD, emphysema
Heart disease
Chronic kidney disease (stage 1 to 4)
Chronic liver disease
Neurological disease:
Parkinson's, Amyotrophic Lateral Sclerosis, Multiple Sclerosis, cerebral palsy
Diabetes
Immunosuppression
Overweight (BMI >40)
Pregnancy

fection has been identified as one of the main protective factors against severe COVID [12]. Male subjects have also worse prognosis than women, in part due to men concentrating other comorbidities.

COMORBIDITY

Overweight and increasing degrees of obesity augments the probability of hospitalization for COVID; although the body mass index seems to play a role of its own, other factors associated with obesity such as glucose intolerance, hyperlipidemia or sedentarism also have a contribution [13]. Type 1 (14) and 2 (15) diabetes are associated with greater severity from COVID, and predispose to hospital and ICU admission, and higher mortality.

There is evidence that COVID is more severe in patients with other underlying illnesses. Within the pulmonary processes, a worse prognosis can be expected in the case of chronic obstructive pulmonary disease, severe asthma, cystic fibrosis in adults, interstitial lung disease, history of pulmonary hypertension, pulmonary thromboembolism or tuberculosis. The presence of respiratory failure at the time of diagnosis of COVID is a data of very poor prognosis. In general, the negative effect of these pathologies is more evident if the risk of death is considered [16,17]. Cardiovascular alterations such as arterial hypertension, ischemic heart disease, cardiomyopathies, heart failure or cerebrovascular disease have also been related to severe COVID [18].

Chronic kidney or liver disease, due to the organic dysfunction they entail and regardless of their cause, and certain degenerative neurological diseases (e.g. Down syndrome, dementia) or mental disorders contribute to severe COVID [19]. The degree of frailty and the need for care that it entails, to which age and various chronic debilitating diseases contribute, may be the common reason for this worse prognosis [20].

Immunosuppression is a recognized risk factor for severe COVID-19 or death [21], with many clinical situations contributing to this situation (primary or acquired immunodeficiencies, immunosuppressive treatments for inflammatory diseases or in transplant recipients, cancer treatment, etc.). It is necessary to analyze the specific effect of each disease, of each treatment and the clinical situation of each patient, since, for example, HIV infection under effective antiretroviral treatment does not complicate the evolution of COVID [22]. Oncological disease [23], particularly if it is of hematological origin [24], is one of the most determining factors of severe COVID. Table 1 summarizes the main factors associated with severe COVID.

LIMITATIONS

It is not easy to establish the individual weight of many of these factors on the severity of COVID. In the first place, it would be necessary to establish if each factor only affects the severity of the infection, or if it also contributes to a greater risk of death. On many occasions, comorbidities are analyzed generically, using definitions that include diseases with very different prognoses and in different stages of severity. Many conditions identified as risk factors are associated with other comorbidities, which produces statistical associations that do not always indicate causality. In other cases, it is necessary to analyze whether the effect of a certain disease is due to the pathology itself or to the effect of certain drugs commonly used for its treatment. Chronic diseases that cause frailty and dependency lead patients to more frequent exposure to infection, which in itself can increase severity. Additionally, most of the analyzes on prognostic factors come from retrospective studies, with the limitations that this entails when drawing conclusions.

The effect of certain factors on the pathogenesis of COVID itself is unknown; for example, there is still discussion about the risk-benefit of drugs that interfere at the level of the ACE2 receptor or some immunosuppressants. Finally, in most pa-

Table 2 Limitations to establish risk factors

Discriminate between risk of infection and disease:
Underestimation of the risks of infection caused by asymptomatic individuals
Diagnosis of infection, illness and death:
Differentiation of hospitalization or death "with" vs "by" COVID
Serological diagnosis:
Sero-reversal of humoral immunity
Greater and longer immunity with more severity
Discriminate infection vs vaccination
Effect of variants not assessed
Deceased patients are excluded
Molecular diagnosis:
Only detect symptomatic cases
Do not assess the effect of the vaccine
Interference of vaccination with disease but less with infection
Interference between factors needs description of the pathogenic mechanisms

tients several factors are associated, and it is precisely this sum of risks that probably contributes most to the severity of COVID [5]. Table 2 summarizes the main limitatiosn to detect risk factors related with severe COVID.

Finally, the understanding of the pathways and involved factors that lead to severe COVID is key to identify those populations that are still in need for special attention. It is very likely that universal indication of SARS-CoV-2 vaccination may not be feasible in the future, so that urges to determine most vulnerable populations for whom yearly vaccine may be clearly indicated. The proven efficacy of early antiviral treatment, ether with antimicrobial agents or monoclonal antibodies, is another reason to elaborate a clear list of criteria to indicate any of these very selective treatments.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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Management of hyperinflammation in COVID-19 patients

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ABSTRACT

In response to SARS-CoV-2 infection, the immune system physiologically upregulates to try to clear the virus from the body; failure to compensate for this inflammatory response with an anti-inflammatory response leads to dysregulation of the immune system that ultimately leads to a situation of uncontrolled hyperinflammation called cytokine storm. This cytokine storm can cause ARDS or multi-organ failure leading to patient death. This review exposes the different mechanisms of the inflammatory response in COVID-19 infection and the therapeutic options to treat this process.

Keywords: COVID19, corticosteroids, tocilizumab, immunomodulators.

MECHANISMS OF HYPERINFLAMMATION IN COVID-19 INFECTION

Theoretically, it has been proposed that COVID-19 infection can be divided into three phases: early infection phase involving viral replication and mild symptoms; pulmonary phase involving adaptive immunity stimulation and predominance of respiratory symptoms; and hyperinflammation phase involving hyperinflammatory conditions such as ARDS or multiorgan failure (MOF) [1].

Infection is initiated when the spike glycoprotein of SARS-CoV-2 binds to the human angiotensin-converting enzyme-2 (ACE-2) receptor on the cell surface in the epithelial cells of the nasal cavity, respiratory tract and lungs. The virus is also recognized by pattern-recognition receptors on immune cells, which are responsible for the initiation of the host defence mechanisms. The subsequent production of immune mediators is essential to fight the infection. However, these can be deleterious when produced in excess [2].

Briefly, low levels of the antiviral IFNs and high levels of proinflammatory cytokines (IL-1β, IL-2R, IL-6, IL-7, IL-8, IL-17 and TNF-α) and chemokines (CCL-2, CCL-3, CCL-5, CCL-7, CXCL-10) are produced by various immunological cells. These secretions from pro-inflammatory cells lead to an uncontrolled inflammatory response that plays a key role in the pathogenesis of COVID-19 and worsens the infection (Figure 1) [3].

IMMUNOMODULATORY DRUGS FOR THE TREATMENT OF HYPERINFLAMMATION IN COVID-19 INFECTION

Proinflammatory phase-specific therapeutics include general inflammatory drugs, cytokine inhibitors, JAK-STAT signalling inhibitors, complement pathway inhibitors, immunomodulatory drugs, cell-based therapy, and convalescent plasma therapy [4]. Below we will refer to those treatments that have shown better results to date (Table 1 and Figure 2).

Steroids. Glucocorticoids strongly inhibit the immune system. Glucocorticoids function as glucocorticoid receptor agonists. Binding of the glucocorticoids to the GR activates the receptor to exert anti-inflammatory effects, such as suppressing the production of pro-inflammatory cytokines [5]

The indication for the use of steroids in patients with COVID-19 infection is based on the RECOVERY study, which showed a reduction in 28-day mortality in patients with COVID-19 with mechanical ventilation or oxygen therapy but not in patients without respiratory support [6].

Based on these results, the World Health Organization (WHO) [7] has established two recommendations regarding the use of corticosteroids in COVID-19 patients:

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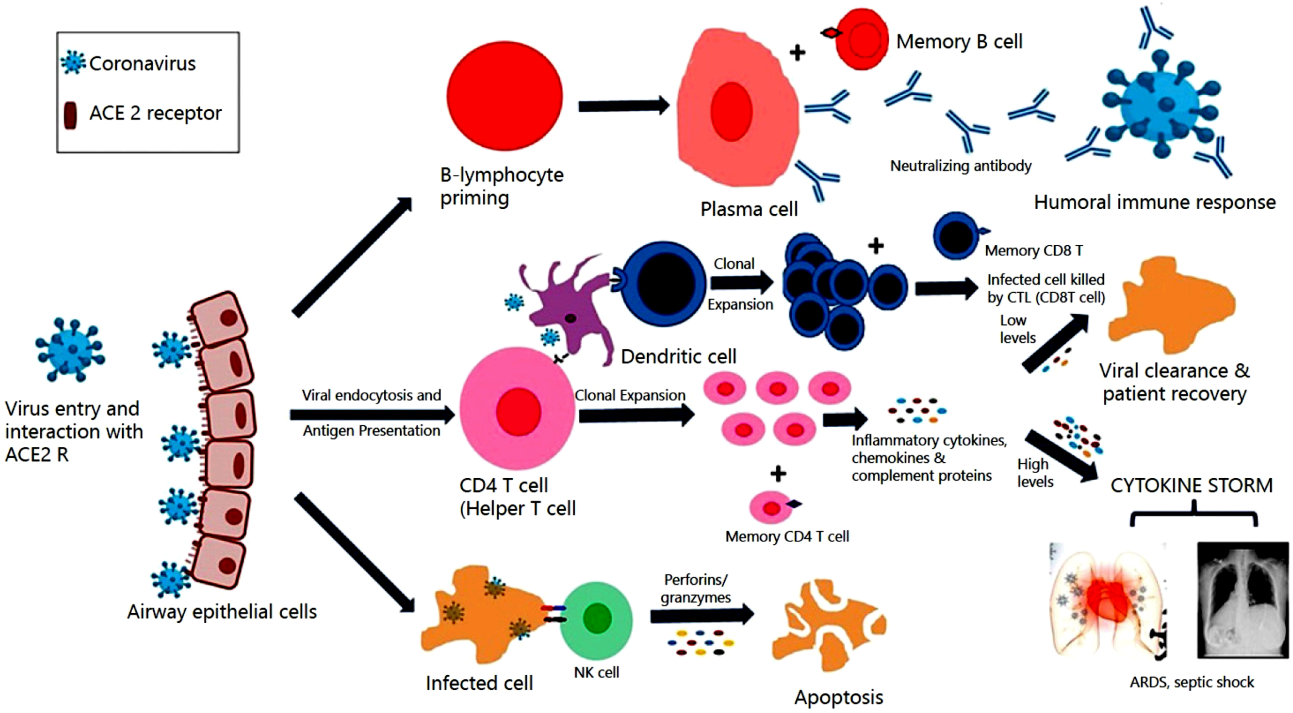

Figure 1 Immune response generation in COVID-19 infection [3] . Reproduced from Mishra KP. Hyperinflammation and Immune Response Generation in COVID-19. © 2020 Karger AG, Basel (https://www.karger.com/Article/FullText/513198)

Table 1 Immunomodulatory drugs investigated in the treatment of COVID-19 (Adapted and modified from García-Lledó A et al [4])

Class	Drugs	Currently recommended drugs in Spain*
Corticosteroids	Dexamethasone Methylprednisolone Hydrocortisone Prednisone	Dexamethasone Methylprednisolone, hydrocortisone and prednisone only if dexamethasone is not available
IL-6 inhibitors	Tocilizumab Sarilumab	Tocilizumab Sarilumab only if tocilizumab is not available
IL-1 antagonists	Anakinra Canakinumab	Anakinra
Bruton`s Tirosin Kinase (BTK) inhibitors	Acalabrutinib	
Janus Kinase (JAK) inhibitors	Baricitinib Tofacitinib Ruxolitinib	Baricitinib
TNF inhibitors	Adalimumab Certolizumab Infliximab Etanercept Golimumab	
Anti CD6 monoclonal antibodies	Itolizumab	
C5 complement inhibitors	Ravulizumab	
GM-CSF inhibitors	Lemilumab	

* At the time this document was written.

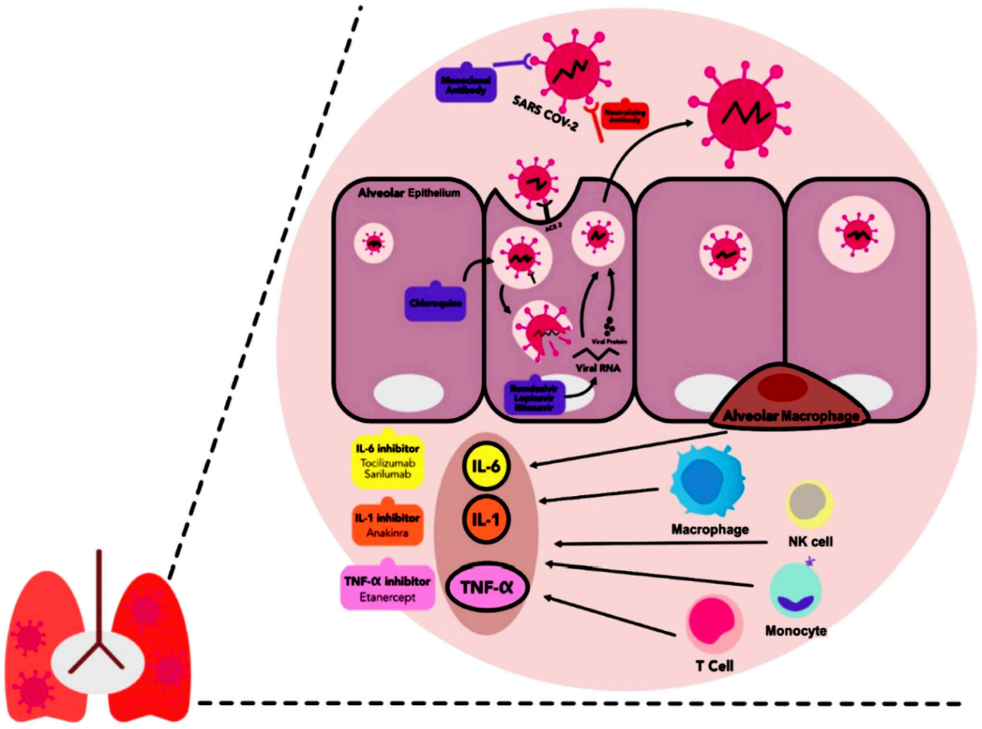

Figure 2 **Mechanisms of action of the main immunomodulatory drugs [14]. Reproduced from Hertanto DM. Immunomodulation as a Potent COVID-19 Pharmacotherapy: Past, Present and Future. 2021 (https://www.dovepress.com/getfile.php?fileID=71836) http://creativecommons.org/licenses/by-nc/3.0/**

1. Administration of systemic corticosteroids in preference to no administration for the treatment of severe and critically ill patients (strong recommendation, based on moderate certainty evidence).

2. Refraining from the use of corticosteroids in the treatment of non-critically ill COVID-19 patients (conditional recommendation, based on low certainty evidence).

Regarding the type of corticosteroid to be used, dexamethasone at a daily dose of 6 mg daily for 10 days or until hospital discharge is the drug of choice. Comparison of higher doses of the drug has shown no difference in the results regarding efficacy and safety [8]. If dexamethasone is not available, other glucocorticoids at equivalent doses (total daily doses of hydrocortisone 160 mg, methylprednisolone 32 mg or prednisone 40 mg) may be considered, although the data supporting the use are limited.

IL-6 inhibitors. Tocilizumab, a recombinant humanized monoclonal antibody for IL-6 receptor (IL-6R), exerts therapeutic effects by blocking the binding of IL-6 to IL-6R. Tocilizumab was previously found be effective against the cytokine release syndrome resulting from chimeric antigen-receptor T-cell therapy.

The possible benefit in terms of survival of the use of tocilizumab in patients with COVID-19 infection has been evaluated in different clinical trials and observational studies. However, the indication of its use is based on two of them, the studies RECOVERY and REMAP-CAP. In the RECOVERY study, patients with oxygen saturation <92% or who required oxygen therapy and who had inflammatory parameters defined as C-reactive protein ≥75 mg/L were randomized to tocilizumab versus standard of care. The mortality of both groups was 29% versus 33% p=0.007, (CI 0.77-0.96). In particular, the greatest benefit in mortality was in those patients who concomitantly received corticosteroids. Among patients not receiving invasive mechanical ventilation at baseline, patients assigned to tocilizumab less frequently met the composite endpoint of invasive mechanical ventilation or death (33% vs. 38%, (95% CI: 0.78-0, 93), p=0.0005) [9]. The REMAP-CAP study was focused on patients in the first 24 hours after starting ventilatory support in the ICU. 93% of the patients had received or received corticosteroids within 48h after tocilizumab. Mortality in the selective IL-6 inhibition group was 27% and in the control group 36% [10].

Sarilumab is another IL-6 inhibitor that has been evaluated in clinical trials. The number of patients treated does not

allow conclusions to be drawn and, at this time, it is recommended for use only in patients who for whatever reason cannot receive tocilizumab [11].

Based on the clinical evidence available at the time of writing, the use of tocilizumab concomitantly with dexamethasone is recommended in patients with SatO2 <92% (basal or with low-flow O_2) and CRP >7.5 mg/dL or if the patient requires high-flow O_2, NIMV or MV. Its use is also recommended in patients with worsening despite treatment with dexamethasone [7].

JAK inhibitors. JAK inhibitors suppress the kinase activity of JAKs by competitively binding to the ATP-binding site of JAKs, thereby inhibiting signal transduction of a wide variety of cytokines.In this group of drugs, baricitinib and tofacitinib have obtained positive results in clinical trials. Both drugs have shown a decrease in progression to mechanical ventilation and mortality, independent of concomitant steroid use [12].

IL-1 inhibitors. An IL-1 inhibitor, anakinra, has also shown beneficial effects on clinical progression and mortality in patients with severe pneumonia [4]. In a double-blind clinical trial, the drug demonstrated benefit especially in patients who had elevated suPAR levels (>6 ng/mL), a marker of severity in patients with COVID-19 [13].

CONFLICT OF INTEREST

Authors declare no conflict of interest

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Antiviral drugs against SARS-CoV-2

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ABSTRACT

The use of antiviral drugs represents an important progress in the therapeutic management of COVID-19, leading to a substantial reduction of SARS-CoV-2-related complications and mortality. In immunocompetent host, peak viral replication occurs around the symptom's onset, and it prolongs for 5 to 7 days that is the window of opportunity for giving an antiviral. Accordingly, early and rapid diagnostic of the infection in the outpatient clinic is essential as well as the availability of oral agents that can be easily prescribe. Remdesivir has demonstrated its efficacy in hospitalized patients requiring oxygen support and in mild/moderate cases to avoid the hospitalization, however, the intravenous administration limits its use among outpatients. Molnupiravir and nirmatrelvir/ritonavir are potent oral antiviral agents. In the present review we discuss the potential targets against SARS-CoV-2, and an overview of the main characteristics and clinical results with the available antiviral agents for the treatment of SARS-CoV-2.

Keywords: oral antivirals, intravenous antivirals, remdesivir, molnupiravir, nirmatrelvir/ritonavir

BACKGROUND

Coronavirus disease 2019 (COVID-19) continues to represent a major health concern worldwide with over 612 million people infected, of whom more than six million have died. The spectrum of infection severity depends on virulence of SARS-CoV-2 variants and underlying host risk factors.

By early January 2022 the B.1.1.529 (omicron) variant of SARS-CoV-2 has widely spread, even among groups with high levels of preexisting immunity. As of September 2022, omicron variants have been divided into five subvariants: BA.1 to BA.5, which are also subdivided into diverse sublineages based on additional mutations that change the genomic viral profile. The initial omicron subvariants, BA.1 and BA.2, are being progressively displaced by BA.5 in many countries. A recent study showed that infection by BA.5 subvariant was less likely among persons with previous SARS-CoV-2 infection, especially due to BA.1 or BA.2 variants, than among uninfected persons [1].

Immune dysregulation related to underlying diseases contributes to COVID-19 severity and immunomodulatory therapy has demonstrated beneficial effect on patients' outcome. In addition, numerous studies have shown that immunocompromised patients have a risk of suboptimal humoral immune response to SARS-CoV-2 vaccines, resulting in increased likelihood for severe illness [2–4].

Therefore, antiviral drugs for COVID-19 may represent a milestone in controlling the progression of the disease into more severe form, particularly in high-risk individuals, including elderly and those with comorbidities such as cancer, cardiovascular disease, and immunosuppression. This review provides a clinical practice overview of potential targets and current available antiviral agents against SARS-CoV-2.

PHARMACEUTICAL TARGETS AND ANTIVIRALS ACTIVE AGAINST SARS-COV-2

Targets for antiviral drugs include molecules involved in life cycle and/or pathogenesis of SARS-CoV-2 that can be divided into two categories: host-derived and viral-derived targets. Different host receptors and enzymes are used by SARS-CoV-2 to entry and maturate in the host cell and represent potential therapeutic targets. The major entry receptor is the angiotensin-converting enzyme 2 (ACE2) that interacts with the S1 subunit of spike protein. In the presence of transmembrane serine protease 2 (TMPRSS2), the S1 and S2 subunits of the spike protein are cleaved and S2 becomes activated to

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Table 1 **Characteristics of main antiviral drugs acting against host-derived targets.**

Drug class	Compound name	Mechanism of action	Current evidence of efficacy
Drugs targeting the ACE Inhibitor pathway	Telmisartan Soluble recombinant ACE2/APN01	Interfering virus and host ACE2 interaction	Most of the clinical and epidemiological studies failed to establish a link between the use of ACEI or ARB and severe COVID-19. No soluble recombinant ACE2/APN01 has entered clinical trial as a candidate for COVID-19 treatment.
Transmembrane serine protease 2 (TMPRSS2) inhibitors	Camostat mesylate	Serine protease inhibitor blocking the conformational changes in spike protein (S2 subdomain) involved in the fusion of the viral with the cellular membrane	A double blind randomized controlled trial with 137 patients showed that camostat mesylate do not affect time to clinical improvement, progression to ICU admission or mortality in hospitalized patients with COVID-19 [23].
Furin inhibitors	Decanoyl-RVKR-CMK a1-antitripsin PDX	Blocking of the proteolytic cleavage site between S1/S2 subunits of the S protein on the viral surface, required for virus cell entry	No molecule of this class has so far entered a clinical trial for treatment of COVID-19.
Cathepsin L inhibitors	Gallinamide Telocinobufagin	Inhibiting of the cleavage at the S1/S2 site of the S protein that enables fusion of viral and cellular membranes	Combination therapy with remdesivir and Gallinamide A or telocinobufagin and molnupiravir has been proposed to optimize the effectiveness of antivirals and to reduce the risk of selection of resistant variants. Clinical and preclinical evidence is still required to assess a possible benefit.
Phosphatidilinositol 3-phosphate 5-kinase (PIKfyve) inhibitors	Apilimod	Blocking of the receptor-mediated endocytosis and the release of the viral genome from a vacuolated endosome	Although apilimod has entered a clinical trial against COVID-19 (NCT04446377), the results have not been published yet.

fuse the viral and host cells lipid bilayers, releasing the viral ribonucleoprotein complex into the cell [5]. An alternative entry pathway, in the absence of TMPRSS2, is via endocytosis. Once within the endosome, catepsin-L play the role of cleaving S1-S1 subunits leading to membrane fusion and releasing the viral RNA into the host cell cytoplasm. During the maturation of the new virions, in Golgi apparatus of the host cells, the S1-S2 subunits should also be cleaved and the main enzymes doing it are furins [6]. As SARS-CoV-2 replication occurs on host membranes, vesicular trafficking machinery is essential for the development of new virions and represents an additional target. The main drugs under development targeting host mechanisms implicated in viral cycle are shown in table 1, however, no one has achieved advanced clinical development yet.

The second group of antiviral drugs include those compounds that target proteins involved in life cycle and/or pathogenesis of SARS-CoV-2: 1) RNA-dependent RNA polymerase (RdRP) inhibitors, 2) viral protease inhibitors, and 3) maturation inhibitors. The main pharmacological characteristics of these molecules are shown in table 2.

Remdesivir is an RNA-dependent RNA polymerase (RdRp) inhibitor approved by the US FDA for the treatment of COVID-19 infection in hospitalized patients in October 2020. Because of its membrane-permeable backbone, remdesivir can easily reach the cytoplasm, where is converted to remdesivir monophosphate and remdesivir triphosphate. The final molecule is an analog of adenosine, and it is incorporated by the RdRP complex into the nascent RNA strands, resulting in termination of RNA synthesis and efficiently stopping viral replication. A recent metanalysis by Lee et al. analyzed the benefits of remdesivir in the treatment of hospitalized patients with COVID-19 [7]. Eight randomized trials with 10,751 participants were considered. The risk ratio of mortality comparing remdesivir vs control was 0.77 (95% CI, 0.5-1.19) in the patients who did not require supplemental oxygen; 0.89 (95% CI, 0.79-0.99) for nonventilated patients requiring oxygen; and 1.08 (95% CI, 0.88-1.31) in the setting of mechanical ventilation. Several observational and retrospective studies have supported these results and have suggested that early administration has been associated with significant reductions in mortality [8,9]. Currently, remdesivir is included as an effective drug in guidelines of different medical societies for hospitalized patients requiring oxygen support. For patients requiring invasive mechanical ventilation, clinical trials do not support the use of remdesivir, however a recent large retrospective analysis shows a signifi-

Table 2 **Main pharmacological characteristics of compounds targeting proteins involved in life cycle and/or pathogenesis of SARS-CoV-2.**

Compound name	Drug class	Route of administration	Dosing regimens	Most common adverse reactions
Remdesivir	Nucleoside analog	Intravenous	200 mg on Day 1, then 100 mg once daily	Diarrhea, rash, renal impairment, hypotension
Molnupiravir	Nucleoside analog	Oral	800 mg twice daily	Diarrhea, nausea, dizziness, embryo-fetal toxicity bone and cartilage toxicity, hypersensitivity to ingredients Male contraception is required during treatment and ³3 months after the last dose
Nirmatrelvir/ritonavir	Protease inhibitor (nirmatrelvir)	Oral	300 mg nirmatrelvir plus 100 mg ritonavir twice daily	Diarrhea, dysgeusia, hypertension, myalgia, hypersensitivity to ingredients; hepatotoxicity

cant reduction of mortality also in this population [10]. In the opinion of the authors, the window of opportunity to use an antiviral drug in patients with severe COVID-19 does not depend on the oxygen support required but on the presence of active viral replication. For SARS-CoV-2, the window of viral replication is 5 to 7 days from symptoms onset in mild-moderate cases, but it can be longer for patients with severe COVID-19 and in immunosuppressed patients. Accordingly, in patients requiring hospitalization an antiviral should always be considered within the first 7 days disregarding the oxygen support. For patients with more than 7 days from symptoms onset, the decision should be based on the presence of active viral replication. Unfortunately, detection of genomic RNA by real-time PCR is not correlated with active viral replication and should not be used as a qualitative test. The evaluation of cycle threshold (Ct) as a surrogate marker has been proposed and a meta-analysis showed a higher mortality rate among hospitalized patients with a Ct <25 [11]. However, the validity of Ct in an heterogenous sample like respiratory secretions is controversial. Alternative, subgenomic RNA detection (qualitative measurement) and antigen test detecting nucleocapsid protein have shown a good correlation with active viral replication and should be considered to guide the antiviral prescription in patients with severe COVID-19 and more than 7 days from symptoms onset [12,13].

The duration of remdesivir treatment is 5 days, and longer treatment has not demonstrated additional benefit in general population. However, the experience in immunosuppressed patients is scarce, and failure after 5 days of treatment is not uncommon with potential to develop resistance [14]. In the future is necessary to evaluate the need of longer courses in this population. Remdesivir is contraindicated in patients with alanine aminotransferase (ALT) levels >5-times the upper limit of normality or severe hepatic dysfunction, and in patients with severe renal impairment (estimated glomerular filtration ≤ 30 mL/min). However, some clinical reports have demonstrated good tolerability an no nephrotoxicity in patients with eGF ≤30 mL/min. A clinical trial in this population is ongoing and results will be available in the future.

Remdesivir has also been evaluated in non-hospitalized mild-moderate COVID-19 to avoid the progression of the infection. The PINETREE study is a randomized, double-blind, placebo-controlled trial involving non-hospitalized patients with COVID-19 at high risk of severe disease [15]. Among 562 patients included most subjects were unvaccinated adults with comorbid medical conditions (diabetes, obesity, and hypertension). The study demonstrated that 279 patients who received a 3-day course of intravenous remdesivir within 7 days of symptoms onset, had a relative reduction of 87% in the need of hospital admission or death compared to placebo. In addition, remdesivir reduced the risk of COVID-19 related medically visits or all-cause mortality by day 28. Nevertheless, it should be noticed that only 5% of patients in Remdesivir group and 3.2% in placebo group were immunocompromised. The need to administer remdesivir through the intravenous route has limited its use in the outpatient settings. An orally bioavailable formulation of remdesivir is currently under development and may have the potential to maximize its availability and prevent progression to severe disease.

Molnupiravir is a small-molecule prodrug of β-d-N4-hydroxycytidine (NHC), a ribonucleoside analog which is finally incorporated to viral RNA by RdRp. Molnupiravir prompts an accumulation of errors in the replicating virus, until the virus can no longer survive. It was originally discovered for Venezuelan equine encephalitis virus (VEEV) infection but was later found to have antiviral activity against several respiratory viruses, including influenza virus and SARS-CoV-2. In a phase-3 study of 1433 patients with mild-to-moderate COVID-19 and at least one risk factor for severe illness, treatment with molnupiravir within 5 days of symptoms onset reduced the chances of hospitalization and death by 30% compared with placebo [16]. This reduction rate is lower than the ones reported with remdesivir or nirmatrelvir/ritonavir in similar clinical trials. Accordingly, molnupiravir has been approved for use in patients with mild to moderate COVID-19 who are at high risk of progression to severe disease, and for whom alterative antiviral

therapies are not accessible or contraindicated. In addition, recent real-world data originating from a large cohort during Omicron BA.2 dominance confirms that molnupiravir reduces the risk of progression and mechanical ventilation [17]. Another recent real-world study conducted in Poland during the dominance of the Omicron variant evidenced that administration of molnupiravir in hospitalized patients within five days from symptoms onset resulted in reduced mortality and less frequent use of oxygen supplementation [18].

The first SARS-CoV-2 protease inhibitor with clinical evidenced of efficacy in preventing a progression to severe disease is nirmatrelvir/ritonavir. Nirmatrelvir inhibits M^{pro} (3CL protease), the main protease of SARS-CoV-2, which catalyzes the cleavage of viral polyproteins into nonstructural proteins that are essential for viral replication. It is combined with ritonavir, a potent cytochrome P450-3A4 inhibitor which prolongs nirmatrelvir half-life, supporting twice-daily administration. Nirmatrelvir/ritonavir has been authorized for treating mild-to-moderate COVID-19 in adults and children aged 12 years and older weighting at least 40 kg, who are at high risk for progression to severe COVID-19, including hospitalization and death. The EPIC-HR trial is a randomized, double-blind study of non-hospitalized adults with COVID-19 who were at high risk of progression to severe disease [19]. In the final analysis, 5/697 (0.7%) of patients who received nirmatrelvir/ritonavir within 5 days from symptoms onset were hospitalized up to day 28 post-randomization, compared to 6.5% of patients who received placebo, resulting in a relative risk reduction of 89% ($p<0.0001$). A population-based study on real world data from Israel shows that nirmatrelvir/ritonavir and adequate vaccination against SARS-CoV2 were associated with significant decrease in the rate of severe COVID-19 or mortality, compared to those not treated, and/or not vaccinated [20]. Of the 180,351 patients included, 4,737 patients were treated with nirmatrelvir/ritonavir, and 135,482 (75.1%) had adequate vaccination status. Nirmatrelvir/ritonavir appeared to be more effective in older patients, immunosuppressed patients, and those with underlying cardiovascular or neurological disease. In addition, a recent real-world retrospective study involving a large inpatients cohort during Omicron BA.2 variant domination confirmed high efficacy of nirmatrelvir/ritonavir in shortening viral replication, reducing disease progression, and preventing death [21]. It should be noted that patients from this cohort were correctly vaccinated and developed COVID-19 at least one month after vaccination. Recently, concerns have been raised about a rebound phenomenon with nirmatrelvir/ritonavir, whereby patients develop symptoms of COVID-19 after taking the drug [20]. A recent study found that rebound occurred in 0.8% of patients, resulted in mild symptoms without requiring additional COVID-19 therapy [22].

Ritonavir has significant and complex drug-drug interactions. Concomitant medications, including over-the-counter medicines, herbals, or recreational drugs, must be reviewed for their potential interactions prior to prescribing nirmatrelvir/ritonavir. Consultation with pharmacologist, COVID-19 guidelines, drug-drug interaction specialized websites, or the drug fact sheet is mandatory for providers prescribing this medication.

CHALLENGES IN CLINICAL PRACTICE

Early administration. The main challenge of antiviral therapies is that they should be administered while the viral replication occurs. This window in the case of SARS-CoV-2 are the first 5 days from symptoms onset in immunocompetent host. Early treatment, by reducing progression to hospitalization, might reduce long-term morbidity and mortality and reduce the burden on healthcare resources. For an early-intervention strategy to work, it is mandatory to accurately identify patients at greater risk of clinical deterioration. Phenotyping studies, incorporating both clinical features and immunological biomarkers and potentially using machine-learning techniques, may allow a better identification of patients requiring early antiviral therapy.

Vulnerable populations. Even with widespread COVID-19 vaccination uptake, specific risk groups remain particularly vulnerable to severe infection. Age ≥ 65 years, obesity, diabetes mellitus, chronic kidney disease, cardiovascular and neurological diseases but most specially the presence of 2 or more of these co-morbidities and being vaccinated >6 months prior to the acute episode are the most relevant risk factors associated with complications among vaccinated population and real-world experience with antivirals has demonstrated a beneficial effect in this population. In contrast, the evidence among patients with immunosuppression (e.g. patients under B cell-depleting therapies, active chemotherapy, or organ transplant) is scarce. These patients have a significantly longer viral shedding with consequences for the management of the patient (delay in chemotherapy), need of prolonged isolation periods, and risk of host evolving viruses leading to the emergence of new variants. It is of utmost important to investigate the efficacy of antivirals in this population and to define the most adequate duration of therapy and even the potential need to combine different antiviral strategies to shorten the viral shedding minimizing the risk of selecting resistant strains.

Pregnant women. Pregnant women are at a greater risk of severe COVID-19. While molnupiravir is not recommended in pregnancy, no data are available with remdesivir and nirmatrelvir/ritonavir safety profile in pregnancy or breastfeeding. These drugs may be considered in pregnant women with mild-to-moderate COVID-19 if one or more additional risk factors are present (e.g., body mass index >25, chronic kidney disease, diabetes mellitus, or cardiovascular disease).

Pediatric population. Remdesivir is approved for patients 28 days and older and weighing at least 3 kg with mild to moderate COVID-19 and at high risk for progression to severe COVID-19, including hospitalization or death. The use of oral antivirals for COVID-19 treatment in the pediatric group may be limited. Molnupiravir is contradicted in children due to potential bone and cartilage toxicity. The use of nirmatrelvir/

ritonavir is not authorized in pediatric patients younger than 12 years of age or weighing less than 40 kg. The clinical trial involving non-hospitalized children with COVID-19 aged 6–12 who are at risk of progression to severe disease is ongoing.

CONCLUSION

The introduction of COVID-19 vaccines has significantly reduced the incidence, the hospitalization rate and the mortality of SARS-CoV-2 infection. However, the emergence of new variants that escape from vaccine immunity, maintains the virus circulation and the risk of infecting vulnerable population that will require hospitalization. The availability of antivirals that have demonstrated a great reduction of the hospitalization rates among patients with mild-moderate COVID-19, particularly among vulnerable population, is one of the most important achievements in the COVID-19 management. Remdesivir, and nirmatrelvir/ritonavir have demonstrated the highest risk reduction and considering the oral formulation of nirmatrelvir/ritonavir, this is the first choice to treat COVID-19 in the community. In the future, it is necessary to enlarge the experience with antivirals in immunosuppressed patients, and to define the duration of therapy or even the need of combine different agents to faster reduce the viral load avoiding the selection of resistant strains.

CONFLICT OF INTEREST

AS has received honoraria for lectures and advisory boards from Pfizer, Merk Sharp and Dohme, Angelini, Shionogi, Menarini and Gilead Sciences. Grants from Pfizer and Gilead Science. CG-V has received honoraria for talks on behalf of Gilead Science, Merck Sharp and Dohme, Pfizer, Jannsen, Novartis, Lilly and a grant support from Gilead Science and Merck Sharp and Dohme.

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Neutralizing antibodies for SARS-CoV-2 infection

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ABSTRACT

The COVID-19 pandemic has boosted significant research in developing monoclonal antibodies (mAbs) to treat and prevent SARS-CoV-2 infection. Clinical trials have shown that mAbs are safe and effective in preventing hospitalization and death in patients with mild to moderate COVID-19 risk factors for progression. mAbs have also been effective for treating severe disease in seronegative patients and preventing COVID-19. So far, studies have been carried out in a largely unvaccinated population at a time when the omicron variant was not described. Future research should address these limitations and provide information on specific population groups, including immunosuppressed and previously infected individuals.

Keywords: Covid-19, SARS-CoV-2, coronavirus, monoclonal antibody.

INTRODUCTION

The use of serum therapy in medicine was initiated by Behring and Kitasato in 1890 with the development of diphtheria antitoxin. Many decades later, the development of monoclonal antibodies (mAbs), derived from a single B lymphocyte clone that recognizes one and only one specific epitope, was a major medical breakthrough. The first mAb used in clinical practice was Muronomab, an anti-CD3 antibody approved in 1975 by the Food and Drug Administration (FDA) for preventing kidney transplant rejection. The first mAb in Infectious Diseases therapeutics was Palivizumab, approved in 1998 to prevent severe respiratory syncytial virus (RSV) disease in high-risk children. Later, other mAbs for anthrax, rabies, HIV, and Ebola were marketed or approved for conditional emergency use [1].

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The mechanism of action of mAbs in viral infections is multiple. It includes the direct binding of the antibody's antigen binding site to free viral particles, neutralizing its ability to infect host cells. In addition, the fragment crystallizable (Fc) region of the antibody stimulates opsonization, antibody-dependent phagocytosis, and antibody-dependent and complement-dependent cytotoxicity [2].

Over the last two years, the COVID-19 pandemic has boosted significant research in developing mAbs against SARS-CoV-2. Clinical Trials have been developed for early treatment in patients with mild/moderate disease at risk of progression to severe/critical disease and late-stage treatment in patients with severe or critical illness. Here we will review the clinical experience of mAbs in these two scenarios. It should be noted that studies with mAbs for SARS-CoV-2 pre-exposure prophylaxis and post-exposure prophylaxis are also being carried out [1].

