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Monkeypox in humans: a new outbreak

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ABSTRACT

Infection caused by Monkeypox Virus (MPVX) has small rodents as its natural reservoir and both monkeys and humans are occasional hosts. The causative agent is an Orthopoxvirus (MPVX) that was isolated in monkeys in 1958 and proved capable of passing to humans in 1970. It remained contained in Africa, causing isolated episodes of infection, until 2003 when an outbreak occurred in the United States following importation of animals from that continent. Since then, anecdotal cases have continued to be reported outside Africa, usually very clearly linked to travelers to those countries, but in May 2022, a broad outbreak of this disease has begun, now affecting several continents, with the emergence of human cases of MPVX (H-MPVX) infection mainly among Men that have Sex with Men (MSM). The disease has an incubation time ranging from 5 to 15 days and is characterized by the presence of pustules, fever, malaise and headache. The presence of significant regional lymphadenopathy is a differential feature with episodes of classical smallpox. Proctitis and pharyngitis, with minimal skin lesions, may be another form of presentation. Diagnosis can be confirmed by PCR testing of lesions or by demonstration of MPVX in other body fluids or tissues, although in the appropriate epidemiologic setting the clinical picture is highly suggestive of the disease. Effective drug treatment has been developed as part of programs to protect against potential bioterrorist agents and smallpox vaccinees are known to have high protection against monkeypox. New vaccines are available, but neither the drugs nor the vaccines are yet freely available on the market. The prognosis of the disease appears, at least in adults in developed countries, to be good, with very low mortality figures and much less aggressive behavior than that described in classical smallpox. Isolation measures, essential for the control of the outbreak, have been published by the health authorities.

Keywords: Monkeypox, MPVX, Poxvirus, outbreaks, vaccines, smallpox, outbreak, sexually transmitted infections

Monkeypox (Viruela del mono) en humanos: un nuevo brote

RESUMEN

La infección causada por el Virus de la Viruela del Mono o Monkeypox (MPVX) tiene como reservorio natural los pequeños roedores y tanto el mono como el hombre son huéspedes ocasionales. El agente causal es un Orthopoxvirus (MPVX) que fue aislado en monos en 1958 y se demostró capaz de pasar a humanos en 1970. Se mantuvo contenido en África, causando episodios aislados de infección, hasta el año 2003 en que se produjo un brote en los Estados Unidos tras la importación de animales desde dicho continente. Desde entonces, han seguido

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comunicándose casos fuera de África, por lo general muy claramente vinculados a viajeros a dichos países, pero en mayo de 2022 se ha iniciado un brote amplio de esta enfermedad que afecta ya a varios continentes, con la aparición de casos humanos de infección por MPVX (H-MPVX) principalmente vinculados a fiestas en las que hay relaciones sexuales de hombres con hombres (HSH). La enfermedad tiene un tiempo de incubación que puede oscilar entre 5 y 15 días