MONOCLONAL ANTIBODIES AGAINST SARS-COV-2

The SARS-CoV-2 particle is surrounded by the spike protein integrated by three monomers, one of which is the receptor binding domain (RBD), that contacts the angiotensin-converting enzyme 2 (ACE2) receptor in the host cell through the receptor binding motif (RBM), its functional site [3,4]. The RBD is the main target of mAbs against SARS-CoV-2, some of which bind directly to the RBM [5,6]. mAbs against SARS-CoV-2 are classified based on their target RBD antigenic sites [1]. There are currently four classes of monoclonal antibodies that bind to four different sites, some of which are more mutable than others. Mutations in the RBD of the different viral variants can affect the antiviral activity of mAbs against SARS-CoV-2. The activity of mAbs against the different SARS-CoV-2 variants is regularly updated on the Stanford University Coronavirus resistance database [7]. Besides, the National Institute of Health (NIH) guidelines also review the activity of the different mAbs

Table 1 **Efficacy of monoclonal antibodies against SARS-CoV-2 in non-hospitalized patients with COVID-19. Modified from reference 9**

	Bamlanivimab Etesivimab	Casirivimab Imdevimab 2400	Casirivimab Imdevimab 1200	Sotrovimab	Tixagevimab Cilgavimab	Regdanvimab
Administration Route	Intravenous	Intravenous	Intravenous	Intravenous	Intramuscular	Intravenous
Clinical trial name	BLAZE-1	na	na	COMET-ICE	TACKLE	CT-P59 3.2
Hospitalizations in mAb group	11/518 (2.1%)	18/1355 (1.3%)	7/736 (1.0%)	3/291 (1.0%)	18/407 (4.4%)	16/656 (2.4%)
Hospitalizations in PLBO group	36/517 (7.0%)	62/1341 (4.6%)	24/748 (3.2%)	21/292 (7.0%)	37/415 (8.9%)	53/659 (8.0%)
% Reduction in hospitalization or death	70.0	71.3	70.4	85.0	50.0	70.0
Number needed to treat	21	30	44	16	22	18

authorized by the FDA [8]. Of note, the neutralizing activity against the omicron variant is significantly reduced for all mAbs except for sotrovimab; an antibody derived from a patient infected with SARS-CoV-1 in 2003 that binds to a highly conserved region of the spike protein away from the RBD.

MONOCLONAL ANTIBODIES AGAINST SARS-COV-2 IN OUTPATIENTS

The information we have on the main pivotal clinical trials with mAbs for the treatment of COVID-19 in outpatients is summarized in Table 1 [9]. All these studies have been carried out in a largely unvaccinated population and, more importantly, at a time when the omicron variant was not described.

Given that sotrovimab is the only mAb active in vitro against the omicron variant, it is worth noting the phase III COMET-ICE clinical trial whose pre-specified interim analysis was published in November 2021 when approximately 40% of the patients had been included [10], and whose final results were communicated in September 2021 in the IDWeek 2021 meeting [11] (Table 2). This clinical trial evaluated the efficacy and safety of sotrovimab in outpatients with mild to moderate COVID-19 at risk of progression to severe COVID. Patients were randomized to sotrovimab 500 mg IV or a dose of placebo. The primary outcome variable was admission or death from any cause in the first 29 days. Patient characteristics were well distributed between groups; 54% were women, the median age was 53, and 87% were white. The duration of symptoms was three days or less in 59%, and the most frequent risk factors for progression were obesity, age greater than or equal to 55 years, and diabetes mellitus. Regarding the primary efficacy analysis, there was hospitalization or death at 29 days in six patients in the sotrovimab group (1.1%) and 30 in the PBO group (5.7%), representing a 79% reduction in the risk of hospitalization or death using sotrovimab. In a post-hoc review, it was found that three of the six admissions in the sotrovimab group were not related to COVID-19: lung cancer, diabetic foot, and intestinal obstruction, while the 30 in the placebo group were related to COVID-19 (29 admissions and one death). Concerning the secondary efficacy outcomes, it should be noted that sotrovimab therapy was associated with a 66% reduction in visits to the emergency department, a 74% reduction in the development of severe or critical illness, and that there were no deaths in this arm while there were two in the placebo group.

MONOCLONAL ANTIBODIES AGAINST SARS-COV-2 IN HOSPITALIZED PATIENTS

The first data about mAb treatment of severe COVID-19 in hospitalized patients were generated on the RECOVERY platform in the United Kingdom, where almost 10,000 patients hospitalized for COVID-19 between September 2020 and May 2021 were randomized 1:1 to the combination of casirivimab with imdevimab (CAS/IMD) or standard treatment [12]. The mean patients age was 62 years, the median time from symptom onset to randomization was nine days, 94% of patients were receiving corticosteroids as part of the standard of care, and 32% had negative serology for SARS-CoV-2. Between 50% and 60% of the patients had some underlying disease, the predominant ones being diabetes, heart disease, and chronic lung disease. The risk of death at 28 days, hospital discharge alive, and need for mechanical ventilation or death were not significantly different between the two groups. However, when a stratified analysis was made according to the SARS-CoV-2 serology result, it was observed that treatment with CAS/IMD provided clear benefits in terms of lower mortality, higher probability of survival, and lower risk of mechanical ventilation. Or death. The number needed to treat (NNT) was 16.7 to prevent one death, 16.7 to be discharged alive, and 14.3 to prevent mechanical ventilation or death.

CAS/IMD was also studied in a randomized, double-blind clinical reported as Late Breaker at the IDWeek 2021 meeting [13]. The inclusion criteria were hospitalization due to COVID with no more than three days of admission and duration of symptoms of no more than ten days. Patients were randomized 1:1:1 to two doses of CAS/IMD or placebo, stratified by the COVID treatment they received: nothing, remdesivir (RDV), corticosteroids, or RDV + corticosteroids. The clinical trial contemplated two primary outcome variables: a virological one (change in viral load from baseline to day 7 in seronegatives)

Table 2 **Efficacy of monoclonal antibodies against SARS-CoV-2 in non-hospitalized patients with COVID-19. Modified from Reference 11**

Outcome	Sotrovimab (n=528)	Placebo (n=529)	Relative risk ratio (95% CI)	P
Primary outcome				
Hospitalization for > 24 h for acute management of illness or death through day 29, No. (%)	6 (0.2)	30 (5.7)	0.21 (0.09 - 0.50)	<0.001
Selected secondary outcomes (through day 29)				
Emergency room visit, hospitalization, or death due to any cause, No. (%)	13 (2)	39 (7)	0.34 (0.19 - 0.63)	<0.001
Progression to severe/critical respiratory COVID-19, No. (%)	7 (1)	28 (5)	0.26 (0.12 - 0.59)	0.002
All-cause mortality, No. (%)	0	2 (<1)		

and a clinical one (death or mechanical ventilation on day 29). CAS/IMD was superior to placebo considering the two outcome variables: virological and clinical, with a relative risk reduction of mechanical ventilation or death at 29 days of 47% in the seronegative group and with an NNT of 11.

A clinical trial by the Therapeutics for Inpatients with COVID-19 Study Group (TICO) has recently been published in which the efficacy of two mAbs was compared in patients hospitalized for COVID-19: sotrovimab and the combination of Amubarvimab/romlusevimab two derivative mAbs of a convalescent COVID-19 patient [14]. Recruitment took place between December 2020 and March 2021 in multiple countries, and the primary efficacy endpoint was time to clinical recovery after a 90-day follow-up. Complete clinical recovery was defined as being discharged home for at least two weeks. A total of 546 patients were enrolled and randomized 1:1:1 to placebo, Sotrovimab, or the combination of amubarvimab/romlusevimab. The patients included had a median age of 61 years, with a slight predominance of women, and approximately 75% of the patients had some underlying disease such as hypertension, diabetes, and less frequently kidney failure, asthma, and heart failure. Of note, approximately one-third of patients were seronegative for SARS-CoV-2. Recruitment was terminated after a protocol-specified interim analysis showed no change in an ordinal scale of lung involvement. Furthermore, neither active treatment arm significantly shortened the time to clinical improvement compared to the placebo. No signal was observed in terms of mortality either, with 14 (8%) dying in the sotrovimab group, 13 (7%) in the placebo group, and 15 (9%) in the amubarvimab/romlusevimab group.

CONCLUSIONS

mAbs treatments are safe and effective in preventing hospitalization and death in patients with mild to moderate COVID-19 risk factors for progression. They also have the potential for the treatment of severe COVID-19 in seronegative patients and as preventive tools against COVID-19. We need more information on the efficacy of mAbs against some variants (omicron) and in some groups of patients (immunosuppressed, vaccinated, previously infected).

CONFLICT OF INTERESTS

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Approach to Infection models

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Important aspects during management of diabetic foot infection

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ABSTRACT

Diabetic foot is a complex disease. One of its most important complications is infection with risk of limb loss. In severe cases it is also a life-threatening condition. Several guidelines are available in order to achieve the implementation of some standard of care strategies. However, these consensus documents do not address all controversial issues arising during diabetic foot infection. The present article aims to review some of these controversial aspects.

Key words: diabetic foot, infection, empirical treatment, osteomyelitis, comorbidity.

BACKGROUND

Diabetic foot is a complex and heterogeneous disease. One of its most important complications is infection with risk of limb loss. In severe cases it is also a life-threatening condition [1].

Management of these types of infections is not easy since many different specialities are involved on it. In addition, perception, knowledge and awareness of this condition is quite heterogeneous between different physicians. Several guidelines are available in order to achieve the implementation of some standard of care strategies [2,3]. However, these consensus documents do not address all controversial issues arising during diabetic foot infection attention. When to treat resistant bacteria empirically, the duration of this treatment and which is the best dose to achieve high concentrations at the site of infection are aspects that guidelines do not always explain. Some comorbidities play also an important role in the management of prognosis of diabetic foot infections. The present article aims to review some of these controversial aspects:

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CHOICE OF EMPIRICAL ANTIBIOTIC

Regarding medical treatment of infection, the choice of an empirical antibiotic is quite difficult, especially in moderate or severe infections, where polymicrobial involvement is frequent. In this clinical scenario, early administration of correct antibiotics improves prognosis and results in lower amputation and mortality rates. On one hand, delay on antibiotic treatment can lead to adverse outcomes, which means that there is often no time to wait until results of cultures are available. But on the other hand, bad evolution and lack of improvement could be the consequence of an incorrect empirical choice.

Mild infection. In general terms, mild infections (less than two centimetres of redness of skin with depth affecting only subcutaneous tissue) of acute lesions with a course of no longer than two weeks and without prior antibiotic are usually caused by gram-positive bacteria and do not need coverage against gram negative bacteria, anaerobes or resistant microorganisms. However, this is only a general approach [2,3]. It is necessary to take into account that some patients are at high risk of methicillin-resistant *Staphylococcus aureus* (MRSA) infections despite having non-complicated disease. So, prior MRSA infections or colonization by this microorganism, nasal carriers, peripheral vasculopathy and chronic kidney disease (most of all in dialyzed patients) are risk factors for being infected by this virulent bacterium and are described in medical literature. It is important to rule out these circumstances for the correct treatment of mild infections because when they are present, coverage against MRSA should be, at least, considered [4-6].

At this point it is important to notice that correct evaluation of both vascular status and infection depth is mandatory. For example, small cellulitis areas are sometimes present in deep lesions including osteomyelitis so that they seem to be less severe infections than they really are. That is why this evaluation should be done by trained professionals. When phy-

sicians have doubts about these facts prompt referral to specialized diabetic foot units is recommended. This shows that correct diabetic foot infection management requires a complex and multidisciplinary approach. In addition, it is important to mention that sharp debridement at site of infection and revascularization when needed is as important as the correct choice of antibiotic, making teamwork crucial to achieve successful outcomes [1,2].

Moderate and severe infection. Moderate diabetic infections have cellulitis areas that are usually bigger than 2 cm and affect deeper structures beyond subcutaneous tissue such as fascia, muscle, joints or bone. In this scenario, wider antibiotic coverage should be considered including gram negative bacteria and anaerobes. However, multidrug resistant bacteria are not always responsible [2,3].

Severe infection with systemic toxicity signs or sepsis, is a life-threatening condition and needs to be treated covering resistant gram-positive and resistant gram-negative bacteria. In these cases, in which broad spectrum antibiotics are prescribed, it is also mandatory to obtain tissue samples correctly in order to switch to a narrow spectrum antibiotic when possible. Swab samples do not offer reliable results and should not be taken.

Following Basetti, M et al. [7] in general population, host factors in general population for multidrug resistant gram-negative infections are older age (more than 70), diabetes mellitus, chronic obstructive pulmonary disease, malignancy, immunosuppression (including neutropenia and corticosteroid use) Charlson comorbidity index greater than 3, indwelling devices, need of haemodialysis, recent surgery or exposure to antibiotics within previous three months. Poor hygiene, recent hospital stay or transfer from another healthcare facility and prior colonization are also epidemiological factors that must be taken into account when assessing treatment for gram-negative bacteria. Probably, the presence of only one of these risk characteristics is not enough to support treatment against resistant Gram-negative bacteria. However, when some of them are present at the same time, wide spectrum antibiotics need to be considered.

Regarding specifically diabetic foot syndrome population, enterobacteria producing extended-spectrum beta-lactamases (ESBL) play an important role. In addition to the risk factors mentioned above, previous treatment with cephalosporines and presence of osteomyelitis are also risk factors for infection [4].

Importance of Osteomyelitis in diabetic foot infection. As mentioned above, ruling out bone infection in diabetic foot patients is always necessary. Long-time non-healing ulcers usually fail to respond to several antibiotic schemes. When this happens, many different causes could be responsible. Wrong local management, need of revascularization, low antibiotic dosages, bad treatment adherence or the failing of discharge strategies are possible causes. However, when ulcers do not heal or infection does not improve despite an apparently correct treatment, it is crucial to rule out diabetic foot osteomyelitis. Bone penetration of antibiotics is a difficult issue and higher doses could be needed. Bone removal, when possible, is another option that helps to improve infections. When not possible, longer antibiotic courses are needed (as long as six weeks). As it has been explained before, osteomyelitis is an independent risk factor for multi-drug resistant bacteria such as MRSA or ESBL enterobacteria. For all those reasons the classical diabetic foot infection classification [2] (Table 1) is presently very much discussed. Although severe infection with

Table 1 | **Classical diabetic foot infection classification by IWGDF in 2019 update [2]**

Definition	Grade
No signs of infection.	1
- Infection limited to skin or superficial subcutaneous tissues without local complication or systemic signs AND - Erythema does not extend > 2 cm around the wound.	2
Infection with no systemic manifestations - Erythema extending > 2 cm form the wound margin AND/OR - Infection deeper than skin and subcutaneous tissues (deep tissue abscess, lymphangitis, tendon, muscle, joint and/or bone involvement)	3 (add "O" if infection involves bone)
Any foot infection with associated systemic signs as manifested by 2 or more of the following: - Temperature > 38 ºC or <36ºC - Heart rate > 90 beats/min - Respiratory rate > 20 breaths/minute or PaCO2 < 32 mmHg - White blood cell count >12.000/mm^3 or < 4000/mm^3 or > 10% immature (band forms)	4 (add "O" if infection involves bone)

IWGDF: international working group of diabetic foot

Table 2 Revised IDSA diabetic foot classification by Lavery et al [8]

Definition	Grade
Diabetic foot ulceration without any manifestation of infection	No infection
Infection limited to skin or superficial subcutaneous tissue without local complication or systemic illness with 2 or more of the following signs: - Local swelling or induration - Erythema (extending <2 cm around the wound) - Local tenderness or pain - Local warmth - Purulent discharge	Mild soft tissue infection
Either systemically stable or unstable patients with 1 or more of the following: - Erythema extending > 2 cm from ulceration - Lymphangitis - Spread beneath fascia - Deep tissue abscess - Gangrene - Can involve muscle tendon and joint but not bone This category includes patients with moderate or severe soft tissue infection. Severe infection is defined by 2 or more of the following: - Temperature > 38 ºC or <36ºC - Heart rate > 90 beats/min - Respiratory rate > 20 breaths/minute or PaCO2 < 32 mmHg - White blood cell count >12.000/mm3 or < 4000/mm3 or > 10% immature (band forms)	Moderate/severe soft tissue infection
Any bone infection of the foot This category includes patients with moderate or severe bone infection. Severe infection is defined by 2 or more of the following: - Temperature > 38 ºC or <36ºC - Heart rate > 90 beats/min - Respiratory rate > 20 breaths/minute or PaCO2 < 32 mmHg - White blood cell count >12.000/mm^3 or < 4000/mm^3 or > 10% immature (band forms)	Moderate/severe diabetic foot osteomyelitis

IDSA: infectious disease society of America.

systemic toxicity is always a reason for concern, it seems that the presence of osteomyelitis has important prognosis implications, and a new classification [8] has been proposed by Lavery et al (Table 2).

One meta-analysis published in 2019 by Pinar Sen et al showed [9] the importance of osteomyelitis as an independent risk factor for amputation. In pooled OR analysis, the presence of gangrene/necrosis (OR: 9.9, 95% CI, 6.243-15.699; $P < 0.001$), presence of osteomyelitis (OR: 4.5, 95% CI, 2.277-8.885; $P < 0.001$), and length of hospitalization (SMD: 0.70, 95% CI, 0.45-0.95; $P < 0.001$) were the main associations with an increased risk of lower extremity amputations in patients with diabetic foot infections. Results showed also that the risk of amputation increased 1.7-fold with an IWGDF grade 3 classification and 2.5-fold with an IWGDF grade 4 classification (95% CI, 1.398-2.061; $P < 0.001$ and 95% CI, 1.647-3.823; $P < 0.001$, respectively)

This study showed that diagnosis of diabetic foot infection is not only based on clinical suspicion and bacterial-isolation. Correct depth assessment and identification of affected structures are obligatory actions to be done before performing any therapeutic strategy. It is particularly important to rule out the presence of gangrene/necrosis and osteomyelitis since they seem to be the most important predictors of amputation.

Bone cultures still remain as an essential procedure in order to diagnose diabetic foot osteomyelitis [10] In addition, in patients with chronic osteomyelitis, which are often overtreated, cultures could be less reliable and bone biopsy should be considered [11].

Table 3 Clinical situations in which nonsurgical treatment could be considered in patients with diabetic foot osteomyelitis [13]

Clinical situation
Infection confined to the forefoot
Adequate limb perfusion
No tissue necrosis
Contraindication to surgery AND/OR high surgical risk
Patient preference to avoid surgery

DURATION OF TREATMENT

Although guidelines make some recommendations about this issue, the decision of stopping antibiotics should be made on the basis of clinical evolution. For example, for mild infections, 1-2 weeks antibiotic courses are recommended [2]. Whether 7 or 14 days of antibiotic treatment are required should be assessed individually taking into account the improvement or absence of infection signs, the possibility of removing infected tissue with sharp debridement, side effects, vascular status, etc. Again, it is particularly important to rule out osteomyelitis because removal of infected bone can reduce dramatically the duration of antibiotic treatment. In cases where bone removal is not possible or surgery is not indicated (Table 3) six weeks of medical therapy alone are recommended [12,13]. However, this medical approach is not preferred since long antibiotic courses are associated with higher risk of side effects such as renal failure, *Clostridioides difficile* infection, candidiasis, bone marrow toxicity, etc. One prospective, randomized non-inferiority pilot trial suggests that 3 weeks of medical treatment could be enough. However, in this study surgical debridement was performed before medical treatment [14].

In addition, in cases of infection that do not improve despite apparently correct medical treatment, just prolonging the same antibiotic scheme seems not to be a good option. Checking treatment adherence and antibiotic dose in order to achieve high concentrations at infection site, revising cultures results, evaluating dressing and offloading devices, assessing vascular status and ruling out bone involvement taking samples are essential aspects to be regarded in order to achieve success during management and avoid amputations, which is the most important objective of diabetic foot infection management.

COMORBIDITIES

Some comorbidities have demonstrated to have prognostic implications in diabetic foot infections [15-21]. Chronic kidney disease, haemodialysis, heart failure, ischaemic heart disease, malnutrition and poor glycaemic control are conditions which are frequently present in diabetic foot patients and have shown to increase the risk of adverse outcomes such as mortality after amputation, amputation risk or delay of the healing process.

If these comorbidities are not detected, treated and controlled, diabetic foot infection will have more systemic repercussion and prognosis will worsen. This shows again the need of a multidisciplinary, collaborative and communicative approach between different specialties involved on diabetic foot management. Nurses, vascular surgeons, podiatrists, orthopaedic surgeons, internal medicine, endocrinology, microbiology and infectious diseases specialists are professionals that contribute to improve diabetic foot infection management and it seems difficult that they can achieve their objectives separately. The objective of successful diabetic foot infection management can only be achieved by efficient teamwork.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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Current treatment of nosocomial pneumonia and ventilator-associated pneumonia

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ABSTRACT

Hospital-acquired pneumonia and ventilator-associated pneumonia are severe nosocomial infections leading to high morbidity and mortality. Broad-spectrum antibiotics with coverage against all likely pathogens are recommended by the international guidelines. Inappropriate empirical treatment is one of the most important prognostic factors. Knowledge of local epidemiology and continuous microbiological surveillance is crucial for improving clinical approaches to empirical antimicrobial treatment. The development of protocols and policies for training healthcare professionals on preventive strategies, such as the "Pneumonia Zero" project, and improved implementation of antimicrobial stewardship practices, will aid early de-escalation of antibiotics and prevent resistance.

Keywords: hospital-acquired pneumonia, ventilator-associated pneumonia, multidrug-resistance, antimicrobial stewardship

INTRODUCTION

Hospital-acquired (HAP), hospital-acquired pneumonia requiring mechanical ventilation or ventilated HAP (vHAP), and ventilator-associated pneumonia (VAP) are three conditions associated with a high risk of death and morbidity. The severity of illness and infections caused by multidrug-resistant (MDR) organisms are two factors associated with the worst outcomes. In general, the negative consequences of initial inappropriate antibiotic therapy may be less pronounced in non-ventilated HAP than in VAP, given that HAP patients tend to be less severely ill. MDR pathogens tend to be less common in HAP patients who develop the infection outside of the intensive care unit (ICU), particularly early in the hospitalization course.

Data from the National Surveillance Program of ICU-Acquired Infection in Europe Link for Infection Control through Surveillance (ENVIN-HELICS) [1], elucidated that the likelihood of receiving inadequate empirical treatment for VAP caused by *Pseudomonas aeruginosa* is around 30%, even in patients receiving combination treatment. Antimicrobial stewardship programs are central to minimize the effects associated with the use of antimicrobials (e.g., drug resistance, toxicity), while promoting the use of cost-effective treatments. Local antibiotic resistance is strongly affected by local antibiotic prescription policies.

Herein we propose an approach to the empirical treatment of HAP and VAP, and targeted use of antimicrobials in the context of MDR organisms. Carbapenem-sparing treatments will be reviewed to provide an approach to new therapies.

EMPIRICAL THERAPY: SEEKING FOR RISK FACTORS TO MDR ORGANISMS

The latest international guidelines in Europe and America serve as tools for the management of HAP and VAP [2-4]. The appropriate antimicrobial regimen for HAP depends upon the presence or absence of risk factors for MDR pathogens, knowledge of the predominant pathogens (and susceptibility patterns) within the health care setting (local ecology), and the individual patient's prior microbiology data (surveillance cultures). Still, the appropriateness of empirical therapy may sometimes be challenging, and the risk of developing infections caused by MDR microorganisms during treatment may lead to adverse outcomes and increased mortality. Clinicians should differentiate VAP from vHAP, as clinical implications, prognosis, and approach to treatment may vary [5,6].

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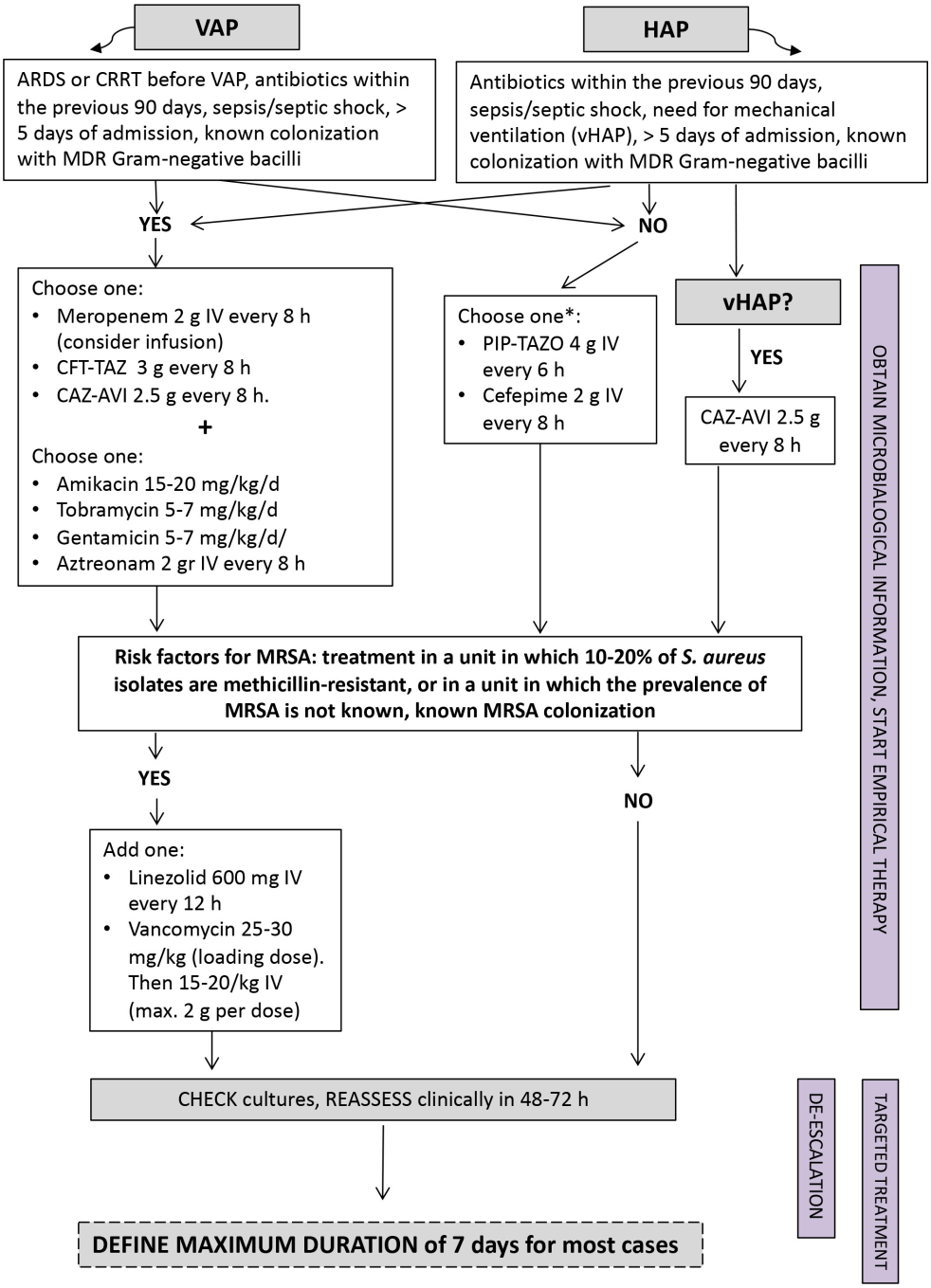

Figure 1 Management of HAP, vHAP and VAP.

Risk factors for MDR gram-negative bacilli: Local prevalence of >10%, unknown local epidemiology, septic shock, acute respiratory distress syndrome prior to VAP, renal replacement therapy, received antibiotics in the last 90 days, ≥ 5 days of hospital stay, known previous colonization (microbiological surveillance).
Risk factors for MRSA: local prevalence >10-20%, unknown prevalence of MRSA, influenza infection.

Table 1 **Most recommended treatment options for HAP, vHAP and VAP.**

Antimicrobial	Characteristics	Use
Piperacillin-tazobactam	In patients with a low-risk for MDR gram-negative bacilli (e.g., ESBL-E, MDR P. aeruginosa, MDR *Acinetobacter* spp.)	Appropriate treatment for early-onset HAP and VAP, in patients with no risk factors for ESBL-E
Meropenem	Coverage to many pathogens, including ESBL-E and P. aeruginosa.	In patients at high-risk for MDR Gram-negative bacilli, is a good option. The recommended standard dose for VAP is 2 gr every 8 h. Prolonged infusions of meropenem can be considered for ventilated HAP and VAP
Ceftolozane/tazobactam (CFT/TAZ)	Various experts recommend this agent to treat *P. aeruginosa* infections	The ASPECT-NP trial demonstrated the non-inferiority of CFT/TAZ when compared with meropenem 1 g every 8 hours. The recommended dose for VAP is 3 g by intravenous infusion within 3 hours every 8 h [12]. The APEKS-NP trial demonstrated cefiderocol was non-inferior to high-dose (2 g), extended-infusion meropenem in terms of all-cause mortality on day 14 in patients with Gram-negative nosocomial pneumonia [17]
Ceftazidime/avibactam (CAZ/AVI)	Third-generation cephalosporin with activity against serin-carbapenemase-producing *P. aeruginosa*, class A, C, and D (OXA-48) beta-lactamases. CAZ/AVI does not have activity against metallo-b-lactamases.	Treatment of infections caused by CPK-like carbapenemase-producing *Enterobacteriaceae*
Cefiderocol	This antibiotic was recently approved by the FDA to treat urinary tract infections. Coverage to metallo-β-lactamases.	VAP: The CREDIBLE-CR trial found that the clinical and microbiological efficacy of cefiderocol was similar to the best available treatments in patients with infections caused by carbapenem-resistant Gram-negative bacteria, including hospital-acquired pneumonia and VAP [13]
Adjunctive aerosolized antibiotics	Controversial, the best available evidence does not support their use. Intravenous colistin is not recommended	-VAP: IASIS and the INHALE trials, did not achieve their primary endpoints [14,15]. The MAGIC-BULLET trial was unable to demonstrate the non-inferiority of colistin compared to meropenem, both in combination with levofloxacin [11]. -VAP caused by MDR *A. baumannii*: aerosolized colistin may be an option as rescue therapy when other systemic treatments fail [16]
Linezolid	For patients admitted in units with a prevalence of MRSA of > 10-20%, or with known colonization by MRSA. Some studies show superior efficacy of linezolid than vancomycin, better lung tissue penetration, and lower incidence of nephrotoxicity	For HAP and VAP cases with known colonization with MRSA, MRSA prevalence > 20% or unknown.

VAP: ventilator-associated pneumonia, HAP: hospital-acquired pneumonia, vHAP: ventilated hospital-acquired pneumonia, ESBL-E : Extended spectrum beta-lactamase *Enterobacteriaceae*, MDR: multidrug-resistant, MRSA: methicillin-resistant *S. aureus*.

Different characteristics predispose individuals to acquired MDR microorganisms, such as the recent use of broad-spectrum antimicrobials, sepsis or shock, known unfavorable local ecology, known colonization with MDR organisms, a recent or current hospitalization for > 5 days [7]. However, there are significant differences in the local prevalence of MDR organisms among centers [8]. Each institution should analyze their local epidemiological data and not rely on national or regional data. In Spain, the 2019 ENVIN-HELICS report quantifies the antibiotic resistance of the most important microorganisms. The report describes all data related to the expected resistance rates in the different ICUs [1].

The MDR organisms most commonly involved in VAP are MDR *P. aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E), *Acinetobacter baumannii*, and carbapenemase-producing *Enterobacteriaceae* (CPE). Once a patient is known to be colonized by an MDR organism, empiric antimicrobial treatment should target such pathogen only if previously described as a potential cause of the suspected infection. The location of colonization is also important.

PRESCRIPTION OF EMPIRICAL THERAPY

Current treatment recommendations for HAP, vHAP and VAP are summarized in Figure 1 [9-12]. New-generation drugs have been clinically validated for the treatment of increasingly common MDR organisms, such as MDR *P. aeruginosa*, ESBL-E, and CPE. Some drugs are useful for improving carbapenem-sparing policies. The Table 1 summarizes the most recommended treatment options for nosocomial pneumonia, including treatments for patients with risk factors for MDR organisms [10,11,13-17]. Also, antimicrobial coverage against MRSA can be added to empirical regimens [18-20].

ANTIMICROBIAL STEWARDSHIP IN NOSOCOMIAL PNEUMONIA

The indiscriminate use of antibiotic combinations can induce the emergence of highly resistant strains. The reassessment of an individual's clinical status at 48-72 hours of initiation of treatment and the use of procalcitonin kinetics when there is clinical uncertainty, could be useful to guide de-escalation and prevent the development of resistance. Advances in developing tools for the rapid diagnosis of nosocomial pneumonia and improved implementation of antimicrobial stewardship programs will reduce the exposure to unnecessary antibiotics.

A 7-day course of antimicrobial therapy is widely recommended by the American and European guidelines, as prolonged courses of antibiotics promote the emergence of resistance. However, the optimal duration of therapy for MDR organisms has not been clearly defined.

CONCLUSIONS

Nosocomial pneumonia is a health-care related infection with significant consequences for the patient and the healthcare system. Appropriate empirical treatment and early de-escalation should be implemented to increase the chance of survival.

Identifying risk factors for MDR organisms, local policies to improve antimicrobial stewardship, and knowledge of local ecology and previous colonization, are of outstanding importance. Healthcare workers should be trained to implement recommended preventive measures, such as adequate hand hygiene and respiratory devices management.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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Optimization of antimicrobial treatment in sepsis

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ABSTRACT

Sepsis represents a serious risk to the life of any patient, which is why it is crucial to start an effective treatment in all its extremes as soon as possible, that is, the chosen antibiotics must have activity against the pathogen that produces the condition and, in addition, they must be dosed considering the patient's situation in all its extremes. It should be considered that it will be necessary to adjust the dose when there is edema (drugs with reduced volume of distribution), hypoproteinemia (drugs bound to proteins in a high proportion), obesity, and also when they require the use of external techniques such as ECMO or any of the different types of hemodialysis and hemofiltration.

Keywords: antibiotics, treatment, sepsis.

The optimization of antimicrobial treatment in patients with sepsis becomes a priority since the correct choice of drug will lead to beneficial effects, while errors often have dire consequences for the patient. The list of criteria to consider in choosing the drug is extensive and often strictly followed by the prescribing physician, and despite this, it is not uncommon for an inadequate dosage regimen to be prescribed, generally because not all adjustment criteria have been considered and especially, those related to the PK/PD relationship.

Currently antibiotics tend to be grouped into one of the PK/PD models; fCmax/MIC, fT>MIC and fAUC/MIC requiring a different dosage plan. In the case of fCmax/MIC adjustment, a high dose should be administered that allows Cmax values of free drug to be 10-12 times the MIC (aminoglycosides and fluoroquinolones). The drugs whose ajsute corresponds to the efficacy time fT>CMI; Basically, beta-lactams are administered in short intervals or extended and even continuous perfusions, so that the value of the PK/PD parameter is close to 100%. Finally, it is recommended to prescribe doses and intervals that facilitate reaching fAUC/MIC values between 80 and 100 (linezolid, daptomycin) or greater than 400, in the case of vancomycin [1,2].

This type of dosage adjustment must also take into account the multitude of situations specific to the patient that alter the volume of distribution and/or the clearance of drugs, which affects the production of different plasmatic concentrations; high or low, which may justify inefficacy or adverse effects. The patient in a situation of sepsis is an example in which these types of alterations are almost constant. Next, the alterations that generate changes in the volume of distribution will be briefly described; edema, hypoproteinemia, obesity and/or clearance; use of external techniques; ECMO and other purification systems. Renal or hepatic failure will not be included in this description since there is abundant information on the dose adjustment of each of the drugs used in the treatment of patients with sepsis [3].

Edema. Edema is characterized by the increase in water in the extravascular, interstitial space and with it the increase in the volume in which some drugs are dissolved. The impact on drug concentrations is very different depending on the type of distribution that characterizes them. A simple numerical example allows us to appreciate these differences. A drug that has a volume of distribution of 20L and is administered intravenously at a dose of 1 g will reach a maximum plasma concentration of 50 mg/L (1000 mg divided by 20 L). If this drug is administered to a patient with edema, for example 10L of water in the form of edema, the concentration reached will be clearly lower; 1000 mg divided by 30 L; 20+10 L of edema. In this case, the maximum plasma concentration will be 33 mg/L, that is, approximately 60% lower, hence, to reach the same concentration as if there were no edema, it is necessary to increase the dose.

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	Weight Mol.	P- B.(%)	V (l)
Piperacillin	516	30	18
Ceftaroline	744	20	20
Ceftazidime	546	10	20
Ceftriaxone	554	85 - 95	14
Meropenem	383	2	20
Cefiderocol	752	40-60	18
Tazobactam	300	30	20
Avibactam	265	8	22
Vaborbactam	297	30	18
Relebactam	348	22	19
Daptomycin	1619	90-93	10
Dalbavancin	1816	93	13
Vancomyciny	1449	50-60	50
Colistin	1155	10	20

	Peso mol.	F. P. (%)	V (l)
Gentamicin	477	30	20-30
Amikacin	585	10	20-30
Levofloxacin	361	30-40	100
Ciprofloxacin	331	20-30	140-200
Linezolid	337	30	40-50
Tedizolid	370	90	65-80
Tigecycline	585	70-89	500-700
Metronidazole	171	20	40-50
Rifampicin	822	80-90	110
Clarithromycin	722	80	200-400
Clindamycin	424	90	60-70

https://pubchem.ncbi.nlm.nih.gov/compound/ https://go.drugbank.com/drugs/

Figure 1 | **Bound protein**

If we repeat the calculations administering the same dose of another drug that, in relation to its chemical structure, has a greater volume of distribution; for example, 100 L, the plasma concentration that will initially be 10 mg/L, will become, in the patient with 10 L of edema, 9.09 mg/L, that is to say, practically only 10% lower, hence no require dose adjustment.

Therefore, the aforementioned dose adjustment will only apply to those antibiotics that have low distribution volumes, approximately 0.5 L/kg or less; ie beta-lactams, aminoglycosides, glycopeptides, lipopeptides, polypeptides. In this case, estimating the edema volume to add it to the conventional distribution volume of the drug can be a very useful tool to avoid sub-therapeutic concentrations.

Plasma proteins. Some antibiotics circulate in plasma bound to proteins in a high proportion. The protein that most frequently participates in this transport is albumin and, much less frequently, alpha-glycoprotein. The presence of a reduced concentration of this type of protein in plasma means that the free fraction of circulating fixed drugs increases and contrary to what could be deduced, the overall balance is a reduction in concentrations since the increase in the free fraction It implies greater distribution and greater clearance. There is no adequate method to adjust the dose since there is no methodology to estimate the impact of this phenomenon on the concentration, which, on the other hand, only becomes relevant in drugs with very high protein binding (Figure 1); Daptomycin, dalbavancin, tedizolid, ceftriaxone, tigecycline, and rifampin are the most notable examples of drugs with potential use in patients with sepsis [4].

Obesity. It is probably the disease that entails the greatest difficulties in adjusting the dose of any drug. In a generic way, it is usually pointed out that the most fat-soluble drugs are the ones that need to be adjusted to the real weight of people, while the water-soluble ones could be administered at the ideal weight, sometimes called lean weight, that is, the one corresponding to the sex, age and height of the person. person. To assess the degree of lipid solubility of a molecule, it is necessary to know its partition coefficient, which is estimated through LogP; quotient between the concentration of non-ionized solutes in octanol and that in water, both expressed in natural logarithms. The presence of a negative number expresses water solubility, while a positive value indicates lipid solubility (Figure 2). Following this criterion, fat-soluble drugs should be administered according to the actual weight of the patient, but accumulated experience indicates that this type of adjustment carries a risk of overdosing, since the volume of distribution of these drugs is not directly related to weight gain. In fact, the tendency is to make a less aggressive adjustment, using the so-called adjusted weight, which is calculated using the ideal weight, to which is added a proportion of overweight, which is usually 0.3-0.4 [5-8]. Figure 2 includes the description of the type of adjustment that is currently considered appropriate for each of the antibiotics.

ECMO (Extracorporeal Membrane Oxygenation). Extracorporeal membrane oxygenation (ECMO) is an extrapulmonary gas exchange system that allows oxygenation and CO_2 extraction through a membrane connected to an external venovenous or venoarterial bypass system.

There are multiple factors inherent to the circuit, the drugs and the patient's situation that make it difficult to estimate the impact on the drugs and thus the type of adjustment to be made.

hydrophilic	LogP	
Daptomycin	-9,4	ABW
Dalbavancin	-3,8	¿?
Vancomycin	-3.1	ABW
Colistin	-2,4	ABW
Amikacin	-7,9	ABW
Gentamicin	-6,2	ABW
Piperacillin	-0,26	DM
Ceftaroline	-3,7	
Ceftazidime	-1,6	
Ceftriaxone	-1,3	
Meropenem	-3,0	
Cefiderocol	-2,9	
Tazobactam	-2	
Avibactam	-1,8	
Vaborbactam	-1	
Relebactam	-3,6	

lypophilic	LogP	
Levofloxacin	0,65	SC
Ciprofloxacin	-0,57	SC
Linezolid	0,9	SC
Tedizolid	2,12	SC
Tigecycline	1,1	SC
Metronidazole	-0,15	TBW
Rifampicin	4,9	TBW
Clarithromycine	3,2	TBW
Clindamycin	2,2	TBW

DM: Maximun dose

CS : Without changes

ABW: adjustment to excess weight

Dose (ABW): ideal weight + ((Total weight- ideal weght) x 0,3-0,4)

TBW: adjustment to actual weight

https://pubchem.ncbi.nlm.nih.gov/compound/ *https://go.drugbank.com/drugs/*

Figure 2 | **Log P and adjustment of dose to weight**

On the part of the drug, it seems that lipid solubility leads to an increase in the volume of distribution, and a reduction in clearance, probably due to sequestration of the drug in the membrane. In addition, protein binding is a factor associated with reduced clearance and also volume of distribution in patients on ECMO. Based on their protein binding (>70%) and their partition coefficient (>2), tedizolid, rifampicin, clarithromycin or clindamycin are the antibiotics that are most likely to be affected by membrane sequestration (Figures 1 and 2). Other drugs affected in relation to high protein binding are cloxacillin, ceftriaxone, dalbavancin, daptomycin, and tigecycline. It is likely that all these drugs require the administration of higher doses and more frequently, but at the moment there are no clear criteria for making this type of adjustment [9-12].

External debugging techniques. This type of technique is of great importance in the maintenance of critical patients with or without sepsis. Its impact on the pharmacokinetics of antibiotics can become very evident and again this impact depends on a large number of circumstances. For antibiotics, volume of distribution, protein binding, and molecular weight are inversely related to the efficacy of elimination techniques. In other words, the higher the values of these parameters, the lower the amount of drug eliminated. The vast majority of beta-lactam antibiotics (except ceftriaxone, cefonid or ertapenem), vancomycin, aminoglycosides and colistin are found in this situation, drugs that will require the administration of doses higher than the conventional adjustment in relation to the patient's renal function [13,14].

CONFLICT OF INTEREST

Authors declare no conflict of interest

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Global strategy in the treatment of HIV infection in 2022

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ABCTRACT

The treatment of HIV infection has become a cornerstone for the global control of the pandemic due to its benefits on individual health and for preventing the transmission of HIV. It should be started in all people with HIV infection and as quickly as possible. Ideally, it should be started on the same day of diagnosis or, failing that, within the first 7 days. Antiretroviral regimens with excellent efficacy, no significant toxicity, and convenient administration are currently available for initiation of antiretroviral treatment. They can incorporate two or three drugs and are always based on a second-generation integrase inhibitor.

Keywords: HIV, antiretroviral therapy, dolutegravir, bictegravir.

INTRODUCTION

The treatment of HIV infection has become a cornerstone for the global control of the pandemic. Antiretroviral treatment (ART) not only has benefits on the individual health of people, by reducing complications and improving survival, but also prevents the transmission of HIV, significantly reducing the appearance of new infections. For all these reasons, it is crucial to incorporate into clinical practice the results of clinical trials and observational studies that guarantee therapeutic success with the minimum risk for patients.

There are national and international guidelines with recommendations for the treatment of HIV based on the best scientific evidence [1-5]. Surely the most important part of these guidelines is the one that refers to the initial treatment of HIV infection. When and with what to start ART is key, since in many cases it can determine the overall evolution of a patient. For this reason, we will briefly summarize the main recommendations in this regard collected from the most followed guides.

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WHEN TO START ANTIRETROVIRAL TREATMENT

The optimal time to start ART has evolved over time. After the results of randomized clinical trials on the optimal time to start treatment to preserve the health of patients [6] and prevent transmission of infection [7] and large observational studies [8], the recommendation is unanimous: ART should be started in all person with HIV regardless of other factors, such as the presence of clinical symptoms, CD4+ T cell count, and viral load levels. In this way, patients and the community are benefited, the quality and quantity of life of patients is increased and the appearance of new cases is avoided, helping to control the pandemic.

The unanimity in this recommendation has led to pursuing a more demanding objective. The standard of treatment included not starting ART until an initial visit had been made with the patient, the pros and cons of treatment had been discussed, and the necessary tests had been ordered to choose the best therapeutic regimen. From the patient's first office visit, initiation of ART could be delayed by 3 to 6 weeks. Some clinical trials and observational studies showed that this delay could be associated with worse outcomes in patients [9]. For this reason, WHO and other organizations and institutions changed their recommendations and propose starting ART as soon as possible, ideally in the first week after diagnosis (rapid start). Furthermore, if the patient is prepared, the recommendation would be to start ART the same day as diagnosis (immediate start).

Conclusion. Antiretroviral treatment should be started in all people with HIV infection and as quickly as possible. Ideally, it should be started on the same day of diagnosis or, if not possible, within the first 7 days.

WITH WHAT TO START ANTIRETROVIRAL TREATMENT

Also in this regard, considerable unanimity has been reached in the different guidelines. The variety of regimens and drugs that were once part of the recommended regimens for initiating ART have been greatly simplified. It should be clarified that all the guidelines include regimens of choice, understanding that they are applicable to the vast majority of patients, but the regimens considered alternative may perfectly be the regimens of choice for a patient and even a group of patients.

The current recommendations can be summarized in two points:

1. How many drugs should be included in an initial regimen: The magic figure of 3 drugs has been altered by the results of randomized clinical trials [10]. A two-drug regimen alone has been shown in these clinical trials to be noninferior to a three-drug regimen in terms of virologic efficacy, rate of discontinuation due to toxicity, rate of any grade undesirable effects, and rate of resistance. after failure. Since the publication of these trials, all the guidelines include the two-drug regimen made up of lamivudine (3TC) and dolutegravir as the drug of choice for starting ART. The guidelines differ in the limitations for this double regimen as initial therapy, including high viral loads (>500,000 copies HIV RNA/ml), low CD4 count (<200 cells/mm3), active infection by the hepatitis B virus (surface antigen positive), pregnancy, absence of transmitted resistance tests before starting ART, and rapid initiation of ART.

2. With which drugs should ART be started: in both triple and dual regimens, the central element is a second-generation integrase inhibitor (dolutegravir or bictegravir in triple regimens, dolutegravir in dual regimen). Along with the integrase inhibitor, two nucleoside reverse transcriptase inhibitor analogs are associated in triple regimens, or a single analog in double regimens. The constant nucleoside analog is 3TC or FTC in all regimens. Furthermore, in three-drug regimens, some guidelines only include tenofovir alafenamide (TAF) while others also include tenofovir disoproxil fumarate (TDF) or abacavir. Table 1 shows the main initial therapeutic regimens included in the guidelines, which are usually recommended to be administered as a single pill. With these regimens, virological efficacy is achieved in practically all patients, with very low toxicity and the absence of resistance mutations in case of failure.

It should be noted that some scientific societies include two additional drugs: the integrase inhibitor raltegravir and the non-nucleoside analogue doravirine, having demonstrated its efficacy and low toxicity in clinical trials [3].

Conclusion. Antiretroviral regimens with excellent efficacy, no significant toxicity, and convenient administration are currently available for initiation of antiretroviral treatment. They can incorporate two or three drugs and are always based on a second-generation integrase inhibitor.

Table 1 Main therapeutic regimens recommended for initial ART.

Type of regimen	Drugs
Triple (3 drugs)	Bictegravir/FTC/TAF
	Dolutegravir/3TC/abacavir
	Dolutegravir + FTC/TAF
Dual (2 drugs)*	3TC/dolutegravir

FTC, emtricitabina; TAF, tenofovir alafenamide; 3TC, lamivudine
*With caveats (variable according to the different guidelines)

CONFLICT OF INTEREST

Authors declare no conflict of interest

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Antimicrobial resistance and One Health

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ABSTRACT

Antimicrobial resistance is one of the major health problems we face in the 21st century. Nowadays we cannot understand global health without the interdependence between the human, animal and environmental dimensions. It is therefore logical to adopt a "One Health" approach to address this problem. In this review we show why a collaboration of all sectors and all professions is necessary in order to achieve optimal health for people, animals, plants and our environment.

Keywords: One Health, antimicrobial resistance, last-resort antibiotics

Since ancient times, infectious diseases have been one of the greatest health problems faced by humankind. The discovery of penicillin by Alexander Fleming in 1928 represented a worldwide revolution and one of the greatest achievements in medicine, since the use of antibiotics made it possible to deal with infectious diseases that had been fatal until then [1]. In the decades following the discovery of penicillin, numerous new molecules with antimicrobial activity were found, thus initiating the "golden age" of antibiotic discovery between the 1940s and 1960s. Antimicrobial agents were classified into different groups according to their mechanisms of action, e.g. molecules that target the cell wall (beta-lactam and glycopeptides), inhibitors of protein biosynthesis (aminoglycosides, tetracyclines, macrolides), inhibitors of DNA replication (quinolones) and folic acid metabolism inhibitors (sulfonamides and trimethoprim) [2]. From the earliest stages, antibiotics began to be used on a massive scale in both human and veterinary medicine, without full awareness of the implications that their indiscriminate use could entail. After the introduction of antibiotics, the development of resistance to them was assumed to be unlikely, based on the theory that the frequency of mutations that generated resistant bacteria was insignificant. Unfortunately, this was not the case, and almost simultaneously with the discovery and use of new antibiotics, it was observed that bacteria could develop a wide variety of mechanisms that made themselves resistant to them [3]. Nowadays it is well known that bacteria can develop resistance to antibiotics through mutation or acquisition of genetic material by conjugation, transformation and/or transduction, known as horizontal gene transfer (HGT) mechanisms. The rapid emergence and dissemination of these mechanisms of resistance to all antibiotics commonly used in the clinical setting, together with the scarce discovery or synthesis of new antibiotics by pharmaceutical companies in recent years, make antimicrobial resistance (AMR) one of the most serious threats to world health in the 21st century. According to The Review on Antimicrobial Resistance chaired by Jim O'Neill, approximately 700,000 people die each year globally as a result of antibiotic-resistant infections and AMR could kill 10 million people by 2050, surpassing other pathologies such as cancer [4]. For this reason, the World Health Organization (WHO) has recently reviewed the current pipeline and together with the Group of Eight (G8) declared this problem as a priority and are implementing action plans to address [5]. Wherever antimicrobials are used, reservoirs of AMR genes and drug-resistant pathogens emerge, including humans, companion and production animals, food and environment (Figure 1).

ANTIOTICS, ANIMALS AND THE FOOD CHAIN

It is widely known that antimicrobials can be used as growth promoters, e.g., given low doses to animals, giving rise to a 30-40% higher weight gain. This practice is however forbidden in January 2006 in Europe, and strictly controlled. In January 2017, the USA stopped using antimicrobials used in human medicine, as growth promoters. In some parts of the world, however, this is still a common practice. Further, a wide number of people in the society still believe, that antimicrobi-

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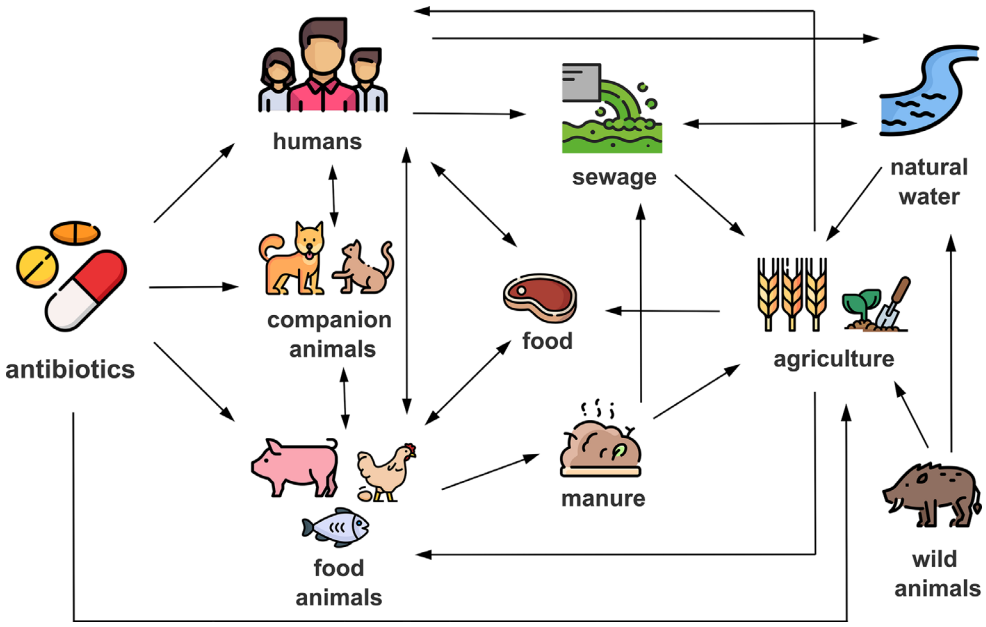

Figure 1 | **Diagram of the transmission routes of antimicrobial resistance between humans, animals and environment.**

als used in animals flow from the animals to the food chain. However, antibiotics and all medicines used in food-producing animals are associated with a withdrawal period. That means that when an animal is treated, depending on the drug, there is a period of time in which none of the animal's food products can be used for human consumption [6]. This is, especially in developed countries, a strictly controlled process, that includes analysis of farms and food throughout the complete food chain in order to ensure that no residue levels of any antibiotic are exceeded. Thus, the use of antibiotics in food-producing animals is a necessity as animals eventually get ill. However, the routine use of antibiotics in animals needs to be controlled because antimicrobial resistant bacteria emerge and may eventually reach humans or accelerate the emergence of resistance [3]. In the One health concept, all actors involved in or with antimicrobials could contribute to delay AMR. In the same line, in many countries worldwide antibiotics can be purchased without prescription, being a major driver of the emergence of antimicrobial resistance in the community.

ANTIBIOTIC-RESISTANT PATHOGENS: A CRITICAL ONE HEALTH ISSUE

Among the drug-resistant pathogens, one of the main concerns nowadays is multidrug-resistant (MDR), extensively drug-resistant (XDR) or even pandrug-resistant (PDR) *Enterobacteriaceae*. Apart from *Enterobacteriaceae*, the WHO has identified bacteria critically important for their resistance and clinical relevance (Table 1).

To illustrate AMR and One Health, over the last 15 years, 16S rRNA methylases (as *armA* gene) that possess the capacity to completely nullify the efficacy of aminoglycosides in clinical practice have been identified worldwide. Although the *armA* gene was initially isolated in a *Klebsiella pneumoniae* from a urinary tract infection of human origin [7], it was subsequently identified in an *Escherichia coli* pig isolate [8]. The importance of coordinated surveillance of human and animal isolates was recognized immediately. Over the following years, the presence of ArmA methylase was reported in *Salmonella enterica* isolates from food [9], in *K. pneumoniae* isolates from companion animals [10] and in members of the *Enterobacteriaceae* family from aquatic environments (wastewater treatment plants) [11]. A real concern is that most of these methylases are often part of mobile genetic elements (MGEs) such as plasmid-mediated transposons, increasing the possibility of horizontal transfer of these elements and their dissemination between bacterial species, genera and families, usually associated with genes encoding resistance to other classes of antibiotics. MGEs (such as phages and plasmids) play a key role in the development and dissemination of AMR. A recent study has shown that phages, viruses that are widespread in all environments and infect and replicate within bacteria, represent a novel high-efficiency transmission route of AMR genes [12]. Phages are capable to encapsidate and transfer AMR genes, and they do this in a highly efficient way when these genes are carried on small plasmids [12]. These elements, plasmids, are the most relevant MGEs in the evolution of bacterial resistance to antibiotics in clinical settings. Plasmids are small circular DNA molecules that replicate independently of the

Table 1 **WHO list of AMR priority pathogens for research and development of new antibiotics.**

Species	Type of Antibiotic Resistance
Critical	
Acinetobacter baumannii	Carbapenem-resistant
Pseudomonas aeruginosa	Carbapenem-resistant
Enterobacteriaceae	Carbapenem-resistant, ESBL-producing
High	
Enterococcus faecium	Vancomycin-resistant
Staphylococcus aureus	Methicillin-resistant, vancomycin-intermediate and resistant
Helicobacter pylori	Clarithromycin-resistant
Campylobacter spp.	Fluoroquinolone-resistant
Salmonellae	Fluoroquinolone-resistant
Neisseria gonorrhoeae	Cephalosporin-resistant, fluoroquinolone-resistant
Medium	
Streptococcus pneumoniae	Penicillin-non-susceptible
Haemophilus influenzae	Ampicillin-resistant
Shigella spp.	Fluoroquinolone-resistant

bacterial chromosome and are transferred through a protein tunnel that directly connects to bacteria in a process known as conjugation. In a One Health context, plasmids bearing AMR genes can be thus selected in bacterial species adapted to a given environment, e.g., river, dog, hospital sink, and serve as a reservoir for genes that can be mobilized through the plasmids to pathogenic bacteria for humans. For this reason, it is not enough to try to isolate disease-causing bacteria in humans in other ecological niches to identify their reservoirs. We need genomic approaches to find mobile genetic platforms, often, but not only, plasmids, in different environments to assess their role in AMR in humans.

In 2015, a plasmid-mediated colistin resistance (*mcr-1*) gene was reported for the first time in food animals, food and humans in China [13]. Why did this report have a big impact? Because colistin is a crucial last-resort option. Colistin has been used, both in human and veterinary medicine, for more than 50 years. The drug was stopped for use in humans because of its side effects (nephrotoxicity and neurotoxicity). However, colistin has recently and increasingly been used to treat patients with infections caused by multidrug-resistant bacteria against which colistin remains effective, despite its side effects [13]. The use of colistin in veterinary medicine has been quite different, where colistin is widely used, especially for controlling diarrheal diseases in pig and poultry production. In 2012, it was estimated that colistin consumption was on average more than 600 times higher in food animals than in humans in the European Union. Data from other parts of the world are scarcer, however, China was reported to be the largest user with an expected 12,000 tons in 2015 [13]. But it doesn't stop there, following the identification of this new gene spread worldwide, bacteria in which *mcr-1* coexists with genes that have the ability to hydrolyze penicillins, cephalosporins, monobactams and carbapenems (carbapenemase genes) were reported [14]. This combination is of particular concern, as carbapenems are also last-resort antibiotics, and these bacteria could cause truly untreatable infections. In Spain, the Spanish Agency of Medicines and Medical Devices (AEMPS) has coordinated efforts from a One Health perspective involving professionals from different sectors (human, animal and environmental health) to fight against AMR. In 2014, AEMPS implemented and approved the Spanish Action Plan on Antimicrobial Resistance (PRAN). Among other achievements, the REDUCE program successfully reduced the use of colistin in swine production by 98.88% (1015-2020) and 97% in poultry production (2015-1019) [15].

AMR AND AQUATIC ENVIRONMENTS

Finally, an important (and sometimes somewhat overlooked) component of One Health concept is the environment. We know, that when antimicrobials are used clinically, most active molecules are secreted by the patient, human or animal, through urine or feces. These active molecules, together with the bacteria and mobile genetic elements flow then into the wastewater, mix, and eventually are shared between bacteria, including environmental bacteria. Thus, we can find antimicrobial resistant genes in clinically important bacteria when we analyze the hosts, but within environmental bacteria when we analyze wastewater and environmental reservoirs [11]. Identi-

fying and characterizing the sources of AMR emissions to the environment is crucial and, for this reason, a great emphasis has been placed in recent years on this issue. This situation is especially notorious in wastewater treatment plants (WWTPs) since they collect residual waters from diverse origins and populations where distinct anthropogenic activities occur and where processes often do not sufficiently neutralize antibiotic resistant bacteria and genes. A recent work demonstrated that wastewater environments promoted the expansion of conserved *E. coli* sequence types (STs), bacteria that share a specific allelic profile, and resistance gene flow through highly disseminated plasmids, leading to specific associations between plasmids and STs [11]. WWTPs allowed the exchange of a diverse genetic repertoire, and therefore, continued close monitoring of these hotspots is needed.

AMR is an increasingly worrisome phenomenon. In this short review, we have shown how this problem involves several actors. Regarding human health, AMR jeopardizes the use of so-called last-resort antibiotics (such as colistin and carbapenems). In recent years, action plans are being implemented involving and coordinating both sectors. We cannot fully understand the problem without considering the environment, which serves as the common link to global health. Therefore, efficient collaboration between all actors involved (humans, animals and environment) is crucial to combat this uncontrolled pandemic.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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Update on antimicrobial pharmacotherapy against multidrug-resistant Gram-negative bacilli

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Controversies in the management of ESBL-producing *Enterabacterales*. Clinical Implications

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ABSTRACT

Extended-spectrum β-lactamases (ESBL)-producing organisms currently represent a major health problem. Although recently published guidelines still consider carbapenems as the treatment of choice for ESBL-producing infections, it is necessary to find non-carbapenem β-lactams as alternatives to reduce the effects associated with their overutilization.

In this review we focus on these alternatives to carbepenem use. It is possible that piperacillin-tazobactam may be an alternative in clinical settings with "low inoculum" infections like urinary tract infections. Newer β-lactam-β-lactamase inhibitors (BLBLIs) are potential options too. The current available data support the efficacy of both ceftazidime-avibactam and ceftolozane-tazobactam against susceptible ESBL-producing *Enterobacterales* (ESBL-E). We are waiting for the results of MERINO-3 study to confirm whether ceftolozane-tazobactam is a good option versus meropenem for treating bloodstream infections caused by ESBL- or AmpC-producing *Enterobacterales*.

Keywords: Extended-spectrum β-lactamases, *Enterobacterales*, management

Extended-spectrum β-lactamases (ESBL) offer resistance to penicillins and cephalosporins. ESBLs are present in several gram-negative organisms, being more prevalent among *Enterobacterales* such as *Escherichia coli* and *Klebsiella species*. ESBL-producing *Enterobacterales* (ESBL-E) were described in the 1980s and they currently represent a global crisis [1-3].

The progressive and worrying appearance in recent years of microorganisms resistant to carbapenems linked, among other causes, to carbapenem overuse has highlighted the need to assess the use of other non-carbapenem β-lactams as therapeutic alternatives to treat infections caused by ESBL-E. However, the different recently published guidelines continue to consider carbapenems as the antibiotics of choice for the treatment of ESBL-causing infections [4-6] as they are stable to ESBL hydrolytic activity and offer favorable results on their clinical efficacy in different studies [7].

In this brief review, we will assess the available data on the use of non-carbapenem β-lactams as therapeutic alternatives to carbapenems for the treatment of ESBL-E producing infections, focusing on the use of piperacillin-tazobactam (PTZ) and the role of newer β-lactam-β-lactamase inhibitors (BLBLIs).

PIPERACILLIN-TAZOBACTAM (PTZ)

According to CLSI [8] and EUCAST [9], the breakpoints for PTZ are ≤16 mg/L y ≤ 8 mg/L, respectively. Although ESBLs are usually inhibited by β-lactamase inhibitors, ESBL-E may present resistance mechanisms to BLIBLs, because β-lactamases are not susceptible to inhibition due to the co-production of Amp-C or OXA-1 type enzymes, overproduction of ESBLs and/or mutations in permeability, and even by a possible "inoculum effect" demonstrated *in vitro*, in animal models and in clinical cases, which would affect PTZ above all [10,11].

Different observational studies have shown contradictory results in patients with infections caused by ESBL-E who were treated with PTZ and carbapenems. One of the initial works that evaluated the difference in mortality in treatment with BLBLIs and carbapenems in ESBL-E bacteremia was a *post hoc* observational study carried out in Spain on 6 cohorts of patients [12]. 70% of the bacteremia had a urinary or biliary origin ("low-inoculum" infections), and only 13% of the patients needed to be admitted to the intensive care unit (ICU). Thirty-day mortality was 10% and 19% in the empiric cohort and 9% and 17% in the definitive cohort for BLBLIs and carbapenems, respectively, although these differences did not reach statistical significance. In the Ofer-Friedman and colleagues'

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study, the mortality was compared between BLBLI and carbapenems for the treatment of ESBL bacteremia, excluding urinary sources [13]. Thirty-day mortality was 60% for the PTZ group and 34% for the carbapenem group, without statistical significance (P = 0.10).

According to these results, carbapenem therapy offers better results than PTZ therapy in critically ill patients with bacteremia caused by ESBL-E.

In another study conducted by Tamma et al., 14-day mortality of patients was compared between those who received PTZ and carbapenems as empiric therapy in a cohort of patients with ESBL bacteremia who all received definitive carbapenem therapy [14]. Only about 40% patients received 4.5 g every 6 h and no patients received extended-infusion therapy. The majority of patients had "high-inoculum" infections, one-third of patients required ICU care, and most ESBL isolates had elevated PTZ MICs. Thirty-day mortality was higher in the PTZ group than carbapenem group (17% vs 8%, p< 0.05).

However, there are several observational studies where no differences in mortality are obtained between the PTZ group and the carbapenem group. The study by Ng et al. was evaluated 30-day mortality in 151 patients with presumed ESBL bloodstream infections. There was no difference found in thirty-day mortality between the groups [15]. Gutiérrez-Gutiérrez et al. conducted a study comparing the effectiveness of BLBLIs and carbapenems for the treatment of ESBL bloodstream infections, including 365 patients in the empiric therapy group and 601 patients in the targeted therapy group [16]. The isolates were from urinary (45%) and biliary (12%) sources ("low-inoculum"). Mortality at 30 days was comparable between the study groups in both the empiric (18% BLBLI group vs 20% carbapenems group) and definitive cohorts (10% BLBLI group vs 14% carbapenems group).

A meta-analysis was performed comparing carbapenem and BLBLIs for ESBL bacteremia for both empiric and definitive therapies [7]. There was no difference in all-cause mortality between therapies. Sfeir et al. conducted a systematic review and metanalysis comparing mortality between BLBLIs versus carbapenem for bloodstream infections due to ESBL-E [17]. There was no significant difference in 30-day mortality between BLBLI, including PTZ, and carbapenems in treating ESBL-E bloodstream infections. The authors concluded that BLBLI, especially PTZ, may be considered as an alternative treatment for ESBL-E bloodstream infections.

Nevertheless, it is still debatable whether BLBLIs can be considered for patients with ESBL-E producing infections. The MERINO trial compared PTZ to meropenem among patients with bloodstream infections due to 3rd generation cephalosporin-resistant *E. coli* and *K. pneumoniae* [18]. Primary outcome was 30-day mortality. The study did not prove the non-inferiority of PTZ, with 30-day mortality rates 12.3% with PTZ vs. 3.7% with meropenem, risk difference (RD) 8.6% (1-sided 97.5% CI, -∞ to 14.5%). The RD was lower in the subgroup of patients with urinary tract infections (RD 3.7%, -∞ to 10.7%) than among patients with other sources of bloodstream infections (RD 14.1%, -∞ to 24.5%). Following the trial, the authors found a high rate of false susceptibility to PTZ among OXA-1 producers with automatic methods or strip-gradient test performed in the trial sites; 60% of isolates were OXA-1 and 10% Amp-C [19]. A further analysis of the trial excluded patients with bloodstream infections caused by non-susceptible strains (PTZ MIC > 16 mg/L; meropenem MIC > 1 mg/L CLSI, or MIC > 2 mg/L EUCAST). The between group difference in mortality decreased and was non-significant: 13/134 (9.7%) with PTZ versus 6/149 (4%) with meropenem; (RD 5.7%, -1 to 11). After excluding non-susceptible strains, the 30-day mortality difference from the MERINO trial was less pronounced for PTZ but according to the authors' conclusions the high prevalence of OXA coharboring ESBLs suggests no recommendation in using PTZ for definitive treatment of ceftriaxone non-susceptible *Escherichia coli* and *Klebsiella*.

The MERINO-2 was a pilot study comparing PTZ to meropenem among patients with bloodstream infections caused by presumed Amp-C β-lactamase producing but 3rd generation cephalosporin-susceptible Enterobacter spp., *Citrobacter freundii*, Providencia spp., *Klebsiella aerogenes*, *Morganella morganii* o *Serratia marcescens* [20]. Seventy patients were included. The difference between groups in clinical failure was no significant, 8/38 (21%) with PTZ vs. 4/34 (12%) with meropenem. There was significant difference between groups with respect to microbiological failure (5/38, 13% with PTZ versus 0/34, 0% with meropenem; p = 0.03), although fewer microbiological relapses were seen in the PTZ group (0/38, 0% with PTZ versus 3/34, 9% with meropenem; p= 0.06).

We are looking forward to seeing the MERINO-3 study. This study will use a multicentre, parallel group open-label non-inferiority trial design comparing ceftolozane-tazobactam and meropenem in adult patients with bloodstream infection caused by ESBL or AmpC-producing *Enterobacterales* [21].

Some authors consider that unfavorable outcomes with PTZ may be due to not using appropriate doses (4.5 g every 6 h or 8 h in continues or extended infusion). However, in a recent study there was no significant difference between patients with therapeutic drug monitoring (TDM) guided dose optimization of PTZ and without TDM in terms of 28-day mortality and clinical and microbiological cure [22].

NEWER BLBLIS (CEFTOLOZANE-TAZOBACTAM AND CEFTAZIDIME-AVIBACTAM)

Ceftazidime-avibactam is usually active against ESBL-E because of the inhibitory ability of avibactam on the ESBLs. A *post hoc* study showed the results from RECAPTURE 1 and 2 trials in ESBL-cases for complicated urinary tract infections comparing ceftazidime-avibactam and doripenem [23]. The clinical cure rates 91.7% and 88%, respectively. A systematic review and meta-analysis showed the results from ceftazidime-avibactam for serious infections due to ESBL- and Amp-C- producing *Enterobacterales* [24]. Clinical response was observed in 91% (224/246) of the patients with ESBL infections

in the ceftazidime-avibactam arm, versus 89% (240/271) of the patients in the carbapenem arm. In patients with Amp-C producing *Enterobacterales* (n=82), clinical response rates were 80% (32/40) and 88% (37/42) in the ceftazidime-avibactam and comparators arm, respectively. Microbiologic response for ceftazidime non-susceptible *Enterobacterales* was 85% in the ceftazidime-avibactam arm and 64% in the carbapenem group. Thus, ceftazidime-avibactam seems like a good option for the treatment of ESBL-E.

Ceftolozane-tazobactam is usually active against ESBL-E. The SUPERIOR multicenter study showed the activity of ceftolozane-tazobactam against *Pseudomonas aeruginosa* (n=80) and *Enterobacterales* (n=400) isolates recovered from intensive care unit patients with complicated urinary tract and complicated intra-abdominal infections in Spain [25]. The activity was excellent against wild-type organisms 100% susceptible. Nevertheless, ceftolozane-tazobactam susceptibility decreased against ESBL producers: *E. coli* (80.4% complicated intra-abdominal infection/84.8% urinary tract infection) and *Klebsiella pneumonia* (59.1% complicated intra-abdominal infection/77.3% urinary tract infection). However, the clinical studies have shown good results against ESBL-E. In a pooled analysis of the pivotal clinical trials performed in patients with complicated urinary tract and intra-abdominal infections that included 2076 patients with 150 infected with ESBL-E [26] the clinical cure rates for patients with ESBL-producing *E. coli* and *K. pneumoniae* with ceftolozane-tazobactam were 98% (49/50) and 94.4% (17/18) respectively. The overall cure rates for complicated urinary tract infections with ceftolozane-tazobactam and levofloxacin against ESBL-E were 98.1% and 82.6%, respectively and for complicated intra-abdominal infection with ceftolozane-tazobactam plus metronidazole and meropenem were 95.8% and 88.5%, respectively. Bassetti et. evaluated ceftolozane-tazobactam for treatment of severe ESBL-E infections in a multicenter real-life study (CEFTABUSE II study) [27]. Ceftolozane-tazobactam treatment was documented in 153 patients: pneumonia was the most common diagnosis (n = 46, 30%), followed by 34 cases of complicated urinary tract infections (22.2%). Septic shock was observed in 42 (27.5%) patients. Favorable clinical outcome was observed in 128 (83.7%) and 30-day mortality was reported for 15 (9.8%) patients. Ceftolozane-tazobactam could be a valid option in empiric and/or targeted therapy in patients with severe infections caused by ESBL-E. Recently, Paterson et al. conducted a retrospective analysis of the ASPECT-NP clinical trial to confirm the efficacy of ceftolozane-tazobactam in treating hospital-acquired/ventilator-associated bacterial pneumonia due to ESBL-producing *Enterobacterales* [28]. The most frequent ESBL-positive and/or AmpC-overproducing *Enterobacterales* isolates (ceftolozane-/tazobactam n=31, meropenem n=35) overall were *K. pneumoniae* (50.0%), *E. coli* (22.7%), and *Proteus mirabilis* (7.6%). Overall, 28-day all-cause mortality was 6.7% (2/30) with ceftolozane-tazobactam and 32.3% (10/31) with meropenem (25.6% difference, 95% CI: 5.54 to 43.84). Clinical cure rate at test-of-cure, 7–14 days after end of therapy, was 73.3% (22/30) with ceftolozane-tazobactam and 61.3% (19/31) with meropenem (12.0% difference, 95% CI: −11.21 to +33.51). These data demonstrate that ceftolozane-tazobactam may be an appropriate option for treatment ESBL- and Amp-C-producing *Enterobacterales*.

Therefore, the available data support the efficacy of both new BLBLIs against susceptible ESBL-E and both antibiotics could be an alternative to carbapenems. We are pending the results of MERINO-3 study to confirm whether ceftolozane-tazobactam is a good option versus meropenem for treating ESBL-producing infections.

CONCLUSIONS

Available data suggest that carbapenems should be the drug of choice for the treatment of ESBL-E severe infections. It is possible that in clinical settings with "low inoculum" infections like urinary tract infections, piperacillin-tazobactam may be an alternative. In fact, it is important to find non-carbapenem β-lactam for the treatment of ESBL-E to reduce the effects associated with their overuse. Newer BLBLIs like ceftolozane-tazobactam and ceftazidime-avibactam are potential alternatives with good clinical results to date although we need more definitive data.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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Update on antimicrobial pharmacotherapy against multidrug-resistant Gram-negative bacilli

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Treatment of infections caused by carbapenemase-producing *Enterobacterales*

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ABSTRACT

Antibiotic resistance is one of the main menaces to public and individual health worldwide. In the last two decades, an increase in the detection of carbapenem-resistant *Enterobacterales* has been reported. The treatment of infections caused by these strains is a therapeutic challenge. The use of carbapenems may be beneficial depending on MIC value and source of infection. New drugs, with different activity against the different classes of carbapenemases, are developed showing significant benefits.

Keywords: Carbapenem resistant *Enterobacterales*, carbapenemases

INTRODUCTION

Antibiotic resistance is one of the main menaces to public and individual health worldwide. *Enterobacterales* can acquire numerous resistance-encoding plasmids, specially *Klebsiella pneumoniae*. Two decades ago, carbapenemase-producing *Enterobacterales* (CPE) began to spread. The most clinically relevant carbapenemases in *Enterobacterales* belong to classes A (i.e., mainly KPC type), B (i.e., metallo-beta-lactamases, VIM, IMP and NDM types) and D (i.e., mainly OXA-48 type). These enzymes exhibit significant variations in hydrolytic efficiency and are characterized by elevated minimal inhibition concentration (MIC) of carbapenems compared to the epidemiological cutoff values. The combination with other resistant mechanisms, like decreased outer membrane permeability or extended spectrum beta-lactamase (ESBL) production, are also frequently described. In addition, CPE often coharbor resistance mechanisms against other antimicrobial classes such as fluoroquinolones, aminoglycosides, tetracyclines, and trimethoprim-sulfamethoxazole.

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CPE cause serious infections, especially in immunocompromised patients, prolong hospital stays and increase mortality rates, ranging from 24% to as high as 70%, depending on the study population. Despite the need to establish effective early treatments, the few therapeutic options available limit the alternatives [1].

EPIDEMIOLOGY OF CARBAPENEMASES

Isolates of carbapenem-resistant *Enterobacterales* (CRE) are increasingly being described. The SENTRY Antimicrobial Surveillance Program analyzed the CRE isolates obtained during the period 1997-2016 in 42 countries from the main geographical regions. A statistically significant increase in CRE rates was reported over time for the overall isolates and breakdowns by all regions and infection sources. CRE rates increased from 0.6% in 1997–2000 to 2.9% in 2013–2016 with gradual increases of 0.8%–0.9% per period since 2005–2008. In this study, a representative number of CRE isolates was analyzed for the presence of carbapenemase encoding genes. KPC producers were the most frequently detected, with rates maintained throughout the study, while a notable increase in MBL (mainly by NDM) and OXA-48 isolates was reported. The detection of double carbapenemases (e.g., KPC+MBL, MBL+OXA-48 or KPC+OXA-48) was only reported during the second period [2].

In the same line, in the epidemiological survey carried out in 2018, all 37 participating European countries reported CPE isolates, whereas in the previous study performed in 2015, three countries had still not identified a single case. Overall, 11 countries reported a worsened epidemiological situation of CPE than in 2015, 25 countries described no change, and one country reported an improvement of the CPE epidemiological situation. Twenty out of 37 countries reported inter-institutional spread of CPE within the country, and compared with 2015, 4 additional countries reported regional or inter-regional spread in 2018, thus increasing the number of countries with regional or inter-regional spread to 16 [3].

In Spain, in a national study involving 42 hospitals conducted over a 5-year period (2010-2014) with isolates of *K. pneumoniae* obtained from blood cultures, resistance to imipenem increased from 0,27% in 2010 to 3,46% in 2014, and 86% of these isolated produced carbapenemases (i.e., mainly OXA-48, followed by VIM, KPC, IMP and GES) [4]. In another prospective multicenter Spanish study (with 83 hospitals participating) from February to May 2013, the impact of CPE from clinical infections and carriers was analyzed. The percentages of CPE isolates significantly diverse between species, being significantly higher in *K. pneumoniae* (75.4%) than in *Enterobacter cloacae* (35.1%) and *Escherichia coli* (33%). Again, the most detected carbapenemase was OXA-48 (71.5%), followed by VIM (25.3%), KPC (2.1%) and IMP (1.6%) [5].

Infections caused by CPE are associated with increased mortality. In the systematic review and metaanalysis published by Falagas et al collecting all data available until 2012, attributable deaths to CRE infections were analyzed. The number of deaths was 2-fold higher among patients with bacteriemia caused by CRE than among patients with bacteriemia caused by carbapenem-susceptible isolates. Those studies showed that many patients with infections caused by CRE had not received adequate empirical treatment, which could explain this increase in mortality [6].

CAN WE STILL USE CARBAPENEMS TO THREAT CARBAPENEM-RESISTANT INFECTIONS?

The use of imipenem or meropenem in monotherapy for the treatment of CPE infections is associated with therapeutic failure when isolates show high MIC values. A large review analyzed 15 studies with 50 patients with carbapenem-resistant (CR) *K. pneumoniae* infections treated in monotherapy with carbapenems. Twenty-nine of these isolates exhibited carbapenem MICs ≤2 mg/L, 13 equal to 4 and 8 mg/L, and 8 isolates ≥8 mg/L. Therapeutic failure increased from 29.4% for MICs ≤1 mg/L to 75.0% for MICs >8 mg/L, showing some therapeutic benefit in infections caused by strains with intermediate susceptibility to carbapenems (28.6% and 33.3% of failure for MICs 4 and 8 µg/ml, respectively) [1]. Carbapenem display time-dependent bactericidal killing when free drug concentrations remain above the MIC for 40 to 50% of the time between dosing intervals. Monte Carlo simulation models of different dosing regimens of carbapenems indicate that prolonging the infusion time from 30 min to 3 h increases the probability of bactericidal target attainment at each MIC value. In addition, for isolates with high MICs, only the high-dose/prolonged-infusion regimen displays a relatively high probability of bactericidal target attainment [7].

COMBINED TREATMENT

A combination therapy with ≥2 active drugs, including a carbapenem, has been reported as the lowest failure rate (8.3%) in the treatment of infections by carbapenemase-producing *K. pneumoniae* in comparison to other regimens, such as combination therapy with ≥2 active drugs not including a carbapenem, monotherapy with an aminoglycoside, monotherapy with a carbapenem, monotherapy with tigecycline, monotherapy with colistin and inappropriate therapy. The highest rate of therapeutic failure was presented by patients with inappropriate therapy, followed by patients receiving monotherapy with colistin, combination therapy with ≥2 active drugs not including a carbapenem and monotherapy with tigecycline [1].

Tumbarello et al reported the survival benefits of non-empirical regimens that include 2 or 3 active drugs (as compared with monotherapy) in the treatment of infections caused by KPC-producing *K. pneumoniae*. In this large multicenter cohort study, combination regimens that include meropenem provided appreciable therapeutic benefits when the meropenem MIC was ≤8 mg/L, but no benefits were obtained when the meropenem MIC exceeds 32 mg/L [8].

In the same line, a prospective cohort study including episodes of bacteriemia caused by colistin-resistant and high-level meropenem-resistant (≥64 mg/L) KPC-producing *K. pneumoniae* showed that the combination therapy (e.g., tigecycline + gentamicin, tygecicline + fosfomycin, gentamicin + fosfomycin, or tigecycline + fosfomycin + gentamicin) was associated with reduced mortality (25%) compared to the use of these antibiotics in monotherapy (43.8%) [9].

Gutiérrez-Gutiérrez et al reported data obtained in a retrospective cohort study of bloodstream infections (BSI) caused by CPE. In this study, 26 tertiary hospitals of 10 countries participated and compared 30-day all-cause mortality between patients receiving appropriate or inappropriate therapy, and among those patients receiving appropriate therapy, combination therapy or monotherapy. Lower mortality was significantly lower in patients receiving appropriate (38.5%) than inappropriate (60.6%) therapy, but overall mortality was not different between those receiving combination therapy (39%) and monotherapy (41%). Combination therapy was associated with improved survival only in patients with high mortality score [10].

NOVEL ANTIBIOTICS FOR THE TREATMENT OF CRE INFECTIONS

Infections caused by CRE/CPE are associated high mortality rates. Although, as it has been shown, combined therapy can be beneficial in the treatment of these infections, new drugs are needed to achieve better clinical outcomes and lower mortality rates. Several antibiotics are recently approved (e.g., ceftolozane/tazobactam, ceftazidime/avibactam, meropenem/vaborbactam, plazomicin, imipenem/relebactam, or cefiderocol) [11].

Several studies showed the efficacy of ceftazidime-avibactam (CAV) –a cephalosporin-beta-lactamase inhibitor combination- in the treatment of infections caused by CRE. Van Duin et al reported lower mortality rates in patients with CRE infections treated with CAV (9%) than in those treated with colistin (32%), and inverse probability of treatment weight-

ing-adjusted efficacy of a better clinical outcome (64%) for CAV [12]. Shields et al compared outcomes of patients with CR *K. pneumoniae* BSI receiving definitive treatments containing CAV or alternative regimens (e.g., carbapenem + aminoglycoside, carbapenem + colistin, or other including monotherapy with aminoglycoside or colistin). CAV was associated with higher rates of clinical success and survival than other regimens [13]. Tumbarello et al analyzed 138 cases of KPC-producing *K. pneumoniae* infections treated with CAV as a salvage therapy after a first-line treatment with other antimicrobials, and most cases (78.9%) CAV was administered with at least 1 other active antibacterial agent (e.g., gentamicin, tigecycline, colistin, fosfomycin, and other drugs). The overall 30-day mortality rate was 34.1%, and the highest rate was recorded in patients with bacteriemia. The 30-day mortality rate among patients with bacteriemia was significantly lower in patients who received CAV (36.5%) than patients without CAV (55.8%), and in patients treated with CAV in monotherapy (40.9%) than patients treated with single-drug (77.8%). A similar difference was observed in patients managed in combination therapy but without statistically significant results. In the multivariate analysis of risk factors for 30-day mortality in patients with bacteriemia, receipt of CAV was the sole independent predictor of survival [14].

Relebactam is a novel non-beta-lactam inhibitor of class A carbapenemases and class C cephalosporinases (e.g., AmpC) which, in combination with imipenem, can restore the activity against many imipenem-nonsusceptible *Enterobacterales*. The efficacy of treatment with imipenem/relebactam (IMI/REL) has been reported. In a randomized, controlled, double-blind, phase 3 trial, hospitalized patients with hospital-acquired/ventilator-associated pneumonia, complicated intraabdominal infection or complicated urinary tract infection caused by imipenem-nonsusceptible (but colistin- and IMI/REL-susceptible) pathogens the efficacy of IMI/REL was evaluated. Favorable overall response was observed in 71% IMI/REL and 70% colistin + imipenem patients, day 28 favorable clinical response in 71% and 40%, and 28-day mortality in 10% and 30%, respectively. No statistically significant differences were observed in favorable overall response, but serious adverse occurred in 10% of patients treated with IMI/REL vs 31% of patients treated with colistin + imipenem patients, and treatment-emergent nephrotoxicity in 10% and 56%, respectively. So, this trial presented IMI/REL as an efficacious and well-tolerated treatment option for CRE infections [15]. Vázquez-Ucha et al analyzed the *in vitro* activity of IMI/REL, and other 16 widely used antimicrobials, against a Spanish nationwide collection of CPE. All isolates showed high rates of susceptibility to colistin (86.5%), IMI/REL (85.8%) and CAV (83.8%). Susceptibility rates to other beta-lactams, aminoglycosides, quinolones and fosfomycin were under 80% in all cases and only amikacin retained activity against >75% of the isolates. Antibiotic susceptibility varied widely depending on the type of carbapenemase detected. While CAV was the most active agent against OXA-48 producers (97.7%) followed by IMI/REL (87.9%), IMI/REL was the most active drug (100.0%) against KPC producers (followed by CAV (93.4%)) [16].

Vaborbactam is a boron-based beta-lactamase inhibitor with activity against class A carbapenemases. Combination with meropenem restores activity against KPC-producers. In a phase 3, multinational, open-label, randomized controlled trial, the efficacy and safety of meropenem/vaborbactam (MER/VAR) was evaluated versus the best available therapy (mono/combination therapy with colistin, carbapenems, aminoglycosides, tigecycline or CAV) for the treatment of CRE infections (i.e., bacteremia, hospital-acquired/ventilator-associated bacterial pneumonia, complicated intraabdominal infections, and complicated urinary tract infection/acute pyelonephritis). Day-28 all-cause mortality was 15.6% and 33.3% for MER/VAR and best available therapy, respectively. Treatment-related adverse events and renal-related adverse-events were 24.0% and 4.0% for MER/VAR, and 44.0% and 24.0% for other treatments. So, monotherapy with MER/VAR was reported with increased clinical cure, decreased mortality and reduced nephrotoxicity compared with other drugs [17]. In a multicenter, retrospective cohort study, Ackley et al compared the efficacy and safety of MER/VAR to CAV in the treatment of CRE infections. No significant difference in clinical success, and 30- and 90-day mortality rates were observed between groups, although in patients with recurrent infection, development of resistance occurred in 3 patients receiving CAV in monotherapy (no resistance was detected in patients with MER/VAR treatment) [18].

Cefiderocol is a novel siderophore cephalosporin designed to threat carbapenem-resistant bacteria, with activity against ESBL, AmpC and class A, B and OXA-48 carbapenemases. An open-label multicenter study assessed the efficacy and safety of cefiderocol and best available therapy for the treatment of patients with serious carbapenem-resistant Gram-negative infection. For patients with hospital-acquired pneumonia, clinical cure rate was very similar in both groups (50% and 53% for cefiderocol and best available therapy, respectively). The same clinical cure rate was achieved for both groups in patients with BSI and sepsis (43%). Cefiderocol achieved higher microbiological eradication in patients with complicated urinary tract infection (53%) than in the best available therapy group (20%). At the end of the study, more patients receiving cefiderocol died (34%) that patients receiving best available therapy (18%) [19].

Plazomicin is a new aminoglycoside with activity against ESBL, AmpC and class A and D carbapenemases. In a multicenter, randomized, open-label trial including patients with bloodstream infection or hospital-acquired/ventilator-associated bacterial pneumonia caused by CRE, efficacy and safety of plazomicin versus colistin were evaluated. Among patients with BSI, death from any cause at 28 days or clinically significant disease-related complication occurred more frequently in patients receiving colistin (53%) than in patients receiving plazomicin (14%). In patients with pneumonia, numerically fewer deaths were observed at day 14 among patients who received plazomicin-based treatment [20].

Infectious Diseases Society of America (IDSA) guidelines recommendations are summarized in Table 1. Although it is expected that bacteria will continue developing resistance

Table 1 **Summary of treatment of carbapenem-resistance *Enterobacterales* according to IDSA Guidelines.**

Source of infection	Preferred treatment	Alternative treatment
Uncomplicated cystitis	Ciprofloxacin, levofloxacin, trimethoprim-sulfamethoxazole, nitrofurantoin or a single dose of an aminoglycoside Meropenem (standard infusion): For cystitis caused by CPE, if ertapenem-resistant, meropenem-susceptible, and no carbapenemases are detected	Ceftazidime-avibactam, meropenem-vaborbactam, imipenem-relebactam, cefiderocol, colistin (when no alternative options are available)
Pyelonephritis and complicated urinary tract infections	Ceftazidime-avibactam, meropenem-vaborbactam, imipenem-relebactam, cefiderocol Meropenem (extended infusion), if ertapenem-resistant, meropenem-susceptible, and no carbapenemases are detected	Once-daily aminoglycosides
Infections outside of the urinary tract caused by ertapenem-resistant meropenem-susceptible and carbapenemase testing are neither available or negative	Meropenem (extended infusion)	Ceftazidime-avibactam
Infections outside of the urinary tract caused by ertapenem and meropenem-resistant and carbapenemase testing are neither available or negative	Ceftazidime-avibactam, meropenem-vaborbactam, imipenem-relebactam	Cefiderocol Tigecycline, eravacycline
Infections outside of the urinary tract caused by carbapenem-resistant and carbapenemase-producer *Enterobacterales*	Ceftazidime-avibactam, meropenem-vaborbactam, imipenem-relebactam	Cefiderocol Tigecycline, eravacycline
Infections caused by metallo-beta-lactamase-producers *Enterobacterales*	Ceftazidime-avibactam + aztreonam, cefiderocol	Tigecycline, eravacycline
Infections caused by OXA-48 -producers *Enterobacterales*	Ceftazidime-avibactam	Cefiderocol Tigecycline, eravacycline

mechanisms against these new antibiotics, their correct use will determine the benefit that we can obtain from them [21].

CONFLICT OF INTEREST

Authors declare no conflict of interest

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Update on antimicrobial pharmacotherapy against multidrug-resistant Gram-negative bacilli

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New treatments for multidrug-resistant non-fermenting Gram-negative bacilli Infections

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ABSTRACT

Ceftolozane/tazobactam, ceftazidime/avibactam and cefiderocol belong to a novel generation of antibiotics that correspond with the β-lactam family. It is necessary to having new options in treating infections caused by Gram-negative, non-fermenting multidrug-resistant bacilli due to the significant increase in multidrug resistance in the last decades. Knowing the main characteristics of each drug is key for correct use.

Keywords: Ceftolozane/tazobactam, Ceftazidime/Avibactam, Cefiderocol, Gram-negative, non-fermenting multidrug-resistant bacilli

INTRODUCTION

Treatment of non-fermenting multidrug-resistant Gram-negative bacilli (NFGNB-MDR) infections is a current challenge for physicians due to both severity and the potential resistance to a high number of antibiotics. The most common and severe NFGNB-MDR includes *Pseudomonas aeruginosa*, which could be involved in a wide variety of nosocomial infections. Despite the severity caused by such infections, 50% of neutropenic patients have been recently reported to have had an infection due to MDR *Pseudomonas aeruginosa* (PAE-MDR) and received inappropriate antibiotic empirical therapy (IAET). This finding is related with higher mortality [1]. Both *Stenotrophomonas maltophilia* and *Acinetobacter baumannii* are not considered highly virulent pathogens [2] Nonetheless, *S. maltophilia* is an emerging nosocomial and MDR pathogen that causes respiratory tract infections and central venous catheter-associated bacteremia [3]. A total of 82% of bloodstream infections due to *S. maltophilia* in neutropenic patients were treated with IEAT, with the source of infection being mostly catheters. The impact of IEAT on outcomes was not significant in this situation due to both the low virulence of bacteria and quick changes to optimal antibiotics and catheter removal [1,3]. Finally, *A. baumannii* is responsible for infections in critically ill patients, mainly ventilator-associated pneumonia and bloodstream infections. Although it is not the most frequently isolated Gram-negative bacillus, the multidrug resistance rate is high, varying per geographic area. Carbapenem resistance rates, for example, exceed 30% in regions like Latin America [4].

Today, there is a new spectrum of promising antibiotics—all of which are β-lactams—to face the most important NF-GNB-MDR: ceftolozane/tazobactam, ceftazidime/avibactam and cefiderocol. We aimed to review the main characteristic of these antibiotics.

NEW BETA-LACTAMS

Use of ceftolozane/tazobactam (TOL/TAZ) for NF-GNB-MDR. Ceftolozane shares structural similarities with ceftazidime, associated with a β-lactamase inhibitor. The main difference between ceftolozane and ceftazidime is the presence of a higher side chain at position 3 of the dihydrothiazine ring [5]. This distinguishing characteristic is relevant as it confers: 1) stability against chromosomal AmpC β-lactamase, which is present in *P. aeruginosa;* 2) better affinity to penicillin-binding proteins (PBP) [5]; and 3) sub-optimal substrate for efflux pumps [6]). It is also not affected by OprD porin as it relates to entrance into the *P. aeruginosa* membrane. Due to all of these characteristics, minimum inhibitory concentration (MIC) values for *P. aeruginosa* are low (86.3% of isolates were inhibited at ≤8 mg/L when compared with the remaining antipseudomonal β-lactams [7]). It remains active even when facing a combined mechanism of resistance like hyperexpression of efflux pumps or loss of porins [8]. Consequently, TOL/TAZ has potent activity in infections caused by *P. aeruginosa*.

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Additionally, the rate of *P. aeruginosa* resistance in our area remains low [9].

It is important to note that the concentration that prevents the selection of resistant mutants (MPC) is fundamental to avoid an increase in resistance to antimicrobials. Remarkably, TOL/TAZ does not only have the lowest MPC values among β-lactams; indeed, these values are not far from those of MIC. This narrow window is what makes the drug important—given its potency in *P. aeruginosa* infections—and capable of preventing the appearance of resistant strains.

On the other hand, TOL/TAZ also has a β-lactamase inhibitor, which is absent in cephalosporins and adds activity to bacteria with β-lactamase. The intrinsic activity of TOL/TAZ to enterobacteria has been demonstrated to have lower MIC values when compared with piperacillin/tazobactam [10]. Of 3,841 cases of enterobacteria, 95.2% were susceptible (MIC ≤8 mg/L), making the drug one of the most currently active antibiotics (alongside meropenem and tigecycline) [11].

The role of TOZ/TAZ in NFGNB-MDR non-*P. aeruginosa*, such as *S maltophilia* isolates, is controversial, with low activity being previously reported (12).

Use of ceftazidime/avibactam (CAZ/AVI) for NFGNB-MDR. This antimicrobial complex combines a well-known cephalosporin with a new β-lactamase inhibitor. The latter is distinct, given that it does not contain the β-lactam ring in its structure. Avibactam is a diazabicyclooctane inhibitor member and can covalently and reversibly bind to β-lactamases, thus reducing their ability to hydrolyze (unlike other inhibitors such as clavulanic or tazobactam) and return to its original form [13–15]. This agent also has activity against producers of β-lactamases from classes A and C per the Ambler classification (and also varying activity against class D, albeit not class B enzymes, i.e., metallo-β-lactamases [MLB]) [16]. It is because of its ability to have good *in vitro* activity—even for KPC-producing strains—that this combination is recommended in recently published guidelines on empirical treatment of patients with severe gram-negative bacilli infections [17]. CAZ/AVI is a very good agent (>99% susceptibility) against more than 8,000 *Enterobacteriaceae* isolates (MIC_{50}/MIC_{90}, 0.12/0.25 mg/L) collected from hospitals in the United States (US) [18]. For NFGNB-MDR, CAZ/AVI recovers activity against *P. aeruginosa* lost by ceftazidime due to derepression of the inducible chromosomal AmpC-type β-lactamase. Of the 7,062 *P. aeruginosa* isolates obtained in four different geographic regions, 92% were susceptible to CAZ/AVI with a MIC_{90} of 8 mg/L, thus recovering up to 65% susceptibility to ceftazidime alone [19]. In another study of US hospitals, CAZ/AVI inhibited 82% of strains that were resistant to ceftazidime. CAZ/AVI is active in approximately 30% of *S. maltophilia*. The remaining strains may produce two types of β-lactamases, one of which is the MLB type—resistant to all β-lactamase inhibitors. Aztreonam (AZT) has activity in these strains. The other β-lactamase is a cephalosporinase, which confers resistance on third-generation cephalosporins and aztreonam; however, it is susceptible to β-lactamase inhibitors such as avibactam (AVI). Thus, the combination of AZT and CAZ/AVI has been successfully tested for the treatment of *S. maltophilia* infections. CAZ/AVI was up to 81.58% more active when compared to CAZ alone, and AVI potentiated the activity of AZT up to 94% [20]. The activity of CAZ/AVI against *A. baumannii* remains limited. More than 50% are resistant to CAZ/AVI.

Use of cefiderocol for NFGNB-MDR. This is a new parenteral cephalosporin that has a complex chemical structure with summatory characteristics of cefepime and ceftazidime, as well as the presence of a catechol-like side chain with siderophore capacity. This allows it to cross iron transport channels present in the GNB outer membrane ("*Trojan Horse*") and enter the periplasmic space at high concentrations, thus evading classical resistance mechanisms such as hyperexpression of efflux pumps or mutations in porin channels [21–23]. It has been shown to have a higher affinity *in vitro* than ceftazidime for PBP3 binding, as well as for PBP1 in *P aeruginosa* or PBP2 in *Klebsiella pneumoniae* [24]. Another characteristic is the high stability present in hydrolysis of most β-lactamases, including those of the metallo-β-lactamase type.

Consistent with these characteristics, cefiderocol confers broad-spectrum coverage against Gram-negative bacilli, even those difficult-to-treat NFGNB-MDR like *Acinetobacter* or *Stenotrophomonas*.

In a recent publication on the SIDERO-WT surveillance program conducted between 2014-2019 that collected clinical samples from hospitals in both Europe and North America, cefiderocol inhibited 99.9% of *P aeruginosa* isolates and 96.0% of *A. baumannii* isolates with MIC ≤4 mg/L. Likewise, 98.6% of *S. maltophilia* were susceptible with MIC ≤1 mg/L [25]. Nowadays, most centers in Spain have limited experience with the use of cefiderocol; however, some trials have been reported as presenting good results [26–28].The role of this antibiotic in such aforementioned NFGNB-MDR infections seems promising.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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COVID-19 in donation and transplant

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ABSTRACT

SARS-CoV-2 infection has had a major impact on donation and transplantation. Since the cessation of activity two years ago, the international medical community has rapidly generated evidence capable of sustaining and increasing this neccesary activity. This paper analyses the epidemiology and burden of COVID-19 in donation and transplantation, the pathogenesis of the infection and its relationship with graft-mediated transmission, the impact of vaccination on donation and transplantation, the evolution of donation in Spain throughout the pandemic, some lessons learned in SARS-CoV-2 infected donor recipients with positive PCR and the applicability of the main therapeutic tools recently approved for treatment among transplant recipients.

Keywords: COVID, donation, transplantation, epidemiology, transmission, immunity, therapy

EPIDEMIOLOGY AND BURDEN OF COVID-19 IN DONATION AND TRANSPLANT

As in the rest of the population and according to multicenter studies, the main risk factors for acquisition and poor outcome after COVID-19 are age over 65 years (OR 6.01) and comorbid conditions, such as cardiovascular disease (OR 4.58) or its risk factors (hypertension (OR 2.95), diabetes (OR 3.07), overweight or smoking (OR 2.04)), chronic obstructive pulmonary disease (COPD) (OR 6.66) or chronic renal failure (OR 5.32) [1-7].

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A retrospective North American study of 482 solid organ transplant (SOT) recipients during the first wave (March-May 2020, 50 centers, 66% kidney, 15% liver, 11.8% hearth and 6% lung recipients), revealed a need for hospital admission of 78%, mechanical ventilation of 27% and mortality of 18% [8]. In this study, the risk factors mentioned above stood out significantly. When specifically analyzing the transplantation process, the worst clinical outcome correlated with lung transplantation and with some immunosuppressive regimens (especially steroids and anticalcineurin drugs). The COVID-19-infected renal recipients were older than 60 years, 5 or more years after transplantation, treated with steroids, tacrolimus, and mycophenolate. Most patients developed pneumonia (81%) and more than 30% had gastrointestinal symptoms. Comorbidities, the elderly, those with grafts younger than 1 year and those with graft dysfunction failed worse outcomes. The infected liver recipients were also older than 60 years. The main risk factor for mortality was liver cirrhosis in patients with Child C or MELD ≥ 15, dyspnea and comorbidities (50%-60%), also immunosuppression with mycophenolate or tacrolimus-free regimens. One third developed gastrointestinal symptoms and one third developed severe COVID-19 (need for ICU admission, mechanical ventilation, or death). Overall mortality was 20-22%. Lung recipients with COVID-19 developed acute respiratory distress syndrome in more than half of the cases (58%) and mortality was 46%, twice as high as in other solid organ transplants. An Indian cohort with 250 patients [9] and the Spanish series with 778 solid organ and hematological recipients from February to July 2020 [10] showed similar results (hospital admission 89%, mechanical ventilation 10%, adult respiratory distress syndrome 36% and mortality 27%).

However, after correction for risk factors associated with mortality in multivariate analysis, only age over 60 years and lung transplantation were significantly correlated with prognosis [10]. A French study of 306 kidney transplant recipients and 795 non-transplant controls again demonstrated no difference in 30-day mortality, when adjusted for age and co-

morbidity [11]. A Spanish comparative study between kidney transplant recipients and non-transplant recipients, which studied prognostic factors in COVID-19 using a matched propensity score, showed that neither being transplanted nor taking immunosuppressants was an independent prognostic factor. However, age over 65 years, creatinine and CRP levels during infection were independent prognostic factors [12]. It is also worth noting that another prognostic factor among transplant recipients was the time of infection during the pandemic. In the Spanish series, as progress was made in understanding the virus, its timeline, in optimizing active or useless drugs, as it could be diagnosed better and earlier, patients were better placed, admitted less frequently to critical care, suffered less ADRS and their mortality decreased [10].

Among patients with hematopoietic malignancies undergoing hematopoietic stem cell transplantation (HSCT), the median time from transplantation to COVID-19 infection was 17 months for allogeneic HSCT recipients and 23 months for autologous HSCT recipients. Fifteen percent in both groups developed severe disease requiring mechanical ventilation. Overall survival for both groups was 68%. Being aged 50 years or older, being male or developing COVID-19 infection within 12 months post-transplant were associated with an increased risk of mortality among allogeneic HSCT recipients. A diagnosis of lymphoma was associated with an increased risk of mortality compared to plasma cell dysplasia or myeloma in autologous recipients [13].

PATHOGENESIS OF SARS-COV-2 INFECTION. RELATIONSHIP TO GRAFT-MEDIATED TRANSMISSION

At the beginning of the pandemic in 2020, detection of SARS-CoV-2 RNA by RT-PCR (*real time protein chain reaction*) was reported in blood (8-15%) and in feces (50%) of patients infected by COVID-19. Also, in *postmortem* studies coronavirus-like inclusions were found in kidney [14-15]). This triggered a universal fear of transplanting organs from donors with suspected or confirmed SARS-CoV-2 infection. Eventually reassuring evidence began to emerge. In China, they detected viremia in only 0.1% of convalescent COVID blood donors, being more likely to be detected in those patients with more severe disease [16]. Also, subsequent studies in cardiac donors with positive PCR at the time of donation failed to detect SARS-CoV-2 in cardiac tissues [17].

Microbiological studies correlated the time at which the detectable virus had no infectious capacity in cell cultures [18], which associated with knowledge of the incubation period and that of symptoms (only 100 out of every 10,000 cases developed symptoms after 14 days [19], made it possible to establish the time of lowest infectious risk in convalescent donors. Thus, if a donor had died 14-21 days after the onset of symptoms, had remained asymptomatic in the last 72 hours and the result of the molecular study (PCR) against SARS-CoV-2 was negative, he could be a donor according to the recommendations of scientific societies, such as the Spanish Transplant Organization (ONT) [20].

However, in clinical practice, the PCR result could be positive for more than 3 weeks in convalescent or clinically resolved patients. Therefore, it was necessary to discriminate which PCR amplification cycle (the Ct or "cycle threshold") could be a surrogate marker of viral viability. In other words, at what Ct there was no longer any growth or cytopathic effect in cell cultures. The first studies placed it at 24 [18,19], although later publications would raise it above 28 [21] and finally above 30 in lower respiratory samples from patients hospitalized in the critical care unit [22]. With these two parameters (more than 14 clinical days and a Ct above 30 in the PCR study) and considering the limitation generated by the variability existing between the different studies reported on infectivity/virulence, the first review study appeared in which no case of SARS-CoV-2 transmission by cell, tissue or organ transplantation was documented [23]. Except for the lung and intestine, some clinical studies, which we will discuss below, have been able to confirm this.

Finally, two other parameters were also assessed individually in the transmission risk screening: the clinical category of severity with which the donor had been infected before death, classified as mild (at home), moderate (hospitalized medical wards) or severe (in the critical care unit), and the presence of IgG antibodies to SARS-CoV-2, which could give an idea of the immune response.

With the parameters described above and knowing that more COVID-infected patients with terminal organ failure were hospitalized and died on the waiting list than transplant recipients convalescing from the infection [24,25], especially in the case of liver and kidney transplantation, a justification was found to reactivate donation and transplantation activity.

IMPACT OF COVID-19 VACCINATION ON DONATION AND TRANSPLANT

The response to immunization in transplant recipients and the risk of acquiring infection after transplantation has been an obsession for transplant teams. Undoubtedly, vaccination has been able to contain the impact of the pandemic in the world population and the transplanted population is no exception. However, the response to immunization is varied depending on whether we value natural or vaccinal immunization and whether we focus on the humoral or cellular response.

The natural cellular response (that acquired after infection) is maintained after transplantation. A small study of 23 infected transplant recipients monitored at 4 and 6 months maintained TCD4 and TCD8 responses as measured by intracellular cytokine staining [26]. This response was also maintained in another group of 31 liver transplant recipients who also had their TCD4 and TCD8 response and fluorospot (IFN- γ) determined [27].

Vaccine immune response in the transplanted patient is not as consistent. A meta-regression analysis of 27 studies evaluated the humoral and cellular response in 1,452 renal transplant patients after vaccination against COVID-19 (all

were seronegative before vaccination). The humoral response was significantly lower, with a gradual increase to 29.98% at week 4 post-vaccination, with the control group maintaining 98%. The cellular response increased from 5% to 59.84%, being higher than 90% in the control group [28]. Another prospective analysis of the immune response at 6 months in 200 patients undergoing SOT (Liver 61 (30.5%), Kidney 102 (51%), Lung 37 (18.5%)) versus 200 healthy controls, showed a humoral response of 36% in the transplanted population versus 97.5% in the control population. The cellular response was performed by measuring interferon-γ (IFN-γ) after whole blood stimulation with SARS-CoV-2 S1 antigen. This response was 13.1% in transplant recipients versus 59.4% in the control group [29].

The median humoral response, measured by antibody determination after mRNA vaccines (e.g., BNT162b2) was 30%-40% depending on published series. In renal transplantation from 30% to 60% [30-32], in liver transplantation from 40% to 60% [30,33,34] and from 10% to 40% in lung transplantation [30,35].

Transplant patients were considered a priority in vaccination programs against SARS-CoV-2. In the same way, vaccination of patients on the transplant waiting list should be prioritized, although not delaying a transplant opportunity because they have not completed a vaccination schedule. Patients on the transplant waiting list should have their vaccination schedule updated to the epidemiological situation in their environment. Vaccination is also indicated in those patients who have passed COVID-19 or who have a positive serostatus against SARS-CoV-2. In the case of patients who have passed the infection (symptomatic or asymptomatic), guidelines indicated by the health authorities should be followed. Vaccination schedules do not differ from those used in the general population. These vaccines have proven to be safe, although they lose effectiveness over time, to a lesser extent than those of viral vectors, especially against new variants such as omicron [36,37]. This type of RNAm vaccine avoids possible alloreactivity phenomena that could have an impact on the transplanted organ. Since there are no studies that define the time of vaccination indication in recently transplanted patients and by analogy with other vaccination strategies against respiratory viruses, vaccination could begin from the first month in the case of solid organ recipients and between three and six months in hematopoietic precursor recipients.

Perhaps the most important thing after taking a measure is to evaluate it. What is the risk of acquiring SARS-CoV-2 infection in vaccinated transplant recipients, and what impact does it have? An United Kingdom retrospective study evaluated the effectiveness of the vaccine in SOT patients in 43,481 transplant recipients aged 16 years and older with full vaccination schedules between September 2020 and August 2021. The vaccines included were Pfizer BioNTech (BNT162b2) and Oxford-Astra-Zeneca (ChAdOx1-S), the predominant variants in the study were Alpha (B.1.1.7) and Delta (B.1.617.2). Of the 43,481 patients, 3,080 were unvaccinated, 1,141 had 1 dose and 39,260 had 2 doses. 4147 (9.53%) were infected, 2,575 of them (83.6%) were not vaccinated, 258 (22.6%) had received 1 dose and 1314 (3.35%) 2 doses. Seventy percent were renal transplant recipients and infection occurred more than one year after transplantation. Of those infected, a total of 407 (9.8%) died on day 28, 269 (10.4%) were not vaccinated, 30 (11.6%) had received 1 dose and 108 (8.2%) had received 2 doses. Vaccination resulted in a 20% reduction in the risk of death in SOT patients (p=0.05) (HR mortality up to day 28 after infection = 0.80 (0.63 - 1.00)). Although this risk reduction is significant, it is lower in the unvaccinated population (90%). Vaccination was not associated with a lower risk of infection (IRR= 1.29 (1.03 - 1.61)) [38]. Another Greek study evaluated the clinical outcomes of SARS-CoV-2 infection in relation to humoral response in fully vaccinated SOT recipients (at least 14 days after second dose). A total of 449 vaccinated SOTs (with more than one comorbidity, more than one year of transplantation and their immunosuppressive treatment) were included. A total of 15 were infected (3.34%) and at that time 6 (40%) were seropositive and 9 (60%) seronegative (< threshold 50 AU/mL). Of the 15 infected 8 were admitted, 7 had severe disease and 2 died [39]. Finally, a third North American, retrospective multicenter study evaluated the risk of infection after vaccination and compared it with the general population. It analyzed 18,215 fully vaccinated SOT patients. A total of 151 (0.83%) were infected, 87 of these (57.6%) required hospitalization and 14 (9.3%) died. The conclusion reached by the authors is that transplant patients have less post-vaccination protection than the general population and that they should continue with barrier measures [40]. This condition of lower protection should have implications for treatment.

EVOLUTION OF DONATION IN SPAIN THROUGHOUT THE PANDEMIC

As in other countries, the situation of donation and transplantation in Spain changed according to changes in the development of the pandemic (waves and variants) and the accumulated scientific evidence. Thus, 2020 was characterized by diagnosis, 2021 by vaccination and 2022 by the start of treatment.

Between March and December 2020 (first and second waves), an increase in the incidence and mortality from COVID-19 began to be observed worldwide, especially in the transplanted population. The international medical community, concerned about the decrease in healthcare resources dedicated to care and the fear of transmission during donation or post-transplant recipient infection, decided to suspend donation and transplantation. Activity was only maintained with death brain donors (DBD) or death cardiac donors DCD) after withdrawing life-staining therapies (WLSTs), in low incidence geographic areas, and after screening with 2 separate PCR tests 24-48 hours apart. Uncontrolled DCD (type II of Maastricht) was discontinued. Protocols were proposed for the transport of potential donors between centers looking for those with lower incidence or greater availability of beds (using continuous veno-venous haemofiltration techniques, ECMO, etc).

Between January and May 2021 (third and fourth waves) there were fewer admissions and mortality in transplant recipients with COVID than in the first wave, there was better diagnostic capacity, vaccination programs were developed worldwide, and scientific evidence allows optimization of remdesivir, dexamethasone, oxygen therapy and anti-inflammatory monoclonal antibodies (tocilizumab, baricitinib). Safety algorithms began to be generated to weigh the risk of COVID transmission (intensity of the disease, time to donation, replicative capacity of the virus at the time of donation measured by the Ct of the PCR, if positive). All this allowed initiation of DCD and DBD activity. The uncontrolled DCD remained suspended.

Between June 2021 and March 2022 (fifth and sixth waves and after) the delta variant was predominant until December and then omicron subvariants (B.A 1, B.A 2, B.A 4/5). The infection rate was higher, although with fewer hospitalizations due to the lower aggressiveness of the strain and vaccine implementation (85-90% of the population). The development of new antiviral drugs such as molnupiravir and nirmatrelvir/ritonavir and monoclonal antibodies such as sotrovimab or casirivimab/imdevimab was completed. The first series of patients transplanted from donors with positive PCR appeared, with informed consent and good results, except in lung transplantation. However, donation and transplantation took a long time to take off for logistical reasons: numerous sick leaves and exhausted among healthcare workers due to work overload, limited availability of beds, the surgical activity No COVID, delayed during the pandemic, competed with the resources of the operating room or critical care beds and there was also a turnover of personnel in all areas (doctors, nurses, assistants, etc.) with less awareness of donation. With all this, donation continues from DCD and DBD if the hospital logistics allow it, and the DCD uncontrolled with triple safety screening (out-of-hospital donor nasal antigen, family questionnaire on COVID and PCR on arrival of the donor at the emergency room) begins to be activated.

Even though donation and transplantation figures are still closer to those of 2017, the report issued by ONT as of January 27th, 2022, reflected an overall growth of 7% in all types of transplantation (1,905 donors, (40.2 donors per million population (pmp) with 4,781 transplants (101 pmp transplants) of which 159 transplants have been to children). On the other hand, 26,347 new bone marrow donors were registered, meaning that our country now has 452,552 registered donors, in line with the objectives of the third phase of National Bone Marrow Plan. At the other extreme is the waiting list, which stands at 4,762 patients, 66 of them of pediatric age. In 2020 the list was 4,794 patients, 92 of them of pediatric age [41].

LESSONS LEARNED AND EXPERIENCE IN SARS-COV-2-INFECTED DONOR RECIPIENTS

There are published references on the absence of SARS-CoV-2 transmission in organ recipients from donors convalescing from infection with 2 months or more prior to donation [42,43]. However, much more relevant is the increasing communication of series demonstrating the absence of SARS-CoV-2 transmission from donors with positive PCR at the time of donation, with ranges from 0 to 30 days from PCR positivity to renal [44,45], hepatic [44,46-48], and cardiac [44,49] donation. However, the results were not similar in lung donation. Of 3 lung implants from positive PCR for SARS-CoV-2 donors (with negative nasopharyngeal PCR and positive bronchioalveolar lavage PCR), 3 developed critical illness and 1 died [50,51].

In the analysis of the cohort of 17,694 donors from the American national database (OPTN), 150 were positive for SARS-CoV-2. Of these, 269 organs were transplanted, including 187 kidneys, 57 livers, 18 hearts, 5 kidney-pancreas, and 2 lungs. The median time from COVID-19 testing to donation was 4 days for positive donors. Survival of patients who received grafts from COVID-19-positive donors and complications of graft dysfunction were equivalent to those who received grafts from COVID-19-negative donors [52]. Similar results were reported by Dhand et al. with 193 COVID+ donors resulting in the transplantation of 281 kidneys, 106 livers and 36 hearts in 414 adult recipients [53]. A systematic review conducted with published from January 2019 to December 2021 collected information from sixty-nine recipients who received 48 kidneys, 18 livers, and 3 hearts from 57 donors with positive RT-PCR for SARS-CoV-2 The investigators concluded that the use of nonpulmonary organs (kidney, liver, and heart) from SARS-CoV-2 positive donors appeared to be a safe practice, with a low risk of transmission, regardless of the presence of symptoms at the time of procurement [54]. Finally, a bioethical analysis addressing the four issues that are considered to constitute the essential structure of individual clinical cases for ethical analysis (medical indications, patient preferences, quality of life and contextual characteristics) concluded that the decision to perform liver transplantation in selected patients shows that the decision is ethically justifiable [55].

In an interesting report, Eichenberger EM et al [56] summarize the lessons learned about donation and transplantation in relation to COVID and could be summarized as follows:

- In non-pulmonary donors (kidney, liver, heart, or pancreas), even with unknown time since infection, without severe disease, no transmission has occurred. Intestinal transplantation is also not indicated due to prolonged viral shedding, which often exceeds 50 days.
- Donors with critical COVID-19, even non-pulmonary, may have organ quality problems due to microvascular disease. Biopsy should be considered in these cases.
- Patients on the waiting list with end-stage organ disease or those with high morbidity might be considered for organ transplantation from a COVID-19-positive donor.
- Recipients should be vaccinated, and transplant teams should encourage vaccination.
- Recipient informed consent should be obtained well in advance of transplantation.
- Although SARS-CoV-2 RNA could be detected in any organ, there is no viable or transmissible virus in organs other than the bowel or lung.

- Most recipients underwent treatment for COVID-19 with remdesivir, neutralizing antibodies or both since there is authorization for its use in transplant recipients.

MAIN THERAPEUTIC TOOLS APPROVED IN THE TREATMENT OF INFECTION. APPLICABILITY IN TRANSPLANT PATIENTS

Given the morbidity and mortality associated with SARS-CoV-2 infection in transplant recipients. It would be advisable to assess the specific treatment alternatives that could benefit these patients. Although experience is limited, some alternatives are presented.

Remdesivir. Remdesivir is a direct-acting nucleotide prodrug of SARS-CoV-2 RNA-dependent RNA polymerase. It has potent activity in primary airway epithelial cells. A phase 3 trial of remdesivir demonstrated that both a 5- and 10-day schedule shortened recovery time in hospitalized patients with COVID-19 [57,58]. Shorter treatment regimens (3 days) have prevented progression to severe disease in ambulatory patients with good adherence and tolerance [59]. In a small Italian case series of 24 patients, 7 treated with remdesivir versus 17 placebos, the authors recognize the usefulness of this same scheme in solid organ recipients, avoiding disease progression and ICU admission [60]. It is currently the most widely used antiviral in the infection of hospitalized transplant recipients, alone or in combination with sotrovimab.

Nirmatrelvir/ritonavir. This is a new orally bioavailable protease inhibitor, which has demonstrated activity against SARS-CoV-2. In a recent phase 2/3 clinical trial in 2,246 SARS-CoV-2 infected patients, Nirmatrelvir (co-administered with ritonavir 100 mg twice daily) reduced the risk of hospitalization or death by 89% compared to placebo [61]. The main problem associated with prescribing this antiviral in the SOT population is that ritonavir is a potent cytochrome P450 (CYP) 3A inhibitor and poses significant drug-drug interaction problems, especially with anticalcineurin drugs. In addition, nirmatrelvir/ritonavir requires dose adjustment in renal insufficiency and its use is not recommended in patients with a clearance of less than 30 ml/min. There is some brief communication in which some precautions for its use are recommended, knowing the interindividual variability in the metabolic activity of P450 (CYP) 3a. Lange et al [62] recommend starting nirmatrelvir/ritonavir from day 1 to day 5. Maintain tacrolimus from day 1 to 5 (do levels on day +3 in case the dose needs to be adjusted). In the case of cyclosporine, reduce the dose to 80% of the usual dose. On day 6-7 do levels of tacrolimus or cyclosporine. In the case of tacrolimus, if the levels are supratherapeutic, maintain the dose and repeat after 2-4 days. If the levels are therapeutic, initiate treatment at a dose of 25-50% of the usual dose and repeat after 2-4 days. If subtherapeutic, start treatment at a dose of 25-75% of the usual dose and repeat after 2-4 days. In the case of cyclosporine, if levels are supratherapeutic, reduce the dose. In other interaction models, cyclosporine has been reduced to 20% of the usual dose. Repeat levels after 2-4 days. If levels are therapeutic, continue and monitor again after 2-4 days. If they are subtherapeutic, the dose should be increased, repeating the dose after 2-4 days. This proposal is indicative. In the case of other concomitant medication such as azoles, anticoagulants, this regimen should be individualized. Other researchers [63] propose reintroducing tacrolimus in partial or full doses between days 8 and 10, ideally guided by drug levels. To avoid elevation of transaminases, it may be prudent to discontinue statins on the day of initiation of treatment.

Molnupiravir. It is a derivative of the synthetic nucleoside N4-hydroxycytidine that exerts its antiviral action through the introduction of copy errors during viral RNA replication, which has been shown to reduce hospitalization and death in SARS-CoV-2 infection [64]. Molnupiravir provides some advantages for use in transplant patients. Since it has low affinity for CYP 3 A (P450), it does not interact with the metabolism of anticalcineurin drugs, as happens with ritonavir. Furthermore, it does not require dose adjustment in patients with renal insufficiency, as is the case with nirmatrelvir/ritonavir or remdesivir. Finally, being an oral drug, it facilitates compliance, sequential therapy after remdesivir or combination therapy. There is little experience in renal transplant recipients with molnupiravir in the early treatment of SARS-CoV-2 infection. In a small Spanish comparative study, no differences were found between molnupiravir (4 patients) and remdesivir (9 patients) in survival, tolerance or poor clinical course, even in very immunosuppressed patients (methylprednisolone bolus, basiliximab, antithymocyte gamma globulin) [65]. In a retrospective American series, the 49 transplanted patients infected with SARS-CoV-2 during the omicron variant and treated with molnupiravir had less hospitalization and death. Four of them had minor side effects (2 rash and 2 gastrointestinal complaints) [66].

Sotrovimab. Monoclonal antibodies have been shown to reduce hospitalization, ICU admission and mortality due to COVID-19 and would be especially useful, alone or in association with specific antiviral treatment, in the immunosuppressed or comorbid population. The most commonly used antibodies in 2021 and 2022 have been casirivimab-imdevimab and sotrovimab. Specifically in solid organ transplant recipients, two studies, with 35 and 28 patients treated with monoclonal antibodies, reduced hospitalization and ICU admission in transplant recipients infected with Delta variant, with no mortality [67,68]. However, the neutralizing capacity of monoclonal antibodies varies according to the viral variants. Thus, there are areas of greater variability in the RBD (receptor binding domain) of viral spike, that modify this "anchor zone" of the monoclonal antibody in the mutational variants, making it less neutralizing. There is an *in vitro* neutralization reference from Stanford University [69] that periodically updates the neutralization capacity of the different synthetic monoclonal antibodies, although there are no references that correlate that *in vitro* neutralization quotient with the greater or lesser beneficial effect *in vivo* or the neutralization breakpoint at which a therapeutic monoclonal antibody should be rejected. In addition, there is also an effect, termed "effector function" that recruits cells of the immune system to facilitate the elim-

ination of infected cells [70]. It is not known whether this is a constant class effect for all monoclonal antibodies or whether it is more intense in some or in others, but even with a reduction in the neutralizing threshold according to the Stanford references, some monoclonal antibodies are able to reduce viral load in experimental models [71].

These modifications in RBD referred above change with the viral variant. For example, after selection of Delta strain and the reduction of the neutralization capacity according to the Stanford references, two antibodies (etesivimab or bamlanivimab) were withdrawn from the market, leaving casirivimab/imdevimad and sotrovimab as the only active drugs. After selection Omicron, casirivimab/imdevimab was also no longer recommended for the same reason. A prospective multicenter real-life cohort study of patients treated with casirivimab/imdevimab (n= 133 vs. Delta) or sotrovimab (n= 116 vs. Omicron), in which 40% were solid organ recipients, has been published with results of reduced hospitalization and ICU admission, and no mortality [72]. A French retrospective observational study conducted between March and June 2021 compared the clinical course of 80 renal transplant recipients treated with sotrovimab for SARS-CoV-2 infection against 155 patients undergoing standard of care. Of the 80 patients treated with the monoclonal antibody, 3 (3.8%) required hospital admission, 2 (2.5%) required ICU admission, none required mechanical ventilation and there were no deaths. In the control group 30 patients (19.4%) were admitted, 24 (15.5%) were admitted to ICU, 18 (11.6%) required mechanical ventilation and all 18 (11.6%) died. All comparisons reached statistical significance [73]. A retrospective study compared the clinical outcomes of the first 25 renal transplant patients treated with sotrovimab against mild-moderate Omicron BA.1 infection versus 100 renal transplant patients who received only the standard of care. In the sotrovimab arm (n = 25) there were 4 hospitalizations (16%), 1 patient was admitted to the ICU, and no deaths. In the standard-of-care arm (n = 100) there were 35 hospitalizations (35%), 17 patients were admitted to ICU (17%), and 11 died (11%) [74].

Omicron variant has also undergone mutations in RBD, changing from BA.1 to BA.2 and currently also to BA.4/5. This has correlated with modifications in the Stanford neutralization standards, leaving sotrovimab and the combination of tixagevimab/cilgavimab as the only recommended monoclonal antibodies. Results of a study comparing the activity of sotrovimab among high-risk BA.1 vs. BA.2 variant-infected patients have recently been reported. In this study, of the 47 BA.2-infected patients, at least 35 were high-risk immunosuppressed (steroid therapy, solid organ or hematopoietic transplantation, chemotherapy, or rituximab immunotherapy). Although the sample size was relatively small, sotrovimab was associated with a low incidence of COVID-19-related hospitalization or death in this very high-risk population with mild to moderate SARS-CoV-2 infection and no new mutations [75]. Sotrovimab is currently an effective alternative, associated with the standard of care to prevent progression of SARS-CoV-2 infection in high-risk patients, such as transplant recipients.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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Norovirus infection as a model of chronic or recurrent infection in common variable immunodeficiency

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ABSTRACT

Common variable immunodeficiency (CVID) is the most frequent symptomatic primary immunodeficiency (PID) in general population. PID are genetic diseases that share a dysfunction in the immune system entailing a greater risk of both chronic and recurrent infections. These patients can also develop chronic gastrointestinal infections caused by norovirus with persistent viral dissemination, which can be detected months after primoinfection. Additionally, a proportion of CVID patients show a typical severe enteropathy presenting with recurrent diarrhoea, intestinal malabsorption, inflammatory lesions, and villous atrophy. Some studies have related this enteropathy with chronic intestinal infection caused by norovirus.

Keywords: common variable immunodeficiency, Norovirus, enteropathy

INTRODUCTION

Primary immunodeficiencies constitute an heterogenous group of more than 450 genetic diseases that share a deficient production in the components of innate and/or adaptative immune system. These disorders entail a higher susceptibility of developing infections which can sometimes be severe, chronic, recurrent, and may be caused by opportunistic agents. Nevertheless, in the last two decades, genomic, biochemical, and cellular analysis have demonstrated that the clinical characteristics of PID are wider than initially thought, and are not only restricted to infections. The immune system dysregulation has been described in many PID and can cause multiple autoimmune disorders, lymphoproliferative diseases, and neoplasms which, when not promptly suspected and diagnosed, will negatively impact the patient prognosis [1,2].

CVID is the most common symptomatic PID, with an estimated prevalence of 1:25.000 to 1:50.000 individuals. It is characterised by decreased blood levels of at least two immunoglobulin (Ig) isotypes (IgG, IgA and/or IgM) together with decreased or absent production of specific antibodies. Diagnosis is made when excluding secondary causes of hypogammaglobulinemia and other well-defined PID, including combined immunodeficiencies with decreased number of CD4 T-cells. CVID patients share a central alteration in the B-cell differentiation to plasmatic Ig secretory cells, and, despite the fact that CVID is classified as a PID with B-cell defect, in the last years a large number of other cellular defects have been discovered. Although the clinical spectrum of CVID is wide, two main phenotypes can be found: a first group of CVID patients that show recurrent infections, and a second group which develops autoimmune/inflammatory manifestations [3]. Within this second phenotype, a small proportion of patients (5-15%) may develop a typical severe enteropathy (called CVID-related enteropathy) of unknown cause. It might present as recurrent diarrhoea, intestinal malabsorption, inflammatory lesions, and villous atrophy in the patients' intestinal mucosa.

The most common infectious manifestations in CVID patients are recurrent airway infections, especially acute bronchitis, sinusitis, and pneumonias. Infections may also less frequently affect the CNS, gastrointestinal tract, and skin and soft tissue. In a subsection of CVID patients, chronic diarrhoea can be the main symptom of disease. Some parasites such as *Giardia intestinalis* can be responsible for the recurrent diarrhoea, but the villous atrophy or the intestinal inflammatory lesions that are seen in these patients have been related to the chronic or recurrent intestinal infection caused by norovirus [4].

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NOROVIRUS INFECTION AND CVID-RELATED ENTEROPATHY

Norovirus is the most common agent of gastroenteritis described both in isolated cases and in outbreaks all along the world. It is a non-enveloped RNA virus of the *Caliciviridae* family. Although it is a unique species, norovirus is divided into six genogroups (GI-GVI) that are subdivided into more than 40 genotypes. Among them, only GI, GII, and GIV can infect humans, being the strain GII.4 the most frequent one, causing more than 80% of intestinal infections worldwide. This virus has faecal-oral transmission, but it can also be transmitted by direct contact or by contaminated water or food. It is highly infectious as very few viral particles are able to cause the disease. Norovirus infection in immunocompetent patients is characterized by intense vomiting, followed by at least 4 days of diarrhoea, reaching the peak of viral excretion in 1 to 3 days after the disease onset. Up to 32% of the infected patients will develop an asymptomatic infection [5]. PID patients infected by norovirus may present the same initial symptoms. Nevertheless, 5 to 20% of these patients can develop severe and prolonged diarrhoea which can last for more than 4 months due to their diminished viral clearance. In addition, the disease can worsen, entailing a higher mortality rate [6]. It is not clear whether the prolonged symptoms are owed to a chronic norovirus infection or to a repeated series of infections, as the incomplete immune response of PID patients imply a higher susceptibility to recurrent infections in all age groups. Despite the cause of the disease, CVID patients develop viral persistent dissemination which can be detected between 9 months to 1 year after the primoinfection. Furthermore, there is no evidence that specific strains are responsible for this persistent infection in human hosts, as the most common genotype in immunosuppressed patients is the strain G-II which is also the predominant genotype in general population [7].

The histopathological findings of the CVID-related enteropathy are similar to those found in coeliac disease: increased number of intraepithelial lymphocytes, severe villous atrophy, crypt hyperplasia and lymphocyte infiltration of the lamina propria. Nevertheless, plasmatic cells may be absent, and, in some severe cases, enterocytes can show important degeneration and vacuolization. In fact, gluten abstinence is rarely beneficial, and most of the patients do not show class II HLA variants (DQ2 or DQ8). It has been recently demonstrated that norovirus infection provokes pathological changes in the duodenal mucosa of immunocompetent patients that resemble those pathological findings of coeliac disease, including villous atrophy, increase of intraepithelial lymphocytes, and permeability increase [8].

In a well-known patient series with CVID-related enteropathy, Woodward et al. [9] proposed that chronic norovirus infection could play an important role in the aetiology of this severe enteropathy. The 8 identified patients of this retrospective series were positive for norovirus in faecal samples, and, interestingly, 3 patients showed clinical resolution and an improvement of villous duodenal atrophy after achieving viral clearance when treated with ribavirin for several months. On the contrary, many patients with this chronic enteropathy seemed to symptomatically respond to immunosuppressor treatment, which included steroids and anti-TNF antibodies, despite the fact no significant histological changes were observed in the intestinal biopsies after this therapy. These findings support a possible role of cytotoxic aberrant immune response to the chronic infection caused by norovirus, and maybe to other enteric infections, in the aetiology of the CVID-related enteropathy.

IMMUNE RESPONSE TO NOROVIRUS IN IMMUNODEFICIENT PATIENTS

When talking about the immune response in CVID patients to norovirus infection and the presence of chronic enteropathy, it is essential to understand two main facts: which the mechanisms that eliminate norovirus from the host are, and which the pathogenicity of the villous atrophy and the inflammation of the intestinal mucosa is. According to the established hypothesis based on experimental animal and human models, norovirus mainly infects antigen-presenting cells (APCs), B-lymphocytes and epithelial cells, where it can produce direct toxicity. The infected cells release type I and type III interferon (IFN). The norovirus antigen is then presented by the infected cells through type I major histocompatibility complex to CD8 T-lymphocytes, or through type II major histocompatibility complex in B-cells and in APCs to CD4 T-lymphocytes. The expression of IL-15, specially in epithelial cells can increase the activation of T-cells. CD8 T-lymphocytes exert their cytotoxic role as intraepithelial lymphocytes, inducing apoptosis of mucosal epithelial cells through the release of granzyme and perforin, union of Fas/Fas ligand, and through the interaction with group 2D natural killer cells. CD4 T-lymphocytes proliferate and release cytokines which improve the activity of APCs, the cytotoxicity induced by CD8 T-lymphocytes, and the antibody production exerted by B-cells and plasmatic cells. This coordinated immune response is able to eliminate norovirus in immunocompetent hosts. Contrarily, in immunodeficiencies such as CVID, B-cell differentiation to plasmatic cells is compromised, and thus, the production of neutralizing antibodies, the interaction among T-cells and B-cells, and the release of cytokines from CD4 T-lymphocytes are impaired. As a result, norovirus clearance is altered, and a persistent and uncontrolled CD8 T-cell response produces epithelial damage and, in the end, causes the typical villous mucosal atrophy [5] (Figure 1).

DIAGNOSIS AND TREATMENT OF NOROVIRUS IN IMMUNODEFICIENT PATIENTS

In the majority of cases, norovirus infection is diagnosed by detecting the presence of viral RNA though PCR in faecal

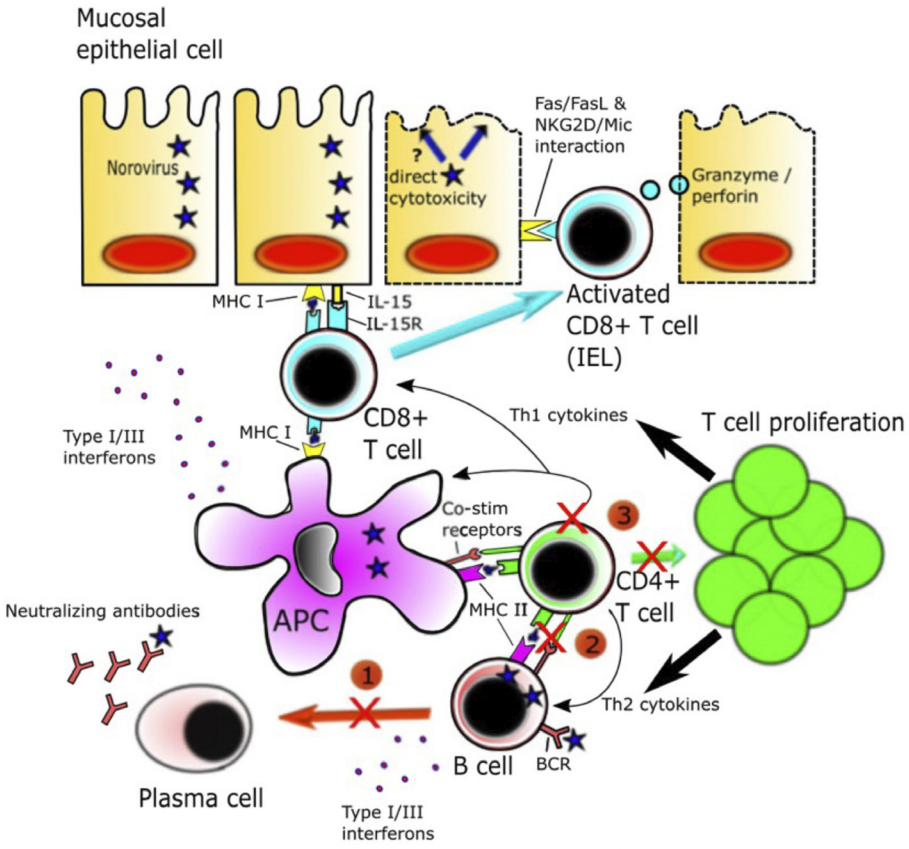

Figure 1 | **Pathogenesis of chronic intestinal infection by norovirus [5]**

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samples. The sample must be processed within the first 48 to 72h after the beginning of the symptoms. Nevertheless, the PCR can still detect viral RNA in faeces during weeks or months after the resolution of the symptoms, especially in patients with PID. This PCR analysis can also genetically classify norovirus strains, which is helpful for epidemiological research.

Nowadays, there is an active debate on whether the presence of norovirus itself implies the need of treatment, whether it is just an innocent spectator, or whether the treatment of norovirus infection should be restricted to the use of immunomodulator agents. Treatment of acute norovirus infection is mainly addressed to the patients' symptoms, and is focussed on fluid therapy for dehydration. To the moment, there are no available vaccines or antiviral targeted therapy. Treatment for patients with chronic infection caused by norovirus remains a therapeutical challenge. There are cases where the use of antivirals, specifically ribavirin and favipiravir, have achieved viral clearance measured through faecal PCR, together with clinical resolution, and improved histopathologic findings [8]. Immunomodulation with oral Ig [10] and breastmilk [11] have also shown some benefits. In addition, several antiparasitic agents such as nitazoxanide have demonstrated antiviral properties with transient benefit [12]. Finally, the use of immunomodulators such as mTOR inhibitors (sirolimus or everolimus) have proved a significant increase in the antiviral properties of the host that should be furtherly and deeply studied [13].

Recently, a unique association *Clostridioides difficile* coinfection has been observed in patients with chronic norovirus infection [14]. This fact has shed light on the role that microbiome modification may play in facilitating enteric replication of the virus and its establishment as a chronic infection. Some works in experimental animal models suggest that commensal bacteria, which are reduced as a consequence of antibiotic treatment, can counter the innate immune response to norovirus, which limits their efficacy in preventing new infections. Another hypothesis is that commensal bacteria may help norovirus in infecting specific cells of the intestinal mucosa [15].

CONCLUSIONS

CVID is the most frequent symptomatic PID in the population, and it is characterized by a dysfunction of the humoral component of the adaptive immune system which leads to a higher risk of repeated, chronic and/or recurrent infections. CVID patients may occasionally develop chronic intestinal norovirus infections with persistent viral shedding that can be detected months after the initial infection. This chronic infection has been related with the presence of a specific enteropathy characterized by an increase in intraepithelial lymphocytes, villous atrophy, crypt hyperplasia, and lymphocytic infiltration of the lamina propria, together with an absence of plasma cells, which poses a differential diagnosis with celiac disease. To date, no effective medical treatment has been described to treat this type of chronic infection.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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Diagnostic and therapeutic approach to pulmonary infiltrates in cancer patients receiving immune checkpoint inhibitors

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ABSTRACT

The advent of immune checkpoint inhibitors (ICIs) targeting cytotoxic T lymphocyte antigen 4 (CTLA-4) and the programmed cell death (PD-1)/PD-1 ligand 1 (PD-L1) axis has transformed the treatment paradigm for multiple cancer types. ICIs are able to restore T-cell-mediated antitumor responses and do not entail an increased risk of infection *per se*. However, immunotherapy is associated to a unique form of toxicity due to the off-target effects on healthy tissues of the excessively enhanced immune response in form of immune-related adverse events (irAEs). Although ICI-induced pneumonitis ranks the fifth of all irAEs in terms of frequency of occurrence, it is associated with a relevant attributable mortality. This review summarizes the incidence, risk factors, clinical and radiological presentation, and therapeutic approach of ICI-induced pneumonitis. Particular focus is on the differential diagnosis of new or worsening pulmonary infiltrates in cancer patients receiving ICI therapy. Finally, the impact on the risk of opportunistic infection of ICIs and immunosuppressive therapy used to treat associated irAEs is reviewed. The diagnosis and management of suspected ICI-induced pneumonitis remains clinically challenging.

Keywords: immune checkpoint inhibitors; pneumonitis; immune-related adverse events; pulmonary infiltrates; diagnosis; cancer.

INTRODUCTION: IMMUNE CHECKPOINT INHIBITORS AND IRAES

Cytotoxic T lymphocyte antigen 4 (CTLA-4 or CD152) and programmed cell death 1 (PD-1) are two co-inhibitory receptors expressed on the surface of CD4+ and CD8+ T-cells that negatively regulate T-cell-mediated responses. In detail, CTLA-4 modulates CD28 co-stimulatory signaling by competing for its activating ligands (CD80 and CD86) on antigen-presenting cells, whereas PD-1 recognizes and binds to its endogenous ligands PD-L1 and PD-L2. Tumor cells exploit these inhibitory pathways to induce T-cell exhaustion and tumor evasion [1]. Accordingly, the disruption of CD28/CTLA-4/CD80/86 and PD-1/PD-L1 axes by monoclonal antibodies is able to restore T-cell-mediated antitumor responses and may induce durable anticancer effects [2].

Since the Food and Drug Administration approval of ipilimumab —a fully human anti-CTLA-4 IgG1 monoclonal antibody— for the treatment of metastatic melanoma in 2011, the use of immune checkpoint inhibitors (ICIs) has experienced a dramatic increase over the past years and revolutionized the therapeutics of solid malignancies. Beyond ipilimumab, six approved ICIs are currently available: nivolumab, pembrolizumab and cemiplimab (anti-PD-1 agents), and atezolizumab, avelumab and durvalumab (anti-PD-L1 agents). In addition, other anti-CTLA-4 (tremelimumab) and anti-PD-1 agents (lambrolizumab and pidilizumab) are being evaluated in phase I and II randomized clinical trials (RCTs) [3]. All of them are humanized or fully human monoclonal antibodies. These agents have been proven particularly effective in malignancies with strong immunogenicity, such as non-small-cell lung cancer (NSCLC) or melanoma, becoming the standard treatment option. In addition, ICI therapy has been approved by US and European regulatory agencies for an expanding range of indications, including renal cell carcinoma, head and neck squamous cell cancer, Hodgkin's lymphoma, gastric cancer, urothelial carcinoma, hepatocellular carcinoma and microsatellite instability-high cancers, among others [4].

Immune-related adverse events (irAEs) are a unique form of toxicity that results from the off-target effects on healthy tissues of an excessively activated immune response induced by ICIs. The most common sites of involvement are the skin, gastrointestinal tract, liver, endocrine organs (mainly hy-

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Table 1 Risk factors for the development of ICI-induced pneumonitis.

Treatment-related factors
Combination anti-CTLA-4 and anti-PD-1/PD-L1 therapy
Anti-PD-1/PD-L1 therapy (versus anti-CTLA-4)
Combination of ICI and conventional chemotherapy (versus ICI therapy alone)
Cancer-related factors
Cancer type (higher risk for NSCLC and RCC)
Histological subtype of NSCLC (higher risk for squamous cell carcinoma than adenocarcinoma)
Patient-related factors
Older age
Preexisting pulmonary conditions (i.e. COPD, interstitial lung disease, pneumothorax, asthma)
Preexisting autoimmune markers (i.e. rheumatoid factor, antinuclear antibody, antithyroglobulin or antithyroid peroxidase)
Male gender and smoking history (less consistent association)
Previous thoracic radiotherapy

COPD: chronic obstructive pulmonary disease; CTLA-4: Cytotoxic T lymphocyte antigen 4; ICI: immune checkpoint inhibitor; NSCLC: non-small cell lung cancer; PD-1: programmed cell death-1; PD-L1: programmed cell death 1 ligand 1; RCC: renal cell carcinoma.

pophysitis) and the lungs. The spectrum of organs affected, however, is very broad (e.g. myocarditis, encephalitis, aseptic meningitis, myasthenia gravis, uveitis or inflammatory arthritis). The overall incidence of irAE is higher for anti-CTLA-4 than for anti-PD-1 or anti-PD-L1 agents [5]. Almost two thirds of ipilimumab-treated patients experience at least one irAE of any grade, with 10-30% being considered serious (grade ≥3). In contrast, about 10% of patients receiving anti-PD-1 agents develop grade ≥3 irAEs [6]. Kinetics of appearance also depends on the type of ICI therapy. Fatal irAEs are rare (0.3-1.3%), with colitis and pneumonitis as the most frequent causes in patients receiving anti-CTLA-4 and anti-PD-1/PD-L1 antibodies, respectively.

ICI-INDUCED PNEUMONITIS

Incidence and risk factors. The most common pulmonary adverse event associated to immunotherapy is ICI-induced pneumonitis (also occasionally termed as ICI-induced interstitial lung disease). The development of pneumonitis in the setting of pivotal RCTs, however, was uncommon (<5%), and this irAE ranks the fifth after skin toxicity, hepatitis, thyroiditis and colitis. The incidence depends on the type of malignancy, with NSCLC patients being at the highest risk [7]. The incidence of any-grade pneumonitis in phase III trials ranged from <0.5% to 10%, whereas the corresponding figure for severe events (grade ≥3) varied from 0.5% to 3%. The majority of cases occur within the first 6 months from the initiation of treatment, although late-onset pneumonitis may appear up to 2 years later [8]. The median interval to the onset of pneumonitis in patients receiving anti-PD-1/PD-L1 therapy varies according to the type of cancer, from 7.8 weeks in melanoma to 15-30 weeks in NSCL [9], and is usually longer than in irAEs that affect other organs (skin, digestive tract or endocrine glands). It should be noted that restrictive enrollment criteria in RCTs may have underestimated the true incidence of this complication in clinical practice. Indeed, observational studies have usually reported higher incidence rates (3.5% to 19% of ICI-exposed patients) [8,10]. Despite its relative rarity, pneumonitis constitutes the most common pulmonary complication during the course of ICI therapy, as well as the leading cause of immune-related death.

Various risk factors for the development of ICI-induced pneumonitis have been identified (Table 1). The presence of preexisting pulmonary conditions —such as chronic obstructive pulmonary disease (COPD), interstitial lung disease, pneumothorax or asthma— acts as a strong predictor of this complication [10-12]. The histological subtype of NSCLC also plays a role, with a higher incidence in patients with squamous cell carcinoma compared with the adenocarcinoma subtype [8]. The association with older age, male gender, and former or current smoking is less consistent [13]. The previous receipt of radiotherapy revealed as a risk factor for pembrolizumab-induced pneumonitis in the KEYNOTE-001 trial [14]. More importantly, different regimens of ICI therapy are associated to distinct incidence rates of pneumonitis in NSCLC patients. A two- to three-fold risk increase has been observed for combination therapy (ICI plus platinum-based chemotherapy) compared with ICI monotherapy [15]. In addition, the combination of different ICIs targeting both CTLA-4 and PD-1 is associated with a higher incidence of pulmonary toxicity [16], as is the use of PD-1/PD-L1 inhibitors compared to CTLA-4 blockade

Table 2 **Differential diagnosis of ICI-induced pneumonitis.**

Conditions	Diagnostic clues and approaches
Infections	
Bacterial pneumonia	Fever, purulent sputum, pleuritic pain, high white blood cell count, increased acute phase reactants
Viral pneumonia	Nasopharyngeal swab for respiratory virus PCR testing
Pneumocystis jirovecii pneumonia	Cumulative corticosteroid exposure, prior use of purine analogs or T-cell-depleting agents, lymphopenia (low CD+ T-cell counts), positive serum β-D-glucan test (typically high levels)
Invasive pulmonary aspergillosis	High cumulative exposure to corticosteroids, severe COPD, positive culture for *Aspergillus* spp. in respiratory tract sample, positive galactomannan in BAL fluid
Pulmonary tuberculosis	History of untreated or partially treated tuberculosis, positive acid-fast bacilli smear or *M. tuberculosis* PCR assay in sputum or respiratory tract specimens, *M. tuberculosis* PCR in gastric aspirate samples (in patients unable to expectorate)
Non-infectious conditions	
Tumor progression	Hemoptysis, weight loss, increasing serum tumor markers, new or increasing nodular shadows and interlobular septal thickening, lung biopsy and histological examination
Pseudoprogression	Stable serum tumor markers, decreasing circulating tumor DNA levels, lung biopsy and histological examination
Radiation pneumonitis	Usually occurs in, or in close proximity to, the irradiated field (while ICI-induced pneumonitis most commonly develops at the edge of the radiation field or in a non-irradiated region)
Drug-induced pneumonitis	Increased eosinophil count in the BAL fluid
Other (congestive heart failure, dermatomyositis, polymyositis, allergic bronchopulmonary aspergillosis)	

BAL: bronchoalveolar lavage; COPD: chronic obstructive pulmonary disease; ICI: immune checkpoint inhibitor; PCR: polymerase chain reaction.

[13]. Finally, a meta-analysis shown that patients receiving PD-1 inhibitors have a higher incidence of any grade pneumonitis than those treated with PD-L1 inhibitors (3.6% versus 1.3%; *P*-value = 0.001) [17]. Although there are no clinically validated biomarkers to predict the occurrence of irAEs, one study showed that NSCLC patients with preexisting autoantibodies (rheumatoid factor, antinuclear antibody, antithyroglobulin or antithyroid peroxidase) were more prone to develop nivolumab or pembrolizumab-induced pneumonitis [18]. Interleukin-17 levels, eosinophil count or the clonal expansion of CD8+ T-cells are other biomarkers explored [19].

The mortality rates observed in real-life studies are often higher than that reported from RCTs, with figures as high as 27% is some series [12,20]. An analysis of the World Health Organization global individual case safety reports database, with data from more than 130 countries, revealed an attributable mortality of 17.5% among 1,694 cases of ICI-induced pneumonitis reported through November 2018. Patients with NSCLC were overrepresented in the group of fatal cases (versus melanoma), as were pembrolizumab treated patients (versus nivolumab) [21]. The timing of onset of ICI-induced pneumonitis also seems to influence outcome, with early events tending to be more severe and be associated with higher fatality rates than late-onset episodes [8,21].

Since the developement of irAEs suggests an enhanced T-cell-mediated immune activation in both healthy and tumor tissues, various studies have reported that patients developing this complication may have a better response to ICI therapy. This association, however, remains controversial and is determined by the type, timing and severity of irAE. A recent meta-analysis involving 12,600 participants from 51 studies showed that the occurrence of irAEs —particularly those with cutaneous and endocrine involvement— exerted a beneficial effect on overall survival and response rates in patients with advanced NSCLC. Although the development of ICI-induced pneumonitis had no significant effect on overall survival (hazard ratio: 1.14; 95% confidence interval [22]: 0.70 – 1.86), it was associated with a better response rate. Nevertheless, treatment discontinuation due to severe pneumonitis led to a poorer outcome [23].

Clinical presentation and radiological features. The majority of cancer patients developing ICI-induced pneumonitis are men (63.6%) with a median age of 65 years at the time of diagnosis [21]. The most common symptoms at presentation are dyspnea (41-80%) and cough (23-53%), and less than one third of the patients may be asymptomatic at diagnosis in the setting of routine surveillance imaging [9]. Hypoxemia and acute respiratory distress syndrome appear in about one third of patients, whereas the presence of fever is relatively uncommon. The underlying cancer is usually controlled at the onset

of pneumonitis, with 23% to 61% of patients having achieved an objective response [9]. Interestingly, other types of irAE may be concurrently present in up to one quarter of cases, mainly with gastrointestinal and endocrine involvement [21].

Chest computed tomography (CT) scan is performed in the majority of patients with clinical suspicion of ICI-induced pneumonitis. The radiological features are variable, since the elementary lesions observed may comprise ground glass opacities (GGO) (66.7% of cases examined in a recent narrative review), consolidations (56.6%), reticular opacities (26.1%), bronchiectasis (10.5%), micronodules (4%), a "crazy-paving" pattern (1.1%), and bronchiolitis (5%). On the other hand, the presence of isolated pleural effusion or hilar or mediastinal lymphadenopathies —other than those related to the underlying cancer— is uncommon [9]. The number of lobes involved varies between one and five, with a median of three [24]. There have been described several patterns of radiological presentation in the CT scan: cryptogenic organizing pneumonia (COP), non-specific interstitial pneumonia (NSIP), hypersensitivity pneumonitis, acute interstitial pneumonia (AIP), sarcoid-type reactions and acute respiratory distress syndrome. The most common radiological pattern is COP —manifested as discrete patchy or confluent shadows with or without air bronchography— followed by hypersensitivity pneumonitis and NSIP [6]. In addition, up to one fifth of cases do not fit into one of these well-defined radiological patterns, and atypical features such as GGO confined to the area around the tumor (peritumoral infiltration), nodules or unclassifiable interstitial changes are described [24]. The prognostic implications of different radiological patterns remain unclear, and some authors have reported that NSCLC patients with peritumoral infiltration had better response to corticosteroids and lower rate of disease progression [20].

Differential diagnosis. The diagnosis of ICI-induced pneumonitis is largely one of exclusion, since no clinical, laboratory or radiological features may be considered pathognomonic. The analysis of the bronchoalveolar lavage (BAL) fluid usually reveals an increased number of lymphocytes and a small number of eosinophils and neutrophils, and some studies have reported a large number of macrophages with high PD-L1 expression in the alveolar space [25]. The median proportion of lymphocytes in the BAL fluid is about 20% to 35% [20,26,27], with an inversion in the CD4+/CD8+ ratio due to the increase of CD8+ T-cell counts [26]. In contrast to sarcoidosis and other connective lung diseases with COP patterns, the neutrophil count in the BAL fluid is not increased in ICI-induced pneumonitis and there is no evidence of foamy macrophages found in hypersensitivity pneumonia. On the other hand, cases of pneumonitis with a NSIP pattern such as idiopathic pulmonary fibrosis are often associated with a paucity of lymphocyte in BAL [26]. None of these findings in the BAL fluid, however, are specific enough to make a diagnosis.

The differential diagnosis of ICI-induced pneumonitis is broad and comprises bacterial or viral pneumonia, active pulmonary tuberculosis, invasive fungal disease (IFD) and *Pneumocystis jirovecii* pneumonia (PCP). Non-infectious alternative diagnoses include tumor progression and pseudoprogression, radiation pneumonitis and other forms of drug-induced pulmonary toxicity (Table 2). In comparison with bacterial pneumonia, ICI-induced pneumonitis is less likely to be associated with fever (which, if present, is usually of low grade) and more prone to have respiratory failure. Pseudoprogression constitutes an atypical response of solid tumors under ICI therapy defined by an increase in the size of the primary tumor or the appearance of a new lesion followed by tumor regression. It is believed that pseudoprogression is due to an ICI-induced lymphocytic infiltration of the tumor or to the edema and necrosis of tumor tissue following therapy rather than real tumor growth [28]. Radiation pneumonitis and ICI-induced pneumonitis may exhibit overlapping symptoms and common radiological features that hamper the differential diagnosis.

Nasopharyngeal swab for respiratory virus testing and sputum and blood cultures must be systematically collected, as well as *Legionella* and pneumococcal urinary antigen. If the patient's respiratory status is acceptable, bronchoscopic examination should be performed to obtain a lower respiratory tract sample (bronchial aspirate, protected specimen brush or BAL fluid). In addition to bacterial culture, acid-fast bacilli smear and respiratory virus PCR testing, the BAL fluid is useful to made the diagnosis of PCP through the detection of ascii or trophic forms of *P. jirovecii* by direct conventional staining (i.e. Giemsa, toluidine blue O or Gömöri methenamine silver) or immunofluorescence (a more sensitive method). The diagnosis of PCP can be ruled out in the presence of a negative *P. jirovecii* real-time quantitative PCR in the BAL fluid, but not in an upper respiratory specimen (such as induced sputum, oral washing or nasopharyngeal aspirate). In case of discordance between both techniques (immunofluorescence-negative, PCR-positive samples), the detection of high fungal load by quantitative PCR would be suggestive of PCP, although diagnostic thresholds have not been established. In patients in whom the collection of a BAL sample is not feasible, a negative serum β-D-glucan result can virtually exclude PCP given the high sensitivity of this biomarker, in particular if the pre-test probability is relatively low [29].

Regarding the diagnosis of IFD —namely invasive pulmonary aspergillosis (IPA)— it should be born in mind the low sensitivity (below 50%) of the galactomannan antigen assay in serum samples in non-neutropenic patients [30]. In addition, the radiological features of IPA in patients with solid cancer patients are often non-specific, and the classical halo sign or air-crescent sign are absent in most of the cases [31]. On the other hand, ICI-induced pneumonitis may present with well-defined nodules or the "reversed halo" sign, resembling IPA or pulmonary mucormycosis [13]. Therefore, the clinical suspicion of IPA in a cancer patient on ICI therapy is most often raised by the isolation of *Aspergillus* spp. in a respiratory sample in the presence of underlying predisposing conditions such as severe COPD with multiple exacerbations or high cumulative corticosteroid doses. The diagnostic performance of the galactomannan assay in the BAL fluid (optical density

Table 3 Management of ICI-induced pneumonitis (modified from Zhou et al [25] and Haanen et al [32]).

Grade of pneumonitis	Clinical manifestations	Immunosuppressive treatment	Management of ICI therapy
Grade 1	No symptoms, radiological changes (GGO, non-specific interstitial pneumonia) limited to a single lobe or <25% lung parenchyma	Not required Monitor symptoms every 2-3 days Repeat chest imaging in 3-4 weeks	Consider holding ICIs
Grade 2	New or worsening symptoms affecting daily life, radiological changes involve multiple lobes and reaches 25-50% of lung parenchyma	Oral prednisone (1 mg/Kg daily or equivalent), with tapering over 4-6 weeks after recovery Monitor symptoms daily Repeat chest imaging every 1-2 weeks If no improvement after 48 hours of oral prednisone, manage as per grade 3	Hold ICIs Reintroduction should be delayed until a daily steroid dose ≤10 mg of oral prednisone
Grade 3 Grade 4	Serious new complications requiring oxygen inhalation and hospitalization, radiological changes involve all lobes or >50% of lung parenchyma, limited personal self-care ability Life-threatening dyspnea, ARDS requiring urgent intervention such as intubation	Intravenous methylprednisolone (2-4 mg/Kg daily or equivalent), with slow tapering over ≥6 weeks If not improving or worsening after 48 hours add: - infliximab IV 5 mg/kg or - MMF IV 1 g BID or - IVIGs for 5 days or - cyclophosphamide	Permanently discontinue ICIs

ARDS: acute respiratory distress syndrome; BID: two times a day; GGO: ground glass opacities; ICI: immune checkpoint inhibitor; IVIGs: intravenous immunoglobulins; MMF: mycophenolate mofetil.

index ≥1.0) in non-hematological patients with immunosuppressive conditions is good in terms of sensitivity and negative predictive value [30].

Therapeutic management. The suspicion of ICI-induced pneumonitis should prompt the initiation of immunosuppressive therapy. Therefore, it is important to rule out the presence of concomitant infection (in particular in the case of grade ≥2 pneumonitis) or, alternatively, to administer a broad-spectrum antibiotic in parallel to immunosuppression. The type and amount of immunosuppressive therapy —oral prednisone, intravenous methylprednisolone or, for steroid-refractory cases, infliximab, tocilizumab, mycophenolate mofetil or cyclophosphamide— depends on the severity of the pneumonitis (Table 3) [25,32]. Since corticosteroid tapering should be performed slowly, PCP prophylaxis should be added in patients who are expected to receive 20 mg of prednisone daily (or equivalent doses) for >4 weeks. In addition, and due to the potential requirement of additional immunosuppressive therapy, conventional screening for latent tuberculosis and chronic hepatitis B virus infection is advisable before initiating ICIs, followed by appropriate prophylaxis or therapy if needed [22].

IMPACT OF ICI THERAPY ON THE RISK OF INFECTION

As discussed above, ICIs enhance T-cell-mediated immunity and this therapy is not associated *per se* with direct immunosuppressive effects. Indeed, pivotal RCTs did not show an increased risk of infection in patients receiving anti-CTLA-4 or anti-PD-1/PD-L1 agents [22]. Nevertheless, the management of irAEs often requires the administration of corticosteroids and other immunosuppressive therapies, which in turn may increase the risk of opportunistic infections such as PCP, IFD, cytomegalovirus disease or reactivation of latent tuberculosis infection [22,33,34]. A recent single-center retrospective study compared the occurrence of infectious complications in patients with advanced NSCLC that received ICIs associated to conventional chemotherapy and those treated with chemotherapy alone. There were no significant differences in the cumulative incidence of infection (15% versus 22%, respectively), with pneumonia as the most common event in both groups. In fact, urinary tract infection was more common among patients receiving only chemotherapy. The diagnosis of COPD and neutropenia and the previous use of corticosteroids (but not ICs) were identified as independent risk factors for infection. Interestingly there were no cases of opportunistic infection within the subgroup of patients with irAE [35]. These findings are in line with those previously reported from a large cohort (n = 740) of melanoma patients treated with ipilimumab, pembrolizumab or nivolumab, 7.3% of which experienced serious infection after a mean interval of 135 days from the initiation of ICIs. Again, the prior or concomitant use of corticosteroids and infliximab for the treatment of irAEs were the only predictive factors identified [36]. It has been recently suggested that PD-1/PD-L1 blockade may lead to

active tuberculosis, and PD-1 knockout mice exhibit impaired immune responses against *Mycobacterium tuberculosis* [37]. A systematic review including 27 studies identified 35 cases of active occurring in patients treated with anti-PD-1/PD-L1 agents (mainly nivolumab). The pooled estimate incidence was 2,000 cases per 100,000 persons, which is 35 times higher than that in the general population [38]. Nevertheless, it is difficult to control for the confounding effect resulting from the use of immunosuppressive therapy for irAE. The relative contribution of anti-PD-1/PD-L1 therapy on the incidence of active tuberculosis remains controversial, and no risk increase has been demonstrated in population-based studies [39]. On the other hand, an alternative explanation proposes that PD-1 blockade may actually unmask latent or subclinical tuberculosis by boosting *M. tuberculosis*-specific T-cell immunity, similar to the immune reconstitution inflammatory syndrome observed in people with human immunodeficiency virus infection that initiate antiretroviral therapy [40].

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CONFLICT OF INTEREST

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Current management of CMV infection in cancer patients (solid tumors). Epidemiology and therapeutic strategies

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ABSTRACT

Little evidence is available regarding the incidence of CMV disease in patients with solid cancers. Latest data show that approximately 50 % of these patients with CMV PCR positivity developed clinically relevant CMV-viremia, and would require specific therapy. In the clinical arena, CMV reactivation is an important differential diagnosis in the infectological work up of these patients, but guidelines of management on this subject are not yet available. CMV reactivation should be considered during differential diagnosis for patients with a severe decline in lymphocyte counts when receiving chemoradiotherapy or immunochemotherapy with lymphocyte-depleting or blocking agents. Monitoring of CMV reactivation followed by the implementation of preemptive strategies or the establishment of early antiviral treatment improves the prognosis and reduces the morbidity and mortality of these patients.

Keywords: Cytomegalovirus, cancer patients, lymphopenia, antiviral preemptive strategy

INTRODUCTION

Cytomegalovirus (CMV) is an important cause of both morbidity and mortality in solid organ or stem-cell transplanted patients and immunocompromised hosts, including cancer patients [1-3]. CMV reactivation especially in immunocompromised patients may rapidly progress to a fatal CMV disease. Patients with CMV infection have a wide variety of clinical manifestations, including fever, enterocolitis, pneumonitis, retinitis, hepatitis, encephalitis, nephritis, and disseminated disease [4]. The exact mechanism of the reactivation of CMV is not well established; however, the disturbance of the host's immune defences plays an important role [5]. Immune impairment in patients with malignancies was considered to be a risk factor for CMV disease. The term "CMV infection" indicates latent and asymptomatic form of infection, whereas the "CMV disease" means symptomatic end-organ involvement [6].

The relevance of infection and reactivation in haematological patients has been a matter of interest, although efforts have fundamentally focused on reactivation in the post-allogenic haematopoietic stem cell transplant (HSCT) patient cohort. Newer transplant modalities have been progressively introduced in the clinical setting, with successively more drugs being used to manipulate graft composition and functionality. Less is known about the effects of CMV in terms of mortality or disease progression in patients with other malignant haematological diseases or solid neoplasms who are treated with immunochemotherapy or new molecules, or in patients who receive autologous SCT. The absence of serious consequences in these groups has probably limited the motivation to deepen our knowledge of this aspect.

However, the introduction of new therapeutic agents for solid and haematological malignancies has led to a better understanding of how natural killer (NK) cells, CD4+ and CD8+ T lymphocytes, and B lymphocytes interact, and of the role of CMV infection in the context of recently introduced drugs such as modern immunochemotherapies, immune check-point inhibitors such as programmed cell death-1 (PD-1) or programmed cell death-ligand 1 (PD-1L) inhibitors and cytotoxic T-lymphocyte antigen 4 (CTLA4) inhibitors, Bruton tyrosine kinase (BTK) inhibitors, phosphoinosytol-3-kinase (PI3K) inhibitors, Janus-kinase (JK) inhibitors, proteasome inhibitors, anti-CD52 blocking agents, purine analogues, anti-BCL2 drugs, and even CAR-T cells therapy [7].

Because of all this, the incidence of CMV infection in patients with malignancies varies widely in different studies [8,9]. However, only limited data is available on the role of CMV reactivation/disease in patients with solid cancers e.g. under

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chemotherapeutic treatment or direct anti-target immunotherapies. In the clinical experience of various centers, CMV reactivation is an important differential diagnosis in the infectological workup of these patients, but guidelines of management on this subject are not available yet.

CYTOMEGALOVIRUS INFECTION AND ITS CLINICAL IMPACT IN PATIENTS WITH SOLID TUMOURS

To date, only a few small case reports, some observational studies or post-mortem analyses are available. A single centre study that analysed 107 patients with CMV disease during 2008-2009, including 75 with solid cancer, reported a mortality rate of 61.3 % in the solid organ malignancy group [10]. With an overall mortality rate of 56.1% (60/107), worse outcomes were observed in patients with solid organ malignancies than in those with haematological malignancies (mortality rate of the haematological malignancy group: 43.8%). Mechanical ventilation, leukocytosis, and lack of appropriate early treatment were independent predisposing factors of mortality. Furthermore, CMV viremia was associated to higher mortality rates in cancer patients. In a retrospective post-mortem analysis of 47 cancer patients with histologically proven gastrointestinal CMV disease, 13 patients had an underlying solid cancer [11]. An older report demonstrated a CMV attributable mortality of 42% in a study cohort including both haematological and solid tumour malignancies [12], although the objective of this study was to estimate the frequency of CMV pneumonia and describe its clinical and radiological presentation in adult non-transplantation patients with cancer. Of the 10,441 autopsies performed during the period of January 1964 through December 1990, 9,029 were evaluable. Twenty histopathologically confirmed cases of CMV pneumonia were found, representing a frequency of 2.2 cases per 1.000 autopsies. When the frequency of CMV pneumonia was compared for the periods 1964-1979 (1.5 cases per 1.000 autopsies) and 1980-1990 (4.6 cases per 1,000 autopsies), it was significantly increased directly related to the number of patients with solid malignancies ($p < .05$). At that time (mid-1990s), the authors from the M.D. Anderson Cancer Center (Houston) concluded that CMV pneumonia was an uncommon diagnosis at autopsy for adult non-transplantation patients with cancer, and that was usually found in conjunction with a disseminated neoplastic process.

These data could suggest that a reliable risk of CMV reactivation/disease exists in solid cancer patients. In a more recent definitive study, the incidence and impact of CMV reactivation in solid cancer patients was investigated by performing a retrospective analysis of a single centre CMV database [13]. The authors retrospectively examined the occurrence of CMV reactivation in patients with solid tumours, resulting in 107 solid cancer patients testing positive for CMV reactivation, out of 890 CMV-positive blood serum samples of mainly haematological and oncological patients. Seventeen patients with solid cancer and a positive CMV-PCR test were identified, of which eight patients had clinically relevant CMV disease and received prompt antiviral treatment. While five patients fully recovered, but despite prompt antiviral treatment three patients died. Of them, two had significant co-infections with another pathogen (Epstein Bar Virus and *Aspergillus*, respectively), which could indicate that CMV reactivation was at least one factor contributing to sepsis. Patients with poor outcomes had progressive underlying neoplastic disease and were receiving adjuvant or salvage chemotherapy. The authors concluded that CMV reactivation and disease might be underestimated in routine clinical practice. In their retrospective analysis they showed that approximately 50% of patients suffering from solid cancers with a positive CMV polymerase chain reaction also had clinically relevant CMV disease requiring antiviral therapy.

The summarized studies show the clinical impact of CMV reactivation and viremia in solid tumour patients. Accumulating data suggest that CMV disease in these patients is more frequent than previously estimated. Furthermore, it must be pointed out that CMV testing is not routinely done in clinical practice and that therefore CMV reactivation or disease may be underreported. Due to the lack of consensus and specific guidelines on CMV infection in patients with solid neoplasms, the positivity cut-off points and significance of CMV viral load (VL) in these patients may vary and differ between different centres and publications. Significant CMV VL was considered to be above >1,000 copies/ml in some studies. However, more recent evidence places the potentially significant viremia above 4,000 copies/ml [13]. Since approximately 50% of patients with CMV PCR positivity would develop clinically relevant CMV-viremia, they would require specific anti-CMV therapy. The early administration of specific antiviral treatment may improve the outcome of these patients and may avoid unsuccessful antibiotic therapy and prolonged hospitalization. Clinicians should be aware of the broad range of potential complications of CMV infection in these patients with solid tumours.

For all these reasons, it would be appropriate to propose the inclusion of routine CMV screening in solid cancer patients presenting with subacute or intermediate duration fever of unknown origin. Larger studies are necessary to identify risk factors for developing CMV disease in this subpopulation. Moreover, the raising number of elderly patients receiving chemotherapy for solid tumours and the fact that CMV incidence increases with age suggests that CMV reactivation and CMV disease are expected to increase in the near future.

A series of unanswered questions and unmet needs in the field of CMV infection in patients with solid tumours deserve to be addressed in the coming years (Table 1).

"UNEXPECTED" CMV INFECTION OR REACTIVATION IN RARE SOLID NEOPLASMS

Beyond merely anecdotal descriptions, series of experiences of CMV reactivation or infection have been reported in patients with very peculiar solid tumours. These special forms have been found mainly in patients with oesophageal cancer

Table 1 CMV infection/disease in solid cancer. Not covered issues

UNMET NEEDS	PENDING QUESTIONS
Scarce information and little evidence of studies or trials; Underestimation of cases	Does it reflect a greater net state of immunosuppression?
Therapeutic guidelines adapted to new non-transplanted immunocompromised hosts (oncohaematological patients, solid tumours)	Is CMV a marker or a consequence of active, uncontrolled neoplasm?
Reduce morbidity and mortality with earlier diagnosis and management	Routine CMV screening against prolonged fevers or unknown origin fever?
Education and training in high clinical suspicion of CMV in groups of emerging patients at risk	Which are the Risk Factors that promote CMV in these patients?
Need to establish consensus cut-off points for CMV viral load in these patients	Solid cancer, age and serostatus of CMV; higher prevalence with aging?
Reduce use of other antimicrobials and antifungals, and specifically treat only viral infection	
Avoid prolonged and unnecessary hospitalizations	

[14], malignant pulmonary mesothelioma [15], and aggressive brain neoplasms undergoing immunochemoradiotherapy protocols that include temozolomide [16,17]. Obviously without forgetting the possible influence of the quintessential anti-CD20 agent, rituximab, in the treatment of multiple oncohaematological neoplasms as a factor that promotes infection or reactivation by CMV [18].

In a retrospective study whose objective was to identify factors associated with CMV reactivation in patients with oesophageal cancer who were receiving chemoradiotherapy, CMV reactivation was not uncommon and was associated with the minimum lymphocyte counts [14]. This study included oesophageal cancer patients receiving definitive or palliative chemoradiotherapy; patients with fever during chemoradiotherapy underwent a systemic work-up to detect the primary focus of infection, and CMV antigenemia (period 2013-2020) was assessed in cases of unidentifiable infection. Among 132 patients, 124 received 5-fluorouracil plus cisplatin and 8 received oxaliplatin–5- fluorouracil–levofolinate chemotherapy. Overall, 19 patients had CMV reactivation, 37 had other infections, and 76 had no identified infection (groups 1, 2, and 3, respectively). Median minimum lymphocyte counts were 81.0/µl (interquartile range: 52.0–144.0/µl) in CMV reactivation group (1), with counts that were significantly lower than in other groups (2 and 3). This retrospective study demonstrated that lymphopenia caused by chemoradiation was associated with CMV reactivation, and that planning target volume had a greater effect on lymphopenia than the chemotherapy itself.

In a consecutive case series of 144 malignant pleural mesothelioma (MPM) patients, one group evaluated two biomarkers of CMV: IgG serostatus (defined as positive and negative) and DNAemia (>100 copies/mL of cell free CMV DNA in serum). Approximately half of the MPM patient population was CMV IgG seropositive (51%). CMV DNAemia was highly prevalent (79%) in MPM and independent of IgG serostatus [15]. DNAemia levels consistent with high level current infection (>1,000 copies/mL serum) were present in 41% of patients. Neither IgG serostatus nor DNAemia were associated with patient survival. In tissues, the authors observed that CMV DNA was present in 48% of tumours and only 29% of normal pleural tissue obtained from individuals without malignancy. These results suggested that nearly half of MPM patients have a high level current CMV infection at the time of treatment and that pleural tissue may be a reservoir for latent CMV infection.

Temozolomide is an alkylating agent, from the triazene family, which is administered orally. It has been used in tumours of the central nervous system (CNS), such as glioblastoma multiforme, refractory anaplastic astrocytoma, and others: brain metastases, refractory primary brain lymphoma, melanoma, etc. It is used in chemoradiotherapy schemes, and in associations with m-TOR inhibitors [16]. Among its adverse effects, it causes myelotoxicity, which leads to profound and prolonged lymphopenia, lasting 2-12 months, which for some groups is a reason to monitor CD4+ T lymphocytes, in order to make predictions about the increased risk of opportunistic infections [17].

In a prospective cohort of patients receiving this drug for neuroendocrine tumors in 2006, the overall incidence of opportunistic infections was 10 percent, while among patients receiving therapy for > or =7 months, the incidence was 20 percent [19]. Among the latter, CMV infections (13% in some series) and severe forms of the disease such as colitis, pneumonitis, and myelitis have been described. Few cases of TMZ-induced cytomegalovirus reactivation have so far been reported, and there are no guidelines regarding the use of chemotherapy after recovery from CMV reactivation. For this reason, many centres recommend monitoring CMV by periodic determination of antigenemia or DNAemia using molecular techniques [20].

In patients undergoing treatment with temozolomide, close surveillance of opportunistic infections (pneumocystosis, varicella-zoster, cytomegalovirosis, candidiasis) should be assessed, in addition to implementing narrow microbiological risk monitoring and pre-emptive treatment or antimicrobial prophylaxis strategies.

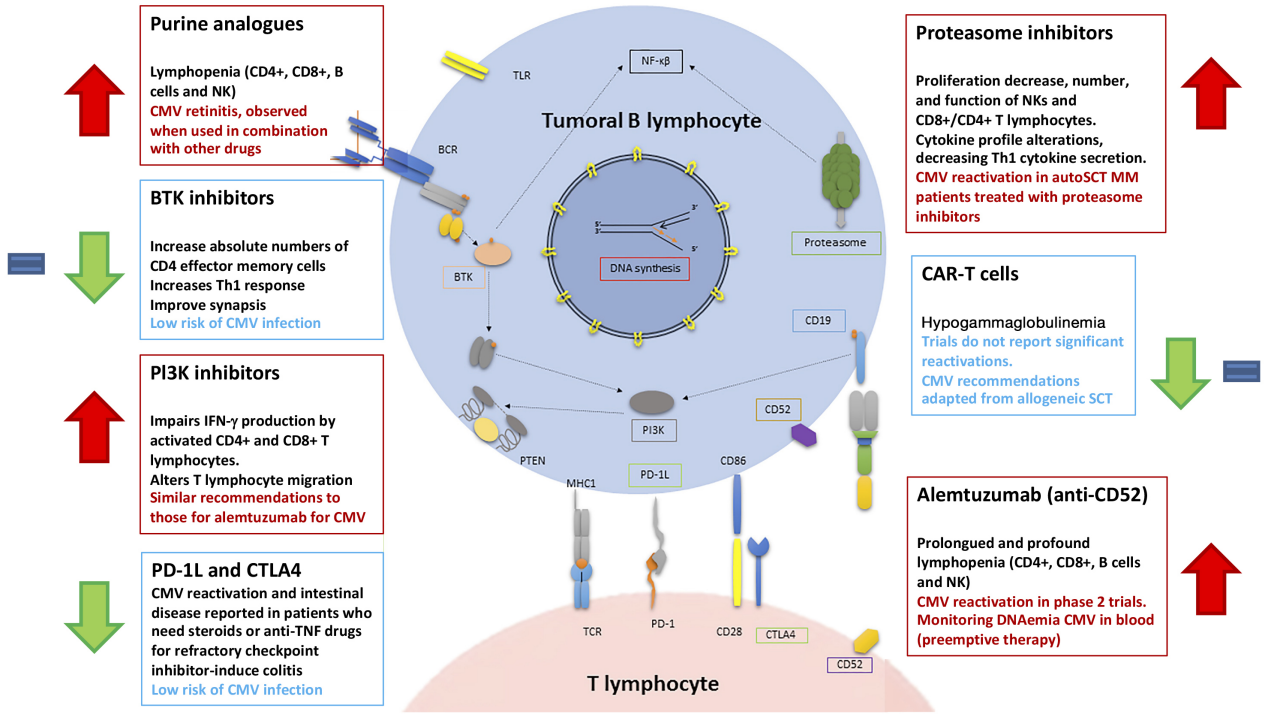

Figure 1 | **CMV infection/reactivation risk in the context of antitumoral drugs used in oncohaematological patients**

Red arrow: symbolizes increased risk of CMV reactivation or infection

Green arrow and equals sign: represent a decreased risk of CMV reactivation or infection or a situation similar to the group of patients related to the diagnosis who do not receive these drugs

BCR, B cell receptor; BTK, Bruton tyrosine kinase; CART cells, chimeric antigen receptor T cell; CMV, cytomegalovirus; CTLA4, cytotoxic T-lymphocyte antigen 4; MHC, major histocompatibility complex; NK, natural killer; PI3K, phosphatidyl inositol 3 kinase; PD, programmed death; PD-1L, programmed death-ligand 1; PTEN, phosphatase and tensin homologue; SCT, stem cell transplantation; TLR, toll-like receptor; MM, multiple myeloma.

Modified and adapted of reference 7 [Alonso-Alvarez S, et al. (2021) Cytomegalovirus in Haematological Tumours. Front. Immunol. 12:703256. doi: 10.3389/fimmu.2021.703256]

CMV IN OTHER ONCOHAEMATOLOGICAL SETTINGS: NEW DRUGS AND NEW THERAPIES IN LYMPHOPROLIFERATIVE SYNDROMES AND MULTIPLE MYELOMA

The introduction of new therapeutic agents for solid and haematological malignancies has led to a better understanding of how immune cells (NK cells, CD4+ and CD8+ T and B lymphocytes) interact, and of the role of CMV infection in the context of recently introduced drugs such as modern immunochemotherapies.

For reasons of extension of this manuscript, this section will be summarized in Figure 1 with additional comments. The figure shows how certain families of drugs or therapeutic strategies favour and increase the risk of CMV reactivation or infection in certain settings of oncohematological disease, and how others do not increase this risk or their influence is less or even neutral [7].

Without forgetting the weight and influence of corticosteroids (their dose and duration), the risk of CMV reactivation or infection is increased in the following groups of oncohaematological patients receiving new treatment modalities, such as: patients with lymphoproliferative syndromes treated with purine analogues, alemtuzumab (anti-CD52 agent) or PI3K inhibitors (idelalisib, e.g.), and patients diagnosed with multiple myeloma undergoing auto-HSCT and previously treated with proteasome inhibitors.

The risk of CMV infection and disease is not increased, and is even comparatively lower, in patients treated with Bruton's tyrosine kinase inhibitors, or with anti-PD-1L or CTLA4 agents, or in those receiving CAR- T [21]. There are documented cases of CMV reactivation in the first month and during the first three months after CAR-T cells therapy. Previous therapies, disease stage, and patient basal characteristics seem to be crucial. Regarding prophylaxis against viral infections, there are no unique international recommendations, and existing ones are heterogeneous. The European recommendations are based

on data from allogeneic transplant recipients. In general, antiviral prophylaxis is established with acyclovir or valacyclovir at least up to one year after CAR-T infusion, or until a CD4+ T lymphocyte count greater than 0.2 x 10^9/L is documented.

CONCLUSIONS

The relationships between CMV infection and oncohaematological pathologies is becoming better known, fundamentally, as a result of the important repercussions from the management of the infection and reactivation of the CMV in the post-transplant patients (post-allogeneic haematopoietic stem cells, or post-solid organ) [22]. The role of CMV in cancer has primarily focused on the presence of virus in tumours [23]. Less well described is the epidemiology of active CMV infection in solid tumour cancer patients. Although rare, CMV infection can be lethal in patients with cancer. However, the criteria for the prevention of CMV reactivation during solid cancer treatment are unclear. CMV reactivation should be considered in the differential diagnosis of patients with a severe decline in lymphocyte counts when receiving chemoradiotherapy or immunochemotherapy with lymphocyte-depleting or blocking agents. Furthermore, there are many other situations that give rise to severe immunosuppression, either due to the oncohaematological pathology itself or to the treatments used, which should prompt a close surveillance concerning the complications derived from infection by this virus. Thus, it is necessary to study the effect of new drugs on the immune system and so adapt CMV prophylaxis and infection monitoring to different treatment schemes and situations, now that new anti-CMV drugs with fewer secondary effects are available for this purpose. Whether CMV, either at the tumour site or as an active infection with positive DNAemia, is present in some solid tumours and contributing to patient outcomes is yet an insufficiently explored area of research [24].

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Current strategies for infectious diseases management

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Rapid techniques for therapeutic optimization. Diagnostic stewardship

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ABSTRACT

Rapid microbiologic methods provide clinicians with information regarding the causative organisms of infections and their resistance to antimicrobials to optimize patient outcomes and antimicrobial use. Diagnostic stewardship requires that appropriate tests are requested and information is translated into appropriate management. The implementation of rapid techniques also provides collaborative opportunities between antimicrobial stewardship and diagnostic stewardship programs contributing to limiting the spread of antimicrobial resistance, and decreasing mortality, hospital length of stay, and healthcare costs.

Keywords: rapid microbiologic methods, antimicrobial stewardship, diagnostic stewardship, PRODIM

INTRODUCTION

The critical role of the microbiology laboratory in infectious disease diagnosis in conjunction with recent advances in microbial diagnostics are revolutionizing clinical microbiology and promise to improve patient outcomes and curb the antimicrobial resistance crisis by improving the use of antibiotics. However, rapid diagnostics only improve clinical outcomes if there is a close relationship between the microbiologists and the infectious disease physicians that properly interpret results and apply them to treatment decisions. This approach may also require expanding the hours of laboratory operation and microbiological assessment 24 h a day, 7 days a week, especially in hospitals with a high health care load and with relevant complexity, which will provide, in addition, an enormous value to the health care team and a cost-effective impact on the clinical management of patients [1-3].

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This minireview focuses on currently available rapid diagnostic microbiologic tests that provide opportunities for antimicrobial stewardship programs to improve antimicrobial use and clinical and economic outcomes. The information presented here is a summary of a lecture given at the XI Updating Course of Antimicrobials and Infectious Diseases last February 2022 in Madrid (Spain).

PROGRAMS FOR OPTIMIZING DIAGNOSTIC MICROBIOLOGY (PRODIM)

The goal of the Programs for Optimizing Diagnostic Microbiology (PRODIM), equivalent to diagnostic stewardship, is to optimize the use of diagnostic techniques and algorithms in order to obtain results that have a tangible and cost-effective impact on the clinical management of patients. These programs, as described by Messacar K, *et al.* aim to select the right test for the right patient, generating accurate, clinically relevant results at the right time to optimally influence clinical care and to conserve health care resources [4].

One of the most important aspects in order to provide a high level of diagnostic quality, is the proper selection of all microbiology specimens as well as their collection and transportation to the microbiology laboratory to optimize analysis and interpretation. Since result interpretation in microbiology depends entirely on the quality of the specimen submitted for analysis, and microbiology specimen selection and collection are the responsibility of the medical personnel, not usually the laboratory, those that collect specimens must ensure its good quality and that specimens arrive at the laboratory for analysis as quickly as possible after collection [5]. Proper specimen management is crucial for an accurate laboratory diagnosis and confirmation, and directly impacts patient care and patient outcomes, patient length of stay, hospital infection control, hospital and laboratory costs and laboratory efficiency, and influences therapeutic decisions and antimicrobial stewardship. In this sense, microbiology laboratory

results that are reported should be accurate, significant, and clinically relevant [2,5,6].

All personnel in charge of the selection, collection, transport and storage of patient specimens must consult the microbiology laboratory to ensure that specimens are adequately managed and call the laboratory to clarify and resolve problems. Some of the relevant aspects to take into account when collecting a specimen include avoiding contamination with commensal microbiota from sites such as lower respiratory tract, nasal sinuses, superficial wounds or fistulae, and sending a specimen (tissues, aspirates, fluids) and not a swab, since these hold small volumes of the specimen (0.05 mL) and make it difficult to get bacteria or fungi away from the swab fibers onto media. However, if flocked swabs are used there is a better release of contents and are more effective. Swabs can be used for collecting nasopharyngeal specimens for the diagnosis of viral respiratory infections. All specimens must be labelled accurately and completely so that interpretation of results will be reliable. In addition, the main criteria for the collection of infected material or blood specimens is that they should be collected prior to the administration of antibiotics, since once antibiotics have been started, the microbiota changes, leading to potentially misleading culture results. Regarding the microbiology laboratory, microbiologists must reject specimens of poor quality and give advice that they will not report everything that grows, since this information is unnecessary and could result in inaccurate diagnosis and inappropriate treatment. Moreover, susceptibility testing should be done only on clinically significant isolates, not on all microorganisms recovered in culture [5].

Selecting the right test for the microbiological diagnosis involves the evaluation of test performance, such as sensitivity and specificity, predictive values, testing volumes, diagnostic yield, laboratory feasibility, cost and clinical impact. Nowadays, the use of nucleic acid amplification and detection techniques, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) and next generation DNA sequencing, has increased the impact of the microbiological diagnosis, but results can be difficult to interpret when conventional culture-based or serological techniques are unable to confirm the presence of infection and it may be necessary to rely on clinical findings. Nevertheless, since standard techniques for identification of microorganisms require at least 48-72 hours for final results, rapid microbiologic tests provide opportunities for antimicrobial stewardship programs to improve antimicrobial use and clinical and economic outcomes [4].

Rapid tests are defined as those providing a same-day turnaround time (TAT). It is important to distinguish the analytic TAT from the actual TAT of a test (the time from when a test is ordered to when the result is translated into a change in patient care). A rapid test with analytic TAT of 2 hours that is batched and performed once daily represents little improvement in TAT over some conventional culture-based methods. Similarly, tests that require an isolated bacterial colony to perform should not be considered really rapid. Thus, the most useful rapid tests are those that can be applied directly to patient samples, however, there is no reason to use a more expensive test with shorter analytic TAT if the actual TAT of the test will not be meaningfully reduced [7]. Once the appropriate test has been selected for implementation, the next step is directing testing toward the right patients. It is important to know that overuse of rapid diagnostic tests can add to health care costs without having a significant impact on patient care, whereas underuse or inappropriate use may lead to suboptimal clinical outcomes. If rapid testing is to provide any benefit, there must be a postanalytic system in place for the results to be translated into action [4,6,7]. The timely communication of test results requires collaboration between the laboratory and antimicrobial stewardship groups, and within this framework, diagnostic stewardship effectively complements antimicrobial stewardship programs in the adequate microbiological diagnosis and selection of antimicrobial treatments with the goal of improving patient management and to decrease unnecessary antimicrobial use [8].

RAPID DIAGNOSTIC MICROBIOLOGICAL TECHNIQUES

Gram-staining. Direct Gram staining of positive blood cultures, respiratory samples, abscesses, urine, and other clinical material, is an inexpensive, simple examination that is used worldwide, including in lower-income countries, that provides immediate information for detecting causative pathogens and may guide the appropriate use of initial antibiotic therapy [9]. In addition to determining Gram reaction and bacterial morphology, Gram stain can assess the suitability for culture of a sputum sample by determining the numbers of squamous epithelial cells and polymorphonuclear leukocytes present in the specimen. The numbers and morphologies of bacteria seen in direct smears of clinical material is very valuable for early clues as to cause of disease, as well as for comparison to the growth resulting after incubation.

Antigen detection assays. Specific microorganism antigens can be rapidly detected from a clinical specimen through immunoassays. At present there is a wide variety of commercialized immunoassays for detecting microbial antigens (bacteria, viruses, parasites, and fungi) and specific antibodies. These assays have been designed in a variety of formats and can be performed as point-of-care tests in as little as 15 to 30 min. Among the most popular formats is the immunochromatographic method (ICT), more commonly referred to as the lateral-flow immunoassay (LFA). These assays are straightforward to perform, are inexpensive, and do not require specialized instrumentation. In addition, multiplexed strip tests are available to detect 3 to 14 pathogens [10]. The most frequently and useful rapid antigen detection assays applied directly from different clinical specimens include the detection of *Legionella pneumophila* serotype 1, and *Streptococcus pneumoniae* from urine, allowing the rapid diagnosis and treatment of pneumonia, the detection of *Cryptococcus neoformans* antigen from cerebrospinal

fluid, the detection of *Streptococcus pyogenes* antigen from pharyngeal swabs and also from wounds, many respiratory viruses from respiratory samples including respiratory syncytial virus, SARS-CoV-2, and influenza virus, fungi like *Aspergillus* spp., *Histoplasma capsulatum* and *Blastomyces dermatitidis* from different clinical samples, enteric pathogens from gastrointestinal specimens such as norovirus, rotavirus, *Helicobacter pylori*, *Closdridioides difficile, Cryptosporidium* spp., *Giardia* spp., among others, and *Plasmodium* spp. from blood [5,10]. These tests are also useful in patients previously treated with antimicrobials and allow rapid diagnosis and implementation of specific treatments, avoiding the use of inadequate antimicrobials. Because immunoassays are simple to perform and give timely results of sufficient sensitivity for routine clinical diagnosis, they will continue to be widely used.

Antibody detection assays. Antibody detection assays are also available for the rapid diagnosis of infectious diseases. These assays are easy to perform and results are available in 5-15 minutes from serum, providing rapid diagnosis and implementation of adequate treatment. Examples of these rapid assays are the highly sensitive rose Bengal test for the diagnosis of brucellosis, the new immunochromatographic strip and dual-point-of-care tests for the detection of both treponemal and nontreponemal antibodies used for the screening and/or diagnosis of syphilis, and the monospot test for the detection of heterophile antibodies in the course of mononucleosis syndrome due to Epstein-Barr virus. Assays for the detection of SARS-CoV-2 antibodies and HIV antibodies are also widely used. The dengue virus can also be tested by using a rapid diagnostic test which detects either IgM and IgG antibodies or IgM antibodies and the NS1 protein. Performance of all these tests is straightforward and does not require technical expertise or special laboratory equipment and must be considered as part of the standard of care [10,11].

Nucleic acid amplification tests and new technologies. Nucleic acid amplification tests are designed for the detection of one or more RNA or DNA sequences specific to a single pathogen and are available for rapid testing as PCR-based techniques and as isothermal nucleic acid amplification techniques (LAMP). They have greater sensitivity than ICT tests but require a higher degree of technicality and training. Since it is of medical interest to simultaneously test the multiple pathogens that may cause signs and symptoms in the patient (bacteremia, pneumonia, gastroenteritis, meningitis), in order to optimize diagnosis, these tests may also adopt a syndromic approach and may include the simultaneous detection of virus, bacteria, fungi, parasites and some resistance genes. With the use of these tests results can be obtained between 20 and 100 min, and allow to implement antimicrobial treatment within a few hours of specimen collection [10].

New technologies provide rapid identification and detection of resistance markers directly from blood (nuclear magnetic resonance testing) or rapid identification and antimicrobial susceptibility from positive blood cultures (peptic nucleic acid fluorescent, morphokinetic fluorescent cellular analysis, nephelometry, syndromic rapid multiplex polymerase chain reaction, microarray-based or nanoparticle probe technology). These diagnostic techniques improve the management of patients with bloodstream infections, particularly those infected with resistant organisms such as extended-spectrum beta-lactamase-producing or carbapenem-resistant Gram-negative bacilli. They are relatively easy to implement and most seem to have a favourable cost-benefit balance. The use of these tests can also reduce unnecessary antimicrobial exposure and increase the appropriateness of empirical antibiotic therapy in bacteremia, pneumonia, central nervous system and gastrointestinal infections [12].

Rapid identification and susceptibility testing from blood cultures. Rapid identification of organisms from blood is a critical component in providing quality healthcare. The combined use of MALDI-TOF from blood-cultures, direct antimicrobial susceptibility testing and real-time antimicrobial stewardship intervention allows early optimisation of antimicrobial therapy and provides significant hospital savings [1,2,13].

The conventional EUCAST (European Committee on Antimicrobial Susceptibility Testing) standardized antimicrobial susceptibility testing method provides results after 16–20 h incubation from colonies grown in culture. However, since rapid antimicrobial susceptibility testing (RAST) is very important, especially in patients with bloodstream infection in which appropriate early therapy improves the clinical outcome, recently, the EUCAST has validated a rapid disc difussion RAST directly from positive blood culture bottles that provides reliable antimicrobial susceptibility testing results for relevant bloodstream infection pathogens after 4-6 h of incubation. The successful introduction of the RAST method in routine microbiology enables rapid evaluation of empirical antibiotic treatment in bloodstream infections [14].

RAPID DETECTION OF RESISTANCE TO ANTIMICROBIALS AND EPIDEMIOLOGICAL SURVEILLANCE OF MULTIDRUG-RESISTANT PATHOGENS

The spread of multidrug-resistant microorganisms has challenged the clinical microbiology laboratory to recognize the presence of responsible resistance mechanisms and develop techniques for their rapid detection. These resistance mechanisms can be detected by conventional antimicrobial susceptibility testing (phenotypic methods) and results will be obtained after 48-72 h after specimen collection, or by genotypic methods in which results can be obtained directly from different clinical specimens or from positive blood culture bottles in few hours. Rapid commercial phenotypic antimicrobial susceptibility tests now are available for laboratory use, and provide results in 5-7 hours directly from positive blood culture bottles. In addition, detection of resistance genes can be rapidly accomplished in cultures

by immunoassays. Nucleic acid amplification testing-based methods can be used to detect resistance genes (methicillin-resistance, vancomycin-resistance, extended-spectrum-beta-lactamases, carbapenemases) directly from clinical specimens. Whole-genome sequencing directly on specimens is being developed for clinical applications [15].

Finally, it is important to highlight that the microbiology laboratory plays a central role in epidemiological surveillance by detecting the multidrug resistant organisms. This laboratory must provide at least an annual cumulative antimicrobial susceptibility report for specific antibiotic-organism combinations, which is critical for the local guidelines for empirical treatment and monitoring over time the local resistance trends. Stratified antibiograms (e.g., by ward or age) can provide significant differences in susceptibility which can help the antimicrobial stewardship team to develop optimized treatment recommendations and guidelines for different wards [7,8].

CONFLICT OF INTEREST

The author declares no conflict of interest

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New definitions of susceptibility categories EUCAST 2019: clinic application

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ABSTRACT

In January 2019, the European Committee for the Study of Antimicrobial Susceptibility (EUCAST) introduced some changes in the definitions of clinical categories for antibiotic susceptibility. The objective of these changes was to improve the credibility of category "I", optimizing and lengthening the survival and use of available antibiotics in the face of increasing antimicrobial resistance. This article aims to describe and explain these changes in the EUCAST criteria as well as make a short review about the factors on which the antibiotic susceptibility criteria depend.

Keywords: breakpoints, antibiogram, new defintiions, EUCAST, pharmacokinetic

The European Committee for the Study of Antimicrobial Susceptibility (EUCAST), belonging to the European Society for Clinical Microbiology and Infectious Diseases (ESCMID), is the responsible of the analysis and study of the cut-off points and the technical issues for antimicrobial *in vitro* susceptibility tests. EUCAST establishes guidelines for the interpretation of antibiotic resistance. Following this purpose, EUCAST standardizes and collects the information provided by each National Antibiogram Committee and thus establishes the susceptibility cut-off points that are used to separate bacterial populations. Recently, the Steering Committee of EUCAST has decided to change the definitions of clinical categories for antibiotic susceptibility, valid since January 2019.

There are several factors that EUCAST analyses and takes into account to define cut-off points for each antimicrobial, including chemical formulation, dosage, pharmacokinetic and pharmacodynamics rules and behaviours, Monte Carlo modelling and others. To understand this complex process, it is useful to be familiar with some basic concepts (figure 1), such as minimum inhibitory concentration, clinical and epidemiological cut-off points and PK/PD parameters [1].

The clinical cut-off point expressed as minimum inhibitory concentration (MIC), distinguishes between a treatable and a non-treatable microorganism, susceptible or not to the antimicrobial. The term MIC is the minimum concentration of an antibiotic (expressed in µg/ml or mg/L) that inhibits the growth of a specific bacterial strain. The MIC pretends to evaluate the *in vitro* response of a microorganism to antimicrobial exposure in order to predict a therapeutic success or failure. Depending on the MIC values, bacteria could be assigned to three different clinical categories: susceptible, intermediate or resistant. It is important to know that MIC values are singular and must be interpreted differently for each antimicrobial and for each microorganism. So that, a lower MIC of one antimicrobial compared to another does not imply higher activity. To understand the MIC value, it is necessary to know how the antibiotic susceptibility techniques are performed. EUCAST considers two main techniques: the broth microdilution method, which provides quantitative results and the agar or disk diffusion test, which provides qualitative results.

Epidemiological cut-off points (ECOFF) distinguish microorganisms with or without phenotypically detectable acquired resistance mechanisms to the targeted microorganism. Wild-type strains are those without intrinsic neither acquired resistance mechanisms and will serve to determine clinical cut-off points. They are able to detect resistance (ie: oxacillin in S. pneumoniae, cefoxitin in MRSA).

PK/PD analyses help to define dose-response relationship in order to identify optimal dosing patterns. Pharmacokinetic (PK) parameters relate the actions of the human body on the antimicrobial and include absorption, distribution, metabolism and excretion. They study the time course of antimicrobial concentrations and their metabolites in different body fluids and tissues. PK parameters depend on the antimicrobial and the patient. Pharmacodynamical parameters (PD) include the

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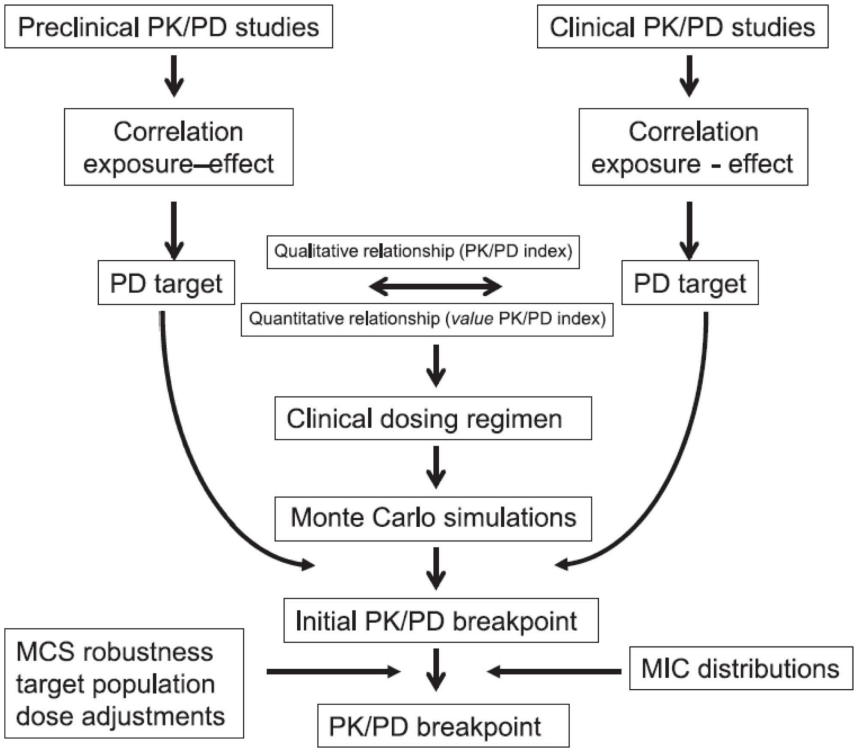

Figure 1 | **PK/PD parameters. Modified from Mouton JW, et al. [1].**

biochemical and physiological effects of the antimicrobial on microorganisms, and they also study the relationship between antimicrobial exposure and clinical or microbiological effects (response, toxicity). They depend on the causative pathogen. PK/PD values are unique and very important for identifying optimal antimicrobial doses and establishing PK/PD cut-off points. Antimicrobial treatment strategies based on PK/PD ratios are designed to maintain a useful concentration for an adequate time in the infective focus, maximizing both, bactericidal action and clinical efficacy, and reducing toxicity too.

Since 2002, EUCAST has used three definitions to categorise microorganisms as treatable or untreatable with each defined antimicrobial agent:

a) Susceptible (S): bacteria are in vitro inhibited by a concentration of an antimicrobial agent that is associated with a high probability of therapeutic success.

b) Intermediate (I): bacteria are in vitro inhibited by a concentration of an antimicrobial agent that is associated with an uncertain therapeutic effect.

c) Resistant (R): bacteria are in vitro inhibited by a concentration of an antimicrobial agent that is associated with a high probability of therapeutic failure.

In 2018, the pressure from a group of researchers and clinicians in favour of optimising antibiotic prescribing without cut-off points, just based only through tools that assess PK/PD targets, together with the indiscriminate rise of multidrug-resistant bacterial infections, made necessary to make some changes, modifying the classification of antibiotic susceptibilities, but keeping the letters "S", "I" and "R".

The previous definition of "Intermediate" generated some confusion and it was often interpreted by laboratories and clinicians as "Resistant", lumping "I" within the "R" category as non-susceptible, i.e. two *Resistant* categories versus a *Susceptible* one. This definition did not help clinical practice because it included some pharmacological, pharmacokinetic and microbiological inaccuracies: an uncertain therapeutic effect, susceptible if higher dosages are used, susceptible if the agent is concentrated at the site of infection, or a buffer zone to reduce miscategorization due to technical factors (natural assay variation [2]. The implementation of the new EUCAST criteria in 2019 had 2 main objectives: to signify and improve the usefulness of antimicrobial susceptibility studies, and to restore the credibility of category "I" to optimise and prolong the survival and use of available antibiotics (old and new).

The new definitions of S, I and R will emphasize the close relationship between the susceptibility of the isolated microorganism and the exposure of that organism to the antibiotic at the site of infection. With these changes there are two categories of *Susceptible* and only a *Resistant* one compared to the previous, and the term non-susceptible will be equated with Resistant.

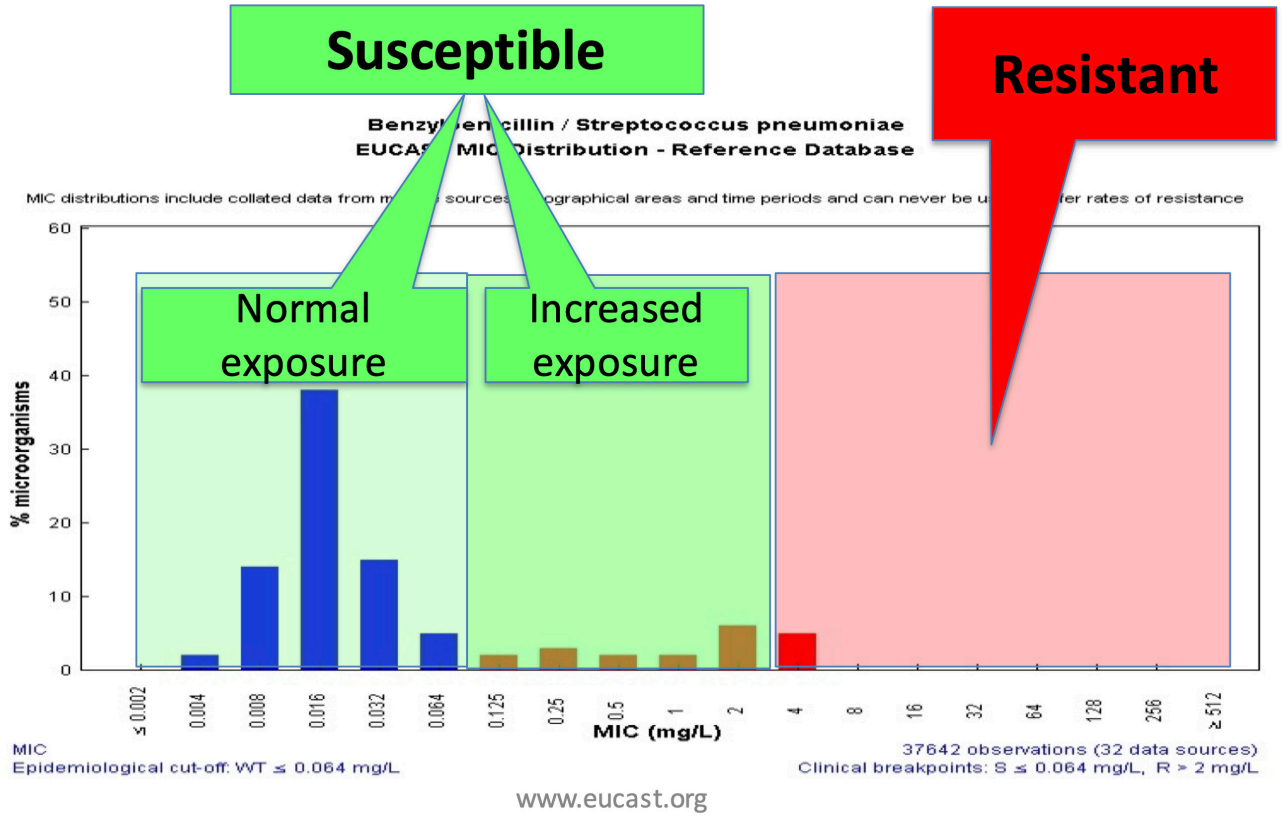

Figure 2 | **New EUCAST Susceptible (normal and increased exposure) and EUCAST Resistant categories (adapted from EUCAST [3]**

The categories of "Susceptible" and "Resistant" were easy to implement due to the changes to the definitions of both categories were minimal. They mainly emphasize the relationship between the clinical category and the level of exposure. S category implies susceptible to standard doses as long as the antimicrobial is the adequate for the type of infection to be treated. While R category discourages its use regardless of dose and mode of administration.

The new definition of the "I" Intermediate category includes situations where there is a high probability of therapeutic success if the exposure of the antimicrobial is increased by adjustment of the dosage regimen or by a higher concentration at the site of infection. The term "Intermediate" is changed to "susceptible-increased exposure"", but the letter "I" in the reports still appears and should be accompanied by an explanatory note [2,3]. With this new definition, the only difference between S and I is the amount of drug that is needed at the site of infection to reach an adequate clinical response (figure 2).

On the other hand, in 2019, the term "ATU" (Area of technical uncertainty) is introduced in susceptibility studies when a warning is needed to alert the laboratory about uncertainty in test results. The warning concerns the laboratory, not the clinician responsible for the patient, and the laboratory needs a strategy to ensure accuracy and to report the uncertainty of the result. This has improved EUCAST's ability to detect areas where technical uncertainty significantly affects the predictive value of the Antibiogram [3].

Because of these new definitions some microorganisms become intrinsically less susceptible to an antimicrobial, and they will never reach S category at standard doses, so it is necessary to remember that they are "Susceptible with increased exposure", i.e. more antimicrobial is needed at the site of infection to achieve clinical success with that strain. For example, treatment of *Pseudomonas* infections requires increased exposure for almost all active antimicrobials (piperacillin-tazobactam, ceftazidime, cefepime, imipenem, aztreonam, fluoroquinolones and aminoglycosides); therefore, wild-type *Pseudomonas* phenotypes fall into the clinical category of "Susceptible with increased exposure" for all relevant antimicrobials (except meropenem).

The recent work of the Swiss group of Munting et al [4] is a retrospective observational study in the hospital of Lausanne where they analyse antibiotic prescriptions, especially meropenem, before and after the new EUCAST criteria. The authors conclude that the new criteria led to increase the meropen-

Table 1 **Therapeutic objectives of the main antibiotics, according to new EUCAST definitions (modified from Cantón R. et al. [5])**

	Concentration-dependent		Time-dependent			
Bactericidal activity	Dependent on focus concentration		Dependent on the duration of exposure			
Post-antibiotiåc effect	Prolonged		Minimum			
PK/PD index	Cmax/CMI AUC24h/MIC		∫T > MIC (%) (% of time with concentration above MIC)			
Antibiotic	Aminoglycosides Fluoroquinolones Daptomycin		Beta-lactams			
Target PK/PD	Aminoglycosides	Cmax/CIM ≥25-30			Bacteriostatic effect	Bactericidal effect
	Fluoroquinolones	Levofloxacin AUC24h/MIC ≥25-30 (non-severe infections and *S. pneumoniae* respiratory infection)	Beta-lactams	Penicillins	<30%	>50%
		Ciprofloxacin AUC24h/CMI ≥125 (Serious infections and immunosuppressed)		Cephalosporins	>30-45%	>60-70%
				Aztreonam	>50%	>60%
	Daptomycin	AUC24h/MIC ≥666		Carbapenems	>20%	>40%
Comments	These antibiotics are used at high doses, and the prolonged PAE allows the use of wide dosing intervals (one dose per day).		- Time to efficacy: time during which concentrations are > MIC - Maximum bactericidal activity at concentrations 4-5 times the MIC value over the whole interval - The shorter the half-life, the higher the frequency of administration - Continuous perfusion is the most effective way of administering these antibiotics, especially if a high T>CMI value is required, and in case of increased clearance			

em prescriptions for the treatment of *Pseudomonas* infections (partially due to uncertain prescription and misinterpretation about other antibiotics defined with category "I" as if were non-susceptible but not due to the ignorance of dosing them according to the new definition). On the other hand, the authors highlighted the fact that consultation with an infectious disease specialist was a protective factor.

Another consequence of these changes requires a revision of the local, national and international antimicrobial susceptibility maps, based on these new definitions, which will be used as a tool to assist in prescribing in various settings and for different purposes.

These changes in Category "I" have a high clinical and technical impact on antimicrobial resistance surveillance and have required a change in some cut-off points. The new definitions reflect the need for correct exposure and for laboratories to take responsibility for technical difficulties and their resolution before finalising antibiogram reports.

These situations requiring "Increased Exposure" (EI) are generally infections that are difficult to treat, either because of the focus (high inoculum or difficult access for the antibiotic such as CNS or biofilms), because of the PK characteristics of the patient (increased volume of distribution, increased or decreased glomerular filtration rate as in burn patients or patients with renal failure), or because of the MIC.

Strategies to achieve IE may be by increasing the dose, in the case of concentration-dependent antibiotics such as quinolone, aminoglycosides or daptomycin, or by increasing the perfusion time or decreasing the interval in the case of time-dependent antibiotics such as beta-lactams (table 1). So the clues for antimicrobial prescription relay on adjusting the dose, the dosing interval, the infusion time or take advantage of concentration at the site of infection [5].

It is convenient to remember that it is important to make a good decision based on the antibiogram. Whenever possible, a beta-lactam should be chosen, especially in severe infections and since it has a better efficacy/toxicity profile, always discard safely a beta-lactam hypersensitivity. "R" antibiotics, consid-

ered resistant, should be ruled out and antibiotics reported as S-susceptible or susceptible-IE should be chosen. In addition, the antibiotic with the lowest possible spectrum should be selected with an adequate diagnostic approach, and a selective antibiogram report should be performed, especially in Primary Care [6,7].

It is important to choose the right dose and mode of administration, and to consult the antibiotic stewardship team in each sector if there is any doubt.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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Current strategies for infectious diseases management

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Models for bacteraemia risk prediction. Clinical implications

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ABSTRACT

Bacteraemia has important consequences for the patient, as it is associated with worse clinical outcomes. On the other hand, unnecessarily obtaining samples for blood cultures increases costs and the workload in the microbiology laboratory. Its diagnosis implies a time delay, but decisions about start antibiotic treatment, discharge, or admits the patient must be taken during the first attention and, therefore, before known the blood cultures results. This manuscript reviews the different strategies based on clinical scores and biomarkers that are useful for predicting bacteraemia and improving initial decision-making.

Keywords: bacteraemia, model, risk, prediction, biomarkers

INTRODUCTION

Bacteraemia has an increasing incidence of up to 1-2 cases/1,000 treatments in hospital emergency departments (ED) and around 6-10 episodes/1,000 hospital admissions [1-3]. When an infectious disease is suspected, blood cultures (BC) are taken in 15% of all microbiological samples obtained [4]. It should be noted that the diagnostic profitability of BC obtained in ED is very variable, between 2-20% [5], of which 3-5% of the positive BC corresponding to "hidden bacteraemia" (BC with significant isolation in patients who have been discharged) and 3% correspond to "contaminated BC" [1,6].

CLINICAL IMPLICATIONS

The main importance of the diagnosis of bacteraemia lies in the fact that it reaches a 30-day-mortality between 10-25% [1], in direct relation to the severity, the site of infection and the characteristics of the patients (age, comorbidity) [7-9]. The highest number of true bacteraemia (TB) are obtained from patients with urinary tract infection and pneumonia, with the most frequent causative agents being *Escherichia coli*, with 35%, and *Streptococcus pneumoniae*, with 75%, for each of them, among the positive BC obtained in the ED. Ten percentage of bacteraemia correspond to an unknown source of infection [10-14].

The key problem is that the certainty diagnosis of bacteraemia will not be obtained until the isolation of the microorganism in the culture, which can lead to a delay of time during which it is necessary to make the first clinical decisions. Being able to predict TB during the initial assessment of patients with suspected infection is very important. The diagnosis, prognosis and initial decisions such as discharge, hospital admission or the early and appropriate administration of an antimicrobial depend on this. Knowing this information can be useful to avoid unfair discharges or unnecessary admissions. Properly establishing diagnostic suspicion and risk stratification during the first evaluation of a patient with an acute event is key to obtaining the best clinical outcome [15-19]. For this reason, these aspects are the focus of numerous research works in emergency medicine [20,21].

Microbiological isolates in patients discharged from the ED can lead to a delay in the start of treatment, as well as an increase in morbidity and mortality. That is why the goal of many authors [22-24] has focused on finding predictive models combining different epidemiological, clinical and analytical variables. These include inflammatory response and infection biomarkers (BM) that increase the predictive power of clinical models [25-28]. Between all BM, procalcitonin (PCT) has been found to be the most sensitive and specific to predict bacteraemia risk [22,3-6], with a high negative predictive value (NPV) that would rule out a TB [29].

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PREDICTIVE MODELS OF BACTERAEMIA

Shapiro model. Shapiro et al. [30] developed, in an already classic study, a prediction model of TB risk. For this purpose, they conducted a prospective observational study on a cohort of adult patients in whom BC were obtained. To develop the model, they divided the sample into a derivation and a validation cohort. They included 3,730 patients with 305 (8.2%) episodes of TB. The model described major criteria (temperature > 39.5°C, presence of permanent vascular catheter or clinical suspicion of endocarditis) and minor criteria (temperature 38.3 to 39.4°C, age > 65 years, presence of chills or vomiting, systolic blood pressure < 90 mm Hg, neutrophil percentage > 80%, white blood cell count > 18,000/mm^3, bands > 5%, platelets < 150,000/mm^3 and creatinine > 2.0 mg/dl). Based on the results obtained, BC were recommended if the patient had at least one major criterion or two minor criteria. Otherwise, patients were classified as "low risk" and it was recommended not to obtain them, since only 4 (0.6%) low-risk patients in the derivation cohort and 3 (0.9%) low-risk patients in the validation cohort had positive BC. The sensitivity of this approach was 98% (95% confidence interval [CI]: 96-100%) in the derivation cohort and 97% (95% CI: 94-100%) in the validation cohort.

Model 5MPB-Toledo. Agustín-Julián et al [31] developed a new predictive model through an observational retrospective cohort study that included all the BC obtained during their attention in a Spanish ED in adult patients (≥ 18 years) with suspected infection. They analysed 38 independent variables (demographic, comorbidity, functional status, clinical and analytical) that could predict the existence of bacteraemia.

They included 2,181 episodes of BC. Between these, 262 (12%) were considered TB. A predictive model of bacteraemia risk was defined with 5 variables (5MPB-Toledo): temperature > 38.3°C (1 point), a Charlson index ≥ 3 (1 point), respiratory rate ≥ 22 breaths per minute (1 point), leukocytes > 12,000/mm^3 (1 point) and PCT ≥ 0.51 ng/ml (4 points). Patients were categorized as low (0-2 points), moderate (3-5 points) and high (6-8 points) risk, with a probability of bacteraemia of 1.1%, 10.5% and 77%, respectively. The area under the operating receiver curve (ABC-COR) of the model after internal validation was 0.946 (95% CI: 0.922-0.969).

Later this model was externally validated through in other research of the infectious diseases group of the Spanish Emergency Medicine Society (INFURG-SEMES) [32]. Seventy-four Spanish hospitals participated in this observational prospective cohort study that was performed to analyse the accuracy of the 5MPB-Toledo model**.**

This study included 3,843 episodes of BC obtained in the ED of the participating hospitals, been TB 839 (21.83%). Patients were categorized as low (0-2 points), moderate (3-5 points) and high (6-8 points) risk, with a probability of bacteraemia of 1.5%, 16.8%, and 81.6%, respectively. The ABC-COR of the model was 0.930 (95% CI: 0.916-0.948). The diagnostic performance for the 5-point cut-off achieved a sensitivity of 94.76% (95% CI: 92.97-96.12), a specificity of 81.56% (95% CI: 80.11-82.92) and a NPV of 98.24% (95% CI: 97.62-98.70). Therefore, the 5MPB-Toledo model could be useful to predict TB in patients with infection in the ED.

Table 1 Bacteraemia Prediction Risk Model of the INFURG-SEMES group (MPB-INFURG-SEMES)

Variable	Score
Procalcitonin ≥ 0,51 ng/ml	4
Respiratory rate 22 rpm	1
Temperature > 38,3ºC	1
Charlson Index ≥ 3	1
Leukocytosis > 12,000/ mm^3	1
Chills-shivering	1
Thrombopenia < 150,000 /mm^3	1

Bacteraemia Risk Prediction Model of the INFURG-SEMES group (MPB-INFURG-SEMES). Subsequently, the INFURG-SEMES group designed its own TB risk prediction model by conducting a prospective and multicenter cohort study [33]. The study involved 71 Spanish ED and included a total of 4,439 adult patients in whom a BC had been requested during their evaluation in the emergency room. Of these, 899 (20.25%) were considered as TB. A predictive model of bacteremia risk with seven variables was defined (Table 1). The model reached an ABC-COR of 0.924 (95% CI: 0.914-0.934) in the derivation cohort and 0.926 (95% CI: 0.910-0.942) in the validation cohort. Based on these results, patients were divided into 10 risk categories based on the probability of having a TB: 0.2% (0 points), 0.4% (1 point), 0.9% (2 points), 1.8% (3 points), 4.7% (4 points), 19.1% (5 points), 39.1% (6 points), 56.8% (7 points), 71.1% (8 points), 82.7% (9 points) and 90.1% (10 points). The findings were similar in the validation cohort. The 5-point cut-off provided the best diagnostic accuracy with a sensitivity of 95.94%, a specificity of 76.28%, a positive predictive value (PPV) of 53.63% and a NPV of 98.50%. Recommendations for making-decisions based on the risk score are expressed in Table 2.

In conclusion, the MPB-INFURG-SEMES model may be useful for the risk stratification of TB in adult patients with infection evaluated in ED. The risk calculation can be done online through the following link: https://mpbscore.urgenciasclinico.com

Usefulness of biomarkers for the prediction of true bacteraemia risk. Several studies have investigated the power of different BM to identify the patient with TB [3-6]. Among them, the literature clearly shows that PCT is the BM that presents a greater diagnostic accuracy. In fact, as we have seen previously, has the greater weight among the variables necessary for the calculation of the risk of TB in the 5MPB-Toledo and MPB-INFURG-SEMES models.

Table 2 Recommendations for making-decision based on the score of the Bacteraemia Risk Prediction Model of the INFURG-SEMES group (MPB-INFURG-SEMES)

Score	Risk classification	Probability of TB (%)	Recommending
0-2	Very low	0.2-0.9	Do not extract blood cultures
3	Low	1.8	Do not extract blood cultures
4	Low-moderate	4.7	The decision to obtain blood cultures and the patient's admission should be made according to the characteristics of the patient
5	Moderate-high	19.1	Blood cultures should be obtained and the patient be admitted
6-7	High	39.1-56.8	Blood cultures should be obtained and the patient be admitted
8-10	Very high	71.1-90.1	Blood cultures should be obtained and the patient be admitted

TB: true bacteraemia

A recently published study [34] analyses and compares the ability of PCT, C-reactive protein (CRP) and leukocytes to differentiate TB from contaminated BC in patients attended in a ED for suspected infectious disease. A retrospective cohort study selected a population with positive BC requested during patient´s evaluation in the ED. A total of 266 BC with any isolation were included in the study. Of these, 154 (57.9%) were considered TB and 112 (42.1%) were considered contaminated BC. The AUC-ROC of the PCT to predict a TB was 0.983 (95% CI: 0.972-0.994) and, considering a cut-off value of 0.43 ng/ml, the PCT achieved a sensitivity of 94%, a specificity of 91%, a PPV of 94% and a NPV of 92%. Moreover, the AUC-ROC obtained for PCR was 0.639 (95% CI 0.572-0.707) and for leukocytes 0.693 (95% CI 0.630-0.756). It is also noteworthy in this study that the mean values of PCT were 3.44 (SD 6.30) ng/ml in TB vs 0.16 (SD 0.18) ng/ml in contaminated BC (P <0.001).

In conclusion, the PCT achieves the best diagnostic performance for TB identification between different BM. Therefore, high PCT values can guide for request or not BC, and in addition to starting antibiotic therapy, especially when its value is above 0.5 ng/ml.

CONCLUSION

The prediction of TB is very important for making-decisions during the initial evaluation of the patients with suspected infection for several reasons. First, to avoid the requested of unnecessary BC, which lead to work overload in the Microbiology laboratory and to increased costs. Second, to avoid unnecessary admission in low-risk patients. Third, to avoid inappropriate discharge of the patient, since TB increases the risk of poor outcomes. Therefore, the suspicion and detection of TB has an important diagnostic and prognostic significance, and forces to change some of the most important decisions that must be taken during the initial assessment of patients. The 5MPB-Toledo and MPB-INFURG-SEMES models are useful tools for predicting TB in patients attended for infection in the ED. Finally, we should note that PCT is a key variable when assessing BC extraction, and the best BM to predict this situation. However, we must remember that both, clinical models and PCT, must be accompanied by the clinical judgment of the attending physician, as well as other variables depending on the process and the patient, for the better making-decision.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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Current strategies for infectious diseases management

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Latent tuberculosis infection: approach and therapeutic schemes

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ABSTRACT

Tuberculosis continues to be a major public health problem. A priority objective is the implementation of early diagnosis, contact investigation and latent tuberculosis infection (LTBI) testing. World Health Organization (WHO) concludes that there is no gold standard for the diagnosis of LTBI; both the tuberculin test and IGRA (interferon gamma release assays) indirectly identify tuberculosis infection; both tests are considered acceptable but imperfect. WHO recommends that regimens that include rifamycins are equally effective but less toxic and more adherent than long regimens with isoniazid.

Keywords: latent tuberculosis, IGRA, Tuberculin test, Isoniazid

INTRODUCTION

Tuberculosis is a global public health problem. According to data from the World Health Organization (WHO), there are approximately 10 million new cases and 1.3 million deaths per year. The global incidence is 142 cases per 100,000 people per year, although 8 countries report >400 cases per 100,000 patients per year [1]. Data in Spain show an incidence of 9.4 cases per 100,000 patients per year, a ratio between children and adults of 0.3%, with HIV-infected patients accounting for 4.8% of all tuberculosis cases [2]. The objectives of the Spanish Plan for tuberculosis include: a) improving information, b) improving the therapeutic success rate and c) maintaining an annual incidence below 4%, through the implementation of early diagnosis, the study of contacts and the analysis of latent tuberculosis infection in certain groups [2].

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LATENT TUBERCULOSIS INFECTION (LTBI)

We define LTBI as a state of persistent immune response to stimulation by *M. tuberculosis* antigens without evidence of clinical manifestations of active tuberculosis disease. Approximately 5-10% of LTBI will develop TB disease (50% in the first 5 years) and the highest risk of progression occurs in the presence of immunosuppression or in children <5 years. Following the WHO recommendation "decision to test is a decision to treat" [1], the Plan for the Prevention and Control of Tuberculosis of the Spanish Ministry of Health recommends ruling out LTBI in contacts of a patient with TB disease, people with HIV infection and patients in the following circumstances [2]: initiation of treatment with biological or immunosuppressive therapies, dialysis, candidates for solid organ or hematopoietic progenitor transplantation, silicosis or in the presence of fibrotic changes in chest X-rays. And it should be evaluated in the following groups: a) health centers, b) microbiology laboratories, c) penitentiary institutions, d) homes for the elderly, e) shelters or refuges for the homeless, f) care centers for immigrants on their arrival in Spain, g) aid workers or military personnel in countries with a high incidence and who have traveled temporarily to countries with a high incidence.

DIAGNOSIS OF LTBI

It is performed by the tuberculin skin test (TST), or Mantoux test, or by interferon gamma release assays (IGRA). A positive TST is considered positive if there is a skin induration greater than 5 mm. It indicates the presence of immune reaction to: a) *M. tuberculosis* complex, including *M. tuberculosis, M. bovis, M. africanum, M. microtii, M. tuberculosis* sups *caprae*, b) non-tuberculous mycobacterial infection, c) previous BCG vaccination (from week +4) [3]. Reactions >15 mm are unlikely to be related to BCG or atypical mycobacteria [3]. IGRA tests include: Elispot or Quantiferon

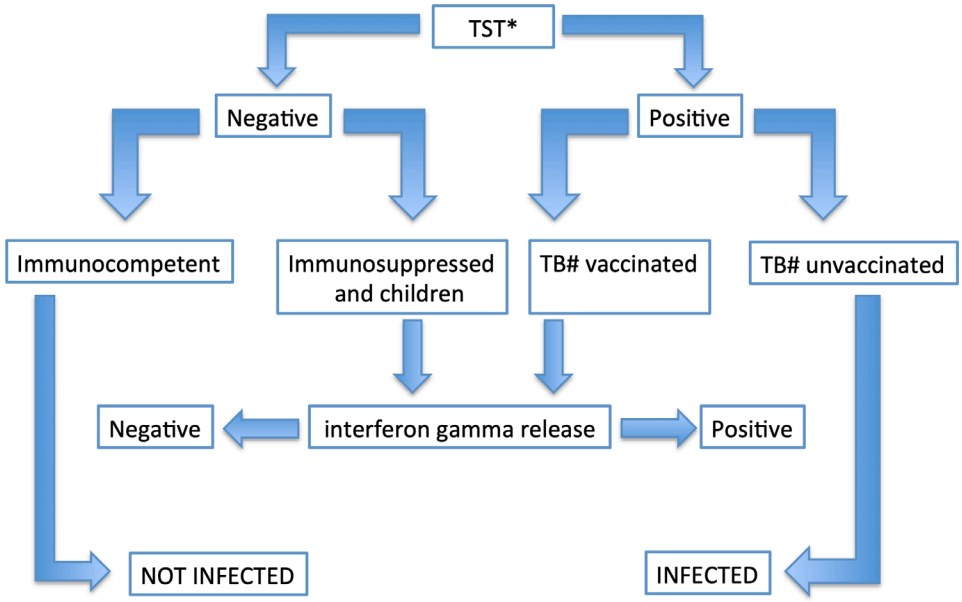

Figure 1 | **Algorithm for prophylaxis in latent tuberculosis infection [9]**

ELISA. The antigens encoded by RD1: ESAT-6 and CPF-10 are highly specific for *M. tuberculosis, M. africanum* and *M. bovis* and are not present in any of the species included in BCG or non-tuberculous mycobacteria, except *M. kansasi, M. marinum* and *M. szulgai* [4,5]. This confers high specificity and negative predictive value to IGRA [6]. Abubakar I et al. in a prospective study conducted with 10,000 patients, contacts of active tuberculosis or migrants from endemic area, confirmed a very high negative predictive value for tuberculin and IGRA testing (<1.9x1000 pac-years with negative tests) and a better positive predictive value with IGRA Elispot (13.2 x 1000 pac-years) or Quantiferon (10. 1 x 1000 pac-years) with respect to TST > 5 mm (6.8 x 1000 pac-years) [7]. WHO concludes that there is no gold standard for the diagnosis of LTBI. Both, TST and IGRA, indirectly identify tuberculous infection; both tests are considered acceptable but imperfect [1]. WHO recommends TST in countries with a high incidence of tuberculosis based on a review of comparative studies between the two tests that show similar prediction, being less costly and technically complex than IGRA. In countries with resources and incidence <100/100,000h, TST or IGRA can be used interchangeably. For the screening strategy, the concentric circles model is applied: high transmission risk (exposure >6h per day with the source) ⇒ intermediate transmission risk (exposure <6h per day with the source) ⇒ low or sporadic transmission risk (non-daily contact). Contact investigation will be expanded until the rate of positive results was indistinguishable from the community. Qantiferon TB-Gold-Plus, which incorporates a fourth tube that collects interferon production by CD4 and CD8 lymphocytes, unlike Quantiferon TB-Gold which only collects CD4, has recently entered the market [8]. Given an earlier CD8 response than CD4, the difference between the two tubes could suggest a recent contact and a greater indication for prophylaxis; however, this advantage has not been confirmed in some studies [8]. In Spain, recently the consensus between the societies of Infectious Diseases (SEIMC) and Pneumology (SEPAR) has reviewed the recommendations for the application of both tests (TST or IGRA) [9]. Given the greater sensitivity of IGRA in children and immunocompromised patients and its greater specificity in BCG vaccinated patients, an algorithm for the application and sequence of both techniques in clinical practice has been proposed (Figure 1).

NEW RISK GROUPS

Biological therapies, in addition to the previously mentioned groups, constitute a new target population for the diagnosis of LTBI and the indication of chemoprophylaxis. In recent years the irruption of biological therapies in the treatment of inflammatory and oncologic pathologies with the consequent risk of tuberculosis has broadened these indications [10]. There are numerous biological therapies and therapeutic targets. The European Society of Infectious Diseases (ESCMID) in a recent consensus document has highlighted the use of the following therapies as a population at higher risk of tuberculosis: anti-TNFs, anti-interleukin 6, anti-interleukin 12-23, anti-interleukin 17, anti-CD52, anti JAK-STAT [11].

GUIDELINES FOR ANTITUBERCULOSIS PROPHYLAXIS

One of the most significant changes in this field in recent years is the recommendation of short regimens with the incorporation of rifamycins. World Health Organization, in 2020 recommendations, using 9- or 6-months isoniazid regimens as a comparator, has analyzed the efficacy and adherence of short regimens of isoniazid + rifamycins (rifampicin or rifapentine) or rifampicin in monotherapy [12]. These recommendations conclude that regimens that include rifamycins are equally effective but less toxic and more adherent than long regimens with isoniazid, and establish the following range of priority in their indications: a) preferred: 3 months of daily isonazid + weekly rifapentine (strong recommendation, moderate evidence), b) preferred: 4 months of daily rifampicin (strong recommendation, moderate evidence, especially obtained in patients without HIV infection), c) preferred: 3 months of daily isoniazid + daily rifampicin (conditional recommendation, very low evidence in patients without HIV infection or low in HIV infection), d) alternative: 6 months of daily isoniazid (strong recommendation, moderate evidence in patients without HIV infection), e) alternative: 9 months of daily isoniazid (conditional recommendation, moderate evidence) [12].

CONFLICT OF INTEREST

Authors declare no conflict of interest

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Current strategies for infectious diseases management

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Treatment of *Clostridioides difficile* infection: from guidelines to clinical practice

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ABSTRACT

The *Clostridioides difficile* Infection (CDI) treatment guidelines were published in 2021; however, the incorporation of these recommendations into clinical practice was rather irregular and inconsistent. The differences in the implementation of these new guidelines were due, in part, to the variety in the different professionals who provided patient care, as well as to the issues involved in either their accessibility or availability or both. The main requirements for implementation include appropriate reflection on patient stratification, drug positioning, accessibility to drugs, as well as the organization of structured clinical pathways that can facilitate the functionality and evaluation of the management of CDI.

Keywords: *Clostridioides difficile* treatment, guidelines, clinical pathway.

INTRODUCTION

After the new evidence related to the *Clostridioides difficile* Infection (CDI) treatment was reported, the main guidelines and recommendations were updated by several scientific societies, including those in America, Europe and Spain (IDSA, ESCMID, ACG and SEQ) in 2021.

The clinical outcomes in different pathologies showed clear optimization when the guidelines were strictly followed, although implementation into regular clinical practice was not always simple or feasible; further, from one center to another, wide variations were noted in terms of the percentage of adherence or follow-up of the recommendations.

In the course of this infection, two major challenges were encountered, the first of which was optimization of the therapeutic objective. Often, in clinical practice, the treatment goal is to clinically resolve the episode, and when the recurrence frequency of this infection is known to be 15-25%, the aim must be to attain sustained cure or cure with no recurrence. Several times, this goal is not achieved, most often because of insufficient follow-up over time and sometimes because the recurrences are not well tracked. The second challenge was that the treatment of this infection was developed from a comparatively stagnant state over the last 20 years. It now includes in its arsenal, new therapeutic methods. But because the clinical care of this infection is performed under a wide variety of settings, from Primary Care to different hospital specialties, these recommendations have not always been sufficiently well incorporated into clinical practice. In fact, a wide plurality of treatment options is available in terms of the approach and treatment of CDI.

Therefore, implementation of these guidelines is crucial, considering CDI is a serious health issue, not only for the individual patient (increasing early and late morbidity and mortality, and compromising the quality of life), but for the health system as well (involving the high cost of primary episodes and recurrences) [1], both of which pose a threat to the sustainability. So, a complete strategy has been put forward to optimize the way this infection is approached, at the level of each specific case, as well as at the collective level, inclusive of a "macro" vision of management planning from epidemiology to prevention, on a global scale.

OPTIMIZATION IN THE CLINICAL EVALUATION AND "INDIVIDUAL" MANAGEMENT

Recent guidelines and recommendations are based on the goal of achieving sustained cure. So, from "classical pharmacological treatment" as metronidazole and vancomycin, new treatment (fidaxomicin (FDX), bezlotoxumab (BZL) and fecal microbiota transplant) or strategies (extended regimens of fidaxomicin, vancomycin in pulsed "taper" regimes) have been

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incorporated FDX (in standard or extended regimen) and BZL provided a percentage of clinical resolution close to that of the comparator (vancomycin), but showed a lower rate of recurrence, thus indicating better therapeutic success over time (sustained cure). Fecal microbiota transplant has been reported as the best treatment in multiple recurrence.

These new treatments have high economic cost and less accessibility, which is a factor that is included in the guidelines for the choice of treatment. On the other hand, it is important to note that CDI and its recurrence also have a high impact on health. CDI recurrence is related to increase in hospital admissions, delay in therapeutic procedures, in quality of life and especially, increasing frailty in elderly patients.

For the reasons cited above, the effective translation of these recommendations into regular clinical practice needs to be done, keeping in mind several c variables namely, proper patient stratification, knowledge of recommended treatments, accessibility-availability concept, and the recurrence factor.

Patient stratification. Patients were stratified for treatment selection traditionally based on the relationship of the infection to clinical severity and number of episodes of infection.

The severity has been evaluated using different scores, but the relevance in distinguishing mild or moderate infection, but the relevance beyond serious infection, is less in currently guidelines because metronidazole is no longer regarded as the first-line of treatment. It is used as an option only for severe episodes, in patients revealing intolerance to the oral route, or the onset of shock or paralytic ileus, in which cases the treatment guidelines remain unchanged.

Distinctions drawn between the first episode, first recurrence and subsequent episodes were made depending upon the higher probability of the recurrence of successive episodes. The earlier guidelines chose the treatments that ensured more efficacy in terms of recurrence for the first or successive recurrences. Although the current guidelines continue to maintain this basic scheme, the best treatments in first episodes have been included, and their prioritization has been proposed, considering the other risk factors for recurrence.

Therefore, a study of the assessment of the risk of recurrence is proposed, incorporating the other risk factors apart from the number of episodes. In fact, 15-25% recurrence risk is estimated in a first episode, with vancomycin as the treatment option. Several works have been published in an attempt to identify the risk factors for recurrence, drawn from highly heterogeneous series, applying different "definitions" of both risk factors and the time span for recurrence. From this angle, a meta-analysis done recently indicates the factors, which present low level of evidence as follows: age (above 65 or 75 years) and a prior episode, as the factors which offer the most evidence (to a moderate extent), as well as an earlier hospitalization, where the episode bears some relationship to care healthcare and prior/concomitant use of proton pump inhibitors [2].

Using predictive models [3] different scores have been proposed, which combine the risk factors with different relative weights, but despite enabling the achievement of a kind of more accurate risk stratification, they include limitations in their predictive ability [4].

New risk factors have been identified as well, namely the quantity of immunoglobulins versus the toxins, intestinal microbiota (in terms of composition and diversity), and the amount of *C. difficile* present in the feces; further, the presence of predisposing genetic factors [5] will likely be a better indicator, in a future time, to identify patients possessing a higher risk of recurrence.

Recommended treatments. Regarding the of the first episode, barring for the ACG guidelines (which assign to vancomycin and fidaxomicin like first choice), the guidelines established by the IDSA and ESCMID show fidaxomicin as the first choice, and vancomycin as an "acceptable" alternative, or when a situation of "limitation of resources" arises. In such events, the fidaxomicin usage is established as a priority for patients who have high risk of recurrence. Metronidazole is not the recommended option and is permitted to be used solely when the vancomycin or FDX is unavailable. The use of BZL is suggested only for patients having a minimum of one risk factor, guaranteeing its accessibility (IDSA), or when FDX is not available.

Controversy continues to exist, both in the literature and in clinical practice, with respect to the removal of any indication for metronidazole, which could still offer a few specially selected opportunities for use in clinical practice.

Despite the fact that the conclusion drawn by the Cochrane review in 2017 indicating the superiority of vancomycin to metronidazole and FDX to vancomycin, the benefit of using metronidazole was noted for "its far lower cost compared to the other two antibiotics" [6].

Data from two series performed recently in real life throw more light on the earlier findings of the lower efficacy of metronidazole. In a Veterans cohort which included 3,566 patients (treated from 2010 to 2014) and showing a recurrence rate of 10.2% after 30 days, the factors related to the recurrence were assessed by employing a propensity score. It was noted that when metronidazole was the medication used, it exhibited behavior that rendered it a risk factor for patients below 65 years of age [7]. In another series [8] patients were treated prior to and post the implementation of the guidelines (1,809 vs 1,799 patients), with 70 vs 20% of patients being given metronidazole, respectively. Of interest, no differences were identified in terms of failure of treatment or appearance of recurrences (mean age 65 years) when compared to vancomycin.

Therefore, it appears that in some patient subgroups (as in patients with low risk of recurrence with mild disease, younger than 65 years and without other risk factors) metronidazole could be given as the alternative treatment. The higher accessibility of metronidazole in our country is not contingent upon its cost but upon the internal dispensing policy of the hospital pharmacy, where vancomycin continues to be subject.

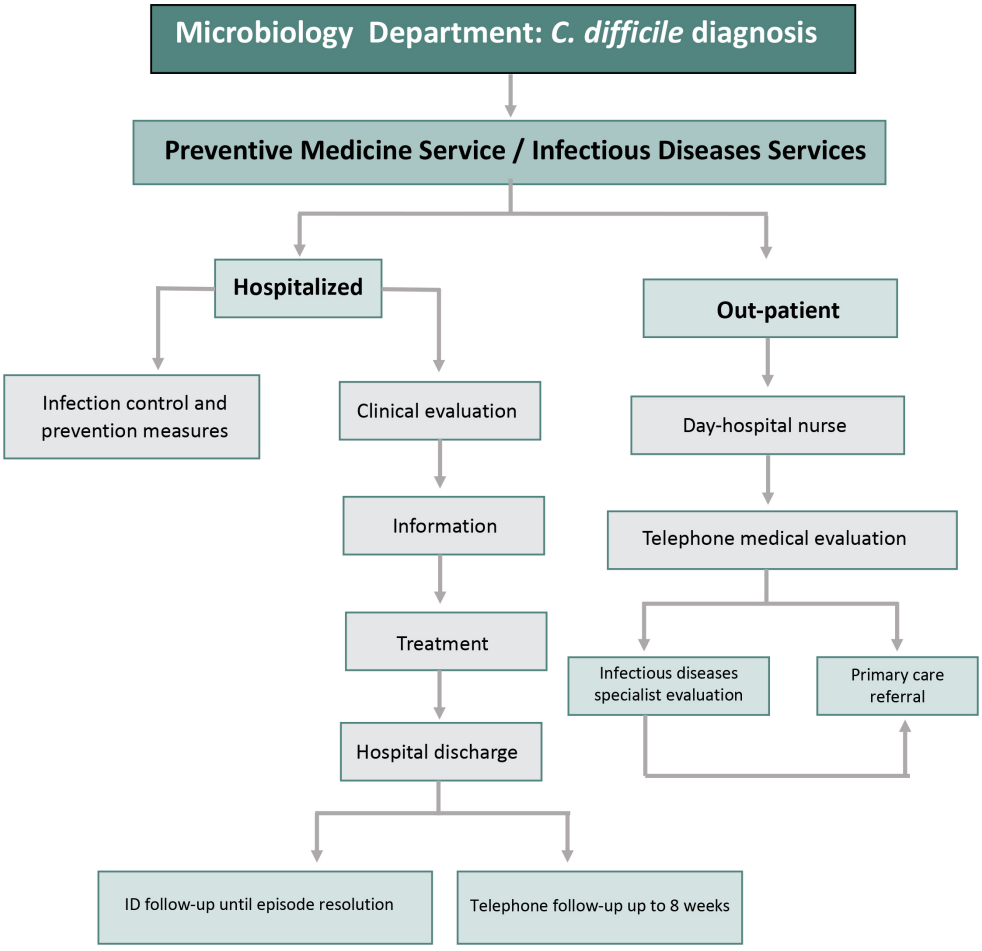

Figure 1 A clinical pathway for CDI evaluation.

With respect to the priority position given to the FDX versus BZL, no comparative studies between the two drugs are available to provide proof as to which one is the better alternative for sustained healing. While FDX is regarded as the more accessible one because it can be orally administered, it must be noted that the direct cost of both medications is similar in our country (although not in the United States). In the post-hoc analyses of the pivotal study [9] the patient group in which BZL was given reveals higher efficacy than the comparator, as seen in its indication for recommendation in the guidelines (age >65 years, immunosuppression, prior episode of CDI, severe intensity of disease or high-risk ribotype).

In terms of the treatment of recurrences, there are a few dissimilarities between the guidelines. The IDSA guidelines list FDX as the recommended drug in the first recurrence, in either the standard or extended regimen; the alternative is to taper vancomycin or BZL, using the identical scheme for the second recurrence, adding rifaximin, and shifting the fecal microbiota transplant from the second recurrence. In the European guidelines, however, the indication proposed is by alternating the medication given in the prior episode, preferably with the inclusion of FDX and BZL; also, vancomycin is recommended on a tapered dosage in the event of the drugs mentioned being absent or unavailable, and with the inclusion of TMF from the second recurrence.

From observational studies, both FDX and BZL show lower efficacy from the first and, definitely, the second recurrence [10-12]; hence, probably positioning these drugs in the second recurrence and thereafter, provides a sign of their lower efficiency. In the event of a second recurrence and particularly in the subsequent ones, the most effective treatment today is FMT. Yet, it remains as a not very accessible treatment option in most centers. Higher accessibility can be attained by ensuring the availability of lyophilized capsules manufactured in reference research centers with stool banks or by using commercialized formulae to facilitate the transfer of microbiota by the manufacturing companies, which can thus assure that all patients get fair access to them [13].

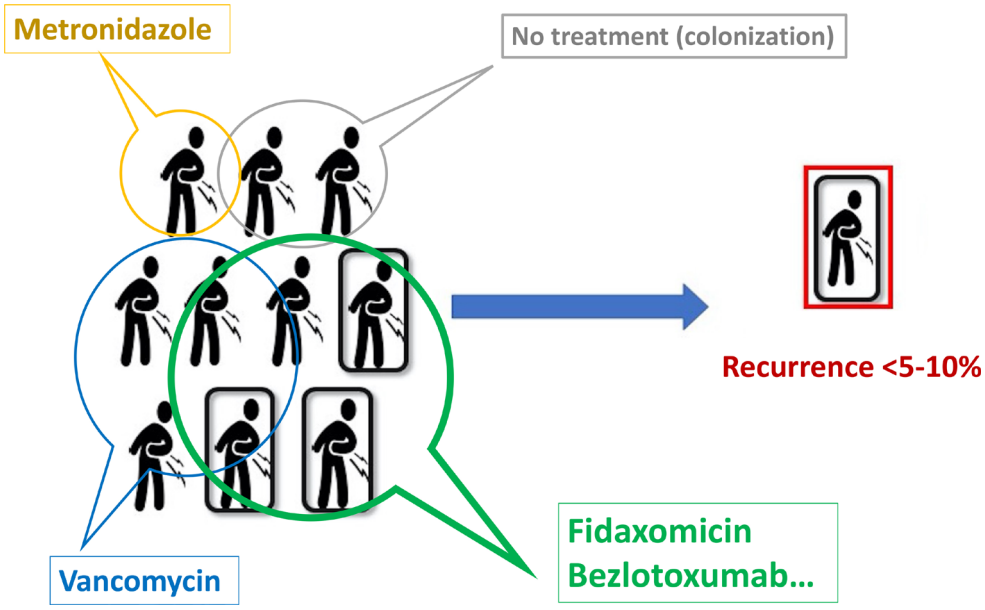

Figure 2 Optimization in the efficiency of the evaluation of *C. difficile* infection: identification of infection-colonization, population identification according risk factors of recurrence

The concept of accessibility/availability. Although the guidelines position FDX and BZL based on data from clinical trials, all the guidelines include the concept of accessibility/availability for choosing treatment. In our country, this "accessibility or availability" concept is governed by the alleged "Therapeutic Positioning Report" determined by the Spanish Agency for Medicines and Health Products (AEMPS, the regulations of the autonomous communities and the Infection Commission Commission/Hospital Pharmacy Services. This report is drawn up after hearing and assessing the suggestions proposed by different scientific societies, and they have reserved these new drugs (FDX or BZL) for use in the first or even second/successive recurrences.

All these regulations are basically built upon the cost evaluation studies (cost-effectiveness) of each drug. Despite that fact that a substantial number of these have been published, one recent review raises the criticism that a majority of these reports are promoted by the pharmaceutical industry and hence are not applicable between different countries and health systems. This is because the price and financing body may differ, as well as the value of the QUALYS/DALYS, and the difference in their time horizon, for which a local assessment is a necessity, with independent analysis [14].

These studies fail to evaluate adequately two factors that can determine the cost-effectiveness of the treatments in the real world. On the one hand, a good estimation by experts is needed, where the distinction is made between colonization and infection; this can decrease the prescription of treatments by around 15-20%. On the other hand, the "non-tangible" influence exerted by the recurrences in some patients, the apparent "lost window of opportunity", must be assessed, which includes the delayed administration of chemotherapy cycles, performance of major surgeries, or reception of transplants, as well as in terms of quality of life (family and domestic, social and work).

OPTIMIZATION IN GLOBAL HEALTH STRATEGIC PLANNING: A CLINICAL PATHWAY

For the proper translation of the recommendations of these guidelines into clinical practice, and assurance of the efficacy and cost-effectiveness of the resources, several key aspects must be considered:

- Correct identification of infection and colonization.

- Therapeutic objective: Sustained cure of the CDI (prevention recurrences).

- Identification the risk of recurrence and the clinical impact and prognosis of the recurrence, to enable the treatment decision.

- Optimization in the indication of the different treatments.

- Clinical follow-up between the various departments and levels of care, which ensure the early identification of new episodes of recurrence.

- Selected, pertinent and practical data to facilitate involving the patient and relatives in managing and following up of the CDI episode (empowerment). An adequate clinical evaluation that allows to identify colonization and infection, select the best treatment according risk factors of recurrence, will optimize the effectivity of treatment well as accessibility and equity throughout the health system (Figure 1).

This approach can be optimally structured by designing a clinical pathway of care for CDI patients.

In different scenarios, clinical pathways have been known to enhance the results of a specific health problem, through clinical care optimization and the speedy and efficient incorporation of available scientific evidence. The impact of this process directly relates to the appropriate design and coordination that facilitates solving any issues that are present, and opening up a way for smooth and efficacious implementation [15].

In order to develop a clinical pathway, four basic pillars have been proposed [1] a structured and normally multidisciplinary intervention plan, [2] transfer of the scientific evidence or general clinical guidelines of the intervention plan to the local structures, [3] presentation of the steps involved in the course of treatment in detail, as a plan and algorithm and, [4] standardization of care for specified populations, as the ultimate goal [16].

These pointers may help in developing a clinical pathway for CDI care, coordinated by the Stewardship teams (Figure 2). This will permit identification of patients having a microbiological diagnosis of CDI, provision of clinical assessment by a team of specialized and competent health care personnel, and availability of pertinent data and accessibility for patients during follow-up, which can thus facilitate early detection of recurrence. Such a clinical pathway will provide an effective route through which several of these recommendations cited in the guidelines or consensus documents can be incorporated into the daily clinical practice in managing CDI, as indicated by the variety of experiences in our country.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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Current strategies for infectious diseases management

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Timing in antibiotic therapy: when and how to start, de-escalate and stop antibiotic therapy. Proposals from a stablished antimicrobial stewardship program

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ABSTRACT

The current morbimortality of serious infections is unacceptable and there is a need to promote the increase in the efficacy of empirical and targeted antibiotherapy. This could be achieved by initiatives coming from ASP teams aimed at promoting increased efficacy of antibiotic therapy .In the optimization of the antibiotic therapy there are several critical points in which an adequate timing could achieve benefits in the survival of patients with severe infections: prompt initiation of empirical treatment; de-escalation performance, appropriate targeted treatment; and finally, curtail antibiotic duration.

Keywords: antimicrobial stewardship program, de-escalate, timing, empirical treatment, targeted treatment

INTRODUCTION

The implementing and promotion of the Antimicrobial Stewardship Programs (ASP) [1] during the last decade into the hospitals is a successful history without any doubt. It improved the control of the infections by the clinicians in a new dialectic context which includes experts in Pharmacy and Microbiology in the generation of fully operative expert teams that are able to provide effective clinical interventions in real time to patients with serious infections or produced by difficult-to-treat bacteria. These interventions also include the safe reduction of antibiotic exposition (de-escalation or reduction of the duration of antibiotic therapy). In addition, have improved registration, control and awareness of the challenges of managing these infections, and established a new educational training in these areas. And with the dissemination of all these measures, an evident improvement in the diagnosis, management and treatment of infectious diseases has been achieved.

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Nonetheless, it has not been possible to demonstrate a clear improvement in the general prognosis of severe infections [2,3], or in the prevention of the appearance and development of the multidrug-resistant (MDR) bacteria [4,5], which are the two main reasons for the creation and dissemination of the ASP.

In general, the ASPs have not changed the primary objective of the old antibiotic policies, which was to restrict the use of antimicrobials (with focus on the new antimicrobials), with the intention of reducing the selective pressure they exert on the development of microbial resistance to antibiotics. With this type of interventions, it has been possible to reduce costs and improve efficiency on a transient and sectorial basis and, eventually, it has been possible to reduce infections by multidrug-resistant bacteria. But they have not substantially improved the prognosis of serious infections [6].

In order to improve the management of the current high morbidity and mortality due to the serious infections, it may be necessary to modify this emphasis on the overuse of antibiotics. Perhaps, it will be necessary to admit with more determination that we need to increase the efficacy of antibiotic therapy in this stage, based on non-restrictive prescribing of new antibiotics and strategies at the time of diagnosis.

The key points for improving the efficacy of the antibiotic therapy. In our opinion the ASP teams could promote initiatives capable of reducing morbimortality associated with serious infections, as:

1. Early and more precise detection of patients with sepsis/severe infections, poor prognosis and high-risk for MDR bacteria colonization in every/all different care setting. This will require the implementation of optimized programs and strategies for Sepsis detection, ideally using new artificial intelligence technologies and computerized programs.

2. Early and more precise microbiological diagnosis, which would enable faster, deeper and better dissemination of

individualized microbiological diagnosis and a more operational knowledge of the local pathogenic flora. This would require the incorporation of technical and strategic innovations in microbiological diagnosis.

3. Improved efficacy of antibiotherapy in both, empirical and targeted treatments; and also the promotion of De-escalation performance and shortening the antibiotic treatment duration [7], which have proven to minimize the development of MDR bacteria. This would require optimized management of new antibiotics agents and new antimicrobial strategies (from antibiotic combination to supplementation with new pharmacological and non-pharmacological products, immunotherapy, phagotherapy, bacterial genetic modification, etc.).

The critical importance of "timing" in these initiatives:

Although in this Review we focus on the importance of timing in the design of the antibiotic strategy, the impact of early diagnosis of Sepsis/Severe Infection and Microbiological Diagnosis is no less important. Therefore, we encourage that all these aspects must always be part of any strategy implemented by the ASP teams in order to attempt a reduction in the morbimortality of Infections.

In the optimization of the antibiotic therapy there are several critical points in which an adequate timing could achieve benefits in the survival of patients with severe infections. On a direct way: 1) In empirical treatment, and 2) In Targeted Treatment and indirectly, in the choice of the moment for 3) De-escalation performance and 4) to curtail antibiotic duration.

TIMING IN EMPIRICAL TREATMENT

When the severity of the patient with suspected infection is greater, it is essential to start antibiotic treatment immediately. There are many studies correlating delayed initiation of empirical treatment with decreased survival. This is a continuous variable, that allows the formulation of a basic principle in empirical antibiotherapy, which is "the earlier it is started, the greater the survival achieved" [8]. Based on the available data, severe infections should be treated within the first hours after diagnosis, and never later than 3-4 h.

This is so important that ASP teams should establish surveillance programs to monitor delays in the initiation of antibiotic therapy in patients with severe infections. The measurement of time from patient admission to the hospital to intravenous antibiotic administration ('door-to-needle time') is a good indicator of promptness or delay of appropriate empirical treatment, and also includes an assessment of the capability of our health system in the early detection and management of sepsis/severe infection. Thus, these indicators would be an achievable and useful tool for improvement of antibiotic management.

The current criteria for Sepsis have a high specificity in the diagnosis of severe infection. But their sensitivity is lower, and there are many patients with severe infections who do not meet these criteria [9,10]. To improve our ability to detect severe infections accelerating or anticipating the initiation of empirical antibiotherapy, with focus again in the most vulnerable patients, new criteria must be adopted. These new criteria, although not sufficiently standardized, has proven to have a good predictive capacity [9,10]. For example, the presence of a systemic inflammatory response syndrome (SIRS), high risk of progression and severity (for instance, a Charlson> 3) and high inflammatory markers (such as CRP > 200 mg/L or Procalcitonin > 5-10 ng/mL) can predict severe infection with high probability [9]. Other criteria such as Age > 65 years, vascular catheters, clinical suspicion of endocarditis, NEWS score, predictive models of bacteremia [10] may contribute in this direction, to facilitate an prompt initiation of empirical antibiotherapy.

Moreover, it would be possible to improve this timeliness if primary care would assume and participate in improving the screening of severe infections in outpatients.

When evaluating these strategies, early initiation of empirical antibiotic therapy in severe infections is a necessary and essential criterion to qualify the treatment as adequate. The other necessary condition is that the choice of antibiotic(s) is appropriate; that is, the antibiotic(s) must be effective (active) against the microorganism causing the infection in each particular patient. Without these two conditions, empirical antibiotic therapy can never be considered adequate. Early and Active is the only choice. Active but Late is associated with worse clinical outcomes, similar to those achieved with Early but Inactive or even Late and Inactive treatment [11,12].

As delay reduces the effectiveness of antibiotherapy, so does the prescription of antibiotics that are not active against the pathogens causing the infection [13]. Surprisingly, in our current clinical practice, the rate of prescribing empirical antibiotic therapy that is inactive or ineffective is very high (up to 20 and 30%) [13–18]. And the rate would be even worse assuming this new strategic concept that appears in recent leading publications: that in severe infections caused by multidrug-resistant bacteria, two active antibiotics improve the survival rate over that achieved with monotherapy [16,19–21]. Furthermore, in the choice of empirical antimicrobials we should to consider several other factors: first of all, the ability of eradicate the infection and its ecological impact, the appropriateness of PK/PD properties to the site of infection, the bacterial inoculum size and the degree of microbial resistance, vulnerability or risk of progression of the patient, and severity of infectious process. Therefore, the optimal empirical antibiotic therapy is considered to be that initiated early, with the highest erradicatory capacity and with the appropriate PK/PD profile, precisely tailored to each individual patient.

For our ASP team, the follow-up of adequate use of empirical treatment has become an important indicator of the use of antimicrobials. And to improve it we have implemented real-time audit programs for all bacteremia and multidrug-resistant isolates in other cultures.

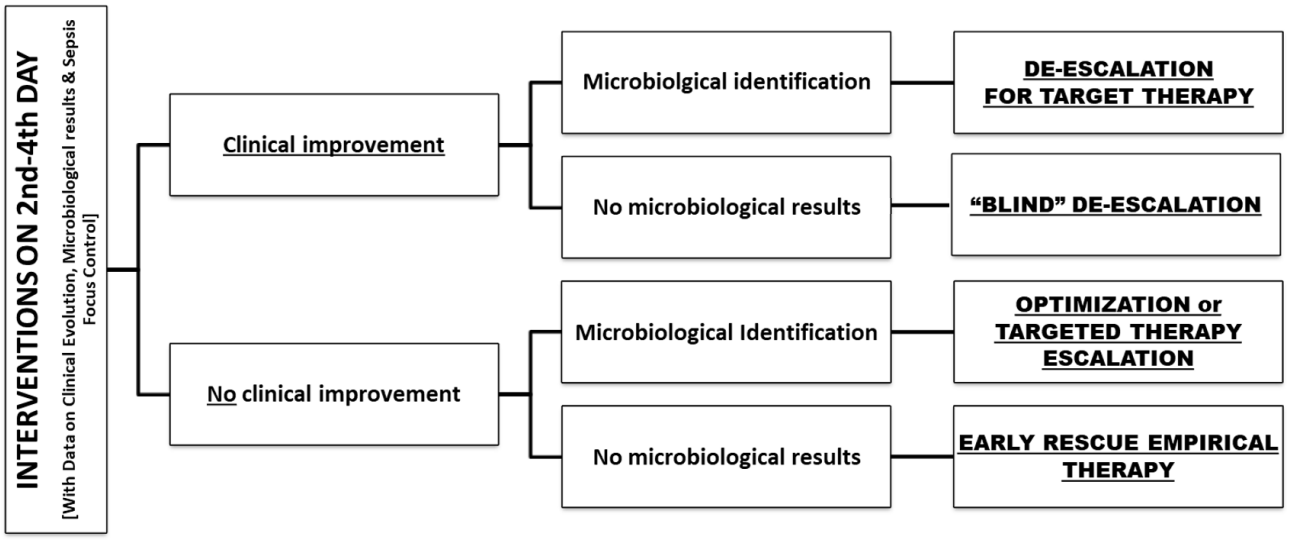

Figure 1 | **Possible Interventions on the 2nd-4th day of antibiotic treatment**

In addition, we adapted our antibiotherapy guidelines, in a timely manner, to the local microbiological map and to the rest of the needs that we have mentioned before.

TARGETED TREATMENT, ACCURACY IN ANTIBIOTHERAPY

The choice of the empirical treatment is only the first step of a complex process of infection management that will require our attention. Then there are still multiple opportunities to further optimize it, to adjust it accurately to the individual characteristics of every infectious process and of each patient.

In antibiotic therapy optimization, the assessment of clinical evolution and the microbiological results are of special relevance, and can already be evaluated typically on the 'third day', between 24 and 72 hours from the start of treatment. And we have here, at this moment, the best opportunity to assess the effectiveness of empirical treatment. The effectiveness of antibiotic treatments is shown very quickly (hours and, therefore, the absence of significant improvement on the third day of treatment (based on the general clinical evolution, the septic focus control, the vital signs, inflammatory biomarkers and other data from complementary tests) is a good predictor of ineffectiveness and poor progression and, consequently, a good reason to reconsider and re-design it uses at that time. The results of microbiological tests (from cultures of clinical specimens and screening of multi-R colonization) allow identification of the etiologic agent causing the infection and, therefore, accuracy of antibiotic treatment (ensuring de-escalation to targeted therapy). The availability of rapid microbiological tests (that are able to provide results within hours) will allow us to reduce that initial period of 'etiological uncertainty' that has an important impact on the prognosis, and therefore contributes to the to the improvement of the clinical outcomes.

The evaluation of antibiotic therapy on the third day is based, accordingly, on clinical evolution data and microbiological results, and should be performed between 24 h and 72 h [9].

Depending on the obtained findings, and with the septic focus successfully controlled (in the event that this requires interventions other than antibiotherapy, such as surgery or the removal of infected prosthetic material, the optimization of antibiotherapy may lead to one of these four options, which may overlap (Figure 1).

De-escalation is a key strategy for antibiotherapy optimization. Generally, defined as the reduction of the initial antimicrobial spectrum based on microbiological results, either by switching from a broad-spectrum antimicrobial to a narrow one, or from combination therapy to monotherapy. In other words, it is no more than the choice of the most appropriate treatment against the identified pathogen, in a phase in which, clinical improvement achieved after an antibiotic 'intensive or induction' phase, would lead to a certain 'maintenance phase', less demanding, in which the reduction or simplification of the antibiotic coverage or potency is possible without negative impact on prognosis, and with ecological advantages (by reducing the duration of exposure to antibiotics and regimens with a critical ecological impact).

Rapid diagnostic microbiological tests and MDR pathogens colonization screenings would allow us to de-escalate from 24-72 h, provided that clinical improvement in the patient has been achieved and empirical antibiotic coverage turns out to be unnecessary [9].

In the absence of microbiological results, de-escalation, in

cases where the clinical evolution is favorable, should be considered [9,22]. It is based on the idea that most of the overall efficacy of antibiotherapy is achieved in the first days of treatment, and once a significant clinical improvement has been observed, a practically complete extinction of pathogenic bacterial inoculum has been done, and microbial regrowth and recurrence of symptoms would not occur in patients without severe immunosuppression, uncontrolled septic foci or prosthetic material with ineradicable inoculums. And this is more likely to be true the more active or effective the chosen of initial empiric antibiotherapy was. In addition, the absence of growth of MDR bacteria in cultures (from clinical samples or in colonization screenings) reduces the need to maintain coverage against them.

For our ASP team, it is a priority to promote and monitor that all patients with severe infections should be assessed for the efficacy of antibiotherapy (based on clinical course and inflammation biomarkers) and microbiological results) between 24 and 72 h after the antibiotic therapy is initiated, allowing an optimization or accuracy of antibiotherapy (Escalation or New empirical rescue therapy, De-escalation -with or without microbiological results, Stopping, if the suspicion of infection disappears).

And this requires economic investments (in the improvement of the healthcare management of severe infections and microbiological diagnosis).

ANTIBIOTIC TREATMENT DURATION

Antibiotic efficacy concerns clinical efficacy (Resolution of symptoms), and could be measured by Time to microbiological eradication (or sterilization of positive microbiological cultures), which under experimental or controlled conditions, would be between 2 and 9 days (according to 'in vitro' studies, microbiological monitoring studies in patients and biological estimates), depending on the bacterial species (e.g., *Escherichia coli 2*-4 days; *Staphylococcus aureus* 4-9 days), the pharmacological or pharmacodynamic properties of Antibiotics (there is a 'Pharmacodynamic Hierarchy' that classifies them according to their activity and eradicatory capacity, and generally places the new antibiotics in the best positions), and the management of these antibiotics (At appropriate doses and based on optimized PK/PD parameters, the time to eradication is reduced; synergistic combination of antibiotics -active against the same bacteria- also decreases time to eradication).

Most of the beneficial effect of appropriate antibiotherapy accumulates in the first 5-7 days. And if the initial antibiotherapy is appropriately optimized, even in the first 2-5 days.

This approach is based on multiple published studies which show us, for example, In vitro, the maximum bactericidal effect is completed on the 7th day (ciprofloxacin vs. BGN) [23]. Biological estimates consider that the time to eradication of *E. coli* is 2-4 days, and of *S. aureus* 4-9 days (compared to 6 months for *Mycobacterium tuberculosis*). Computational biology in experimental models has established that microbiological eradication can be achieved in 3.9 days (with intensified initial treatment -front loading-) and 8.7 days (with standard treatment) [24]. In vivo, rapid eradication of the causal pathogen of severe pneumonia (in BAS cultures) can be observed in most patients [25].

Clinical and microbiological biomarkers of infection and inflammation generally improve in 3-5 days when antibiotherapy is effective [26,27]. There are studies showing significant differences in efficacy, that is, in the time until eradication, between various antibiotics when the comparison is established in those first 2-3 'critical' days of antibiotherapy (meropenem vs. piperacillin/tazobactam [28]; daptomycin vs. vancomycin [29]. In other studies, differences in efficacy are established based on the reduction of the symptom period (moxifloxacin vs levofloxacin) [30].

In the last 20 years, numerous clinical studies were published demonstrating the similar efficacy and safety of 3- to 8-day vs more prolonged (> 10-14 days) antibiotic treatments [31].

On the other hand, the negative ecological impact of antibiotherapy begins after the first few days. Disruption of the ecological balance and overgrowth of MDR flora can occur within the first 2-4 days of treatment, but is significantly delayed with intensive initial antibiotherapy -front loading-, especially if concentration levels of the antibiotic in the septic focus are above the mutations preventive concentration [31–34]. However, when antibiotic therapy is not capable of eradicating the pathogenic microbial inoculum, its prolongation over time greatly increases its ability to select and promote the emergence of resistance. In such a way that the longer the duration of the treatment, the more intense antibiotic activity is required to avoid the emergence of mutations during the treatment [35]. This last point challenges the appropriateness of De-escalation (which reduces antibiotic spectrum when the opposite might be necessary to avoid the emergence of resistance in that scenario. But, in practical terms, the best way to minimize the selection and emergence of resistant microorganisms during antibiotic treatment involves employing a front-loading strategy (early and intensive antibiotherapy, with the maximum achievable eradicatory capacity) and shortening the duration of treatment [36].

Overall, we could assume that practically all common bacterial infections, including severe cases, could be treated successfully for 5-8 days [31,33]. With the exception of certain conditions where the safety of shortening of the duration is not well demonstrated [31,33]:

a) Absence of a rapid and significant clinical response to initial treatment.

b) Major Immunosuppressed patients (neutropenic, cancer under chemotherapy, etc...).

c) Involving infections that affect tissues or structures difficult to access for antibiotics and that cannot be 'withdrawn':

I. Devitalized or abscessified tissues (No control of the septic focus).

II. Bone (osteomyelitis), endocardium (endocarditis), vitreous humor (endophthalmitis)...

III. Prosthetic material, catheters, biofilms…

d) In infections produced by particularly drug-resistant, persistent or latent/quiescent bacteria:

I. *M. tuberculosis* and other infections of slow chronopathology.

II. *S. aureus* (especially MRSA).

III. Non-fermenting gran-negative bacilli, such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, etc.

Finally, to implement all these ideas, the ASP team needs to design specific programs and initiatives that place them in the healthcare surveillance and intervention programs, in the development of the local microbiological map and the local Antibiotherapy Guidelines, and in the educational programs. These should be diffused throughout the hospital, starting with the critical areas and extending to the outpatient setting.

CONCLUSIONS

Assuming that the current morbimortality of serious infections is unacceptable, and if we want to contribute to minimize it, we should work on a reorganization of the ASPs that, mainly but not exclusively, promotes an increase in the efficacy of antibiotic therapy and a reduction of its negative ecological impact through improvement of the design of empirical and targeted antibiotherapy (to maximize its efficacy). Numerous studies indicate that improvements can be made in both directions with earlier, more accurate and optimized treatments against the specific infection-causing bacteria in the particular patient, and with the highest possible eradication capacity. The timing of antibiotherapy would be of decisive importance in this design. A very important part of the current and future efficacy of antibiotherapy of severe infections involves the search for earlier antibiotherapies (empirical, targeted and rescue), which should be de-escalated when possible and at the optimum time, and stopped after the shortest time possible with proven efficacy and safety.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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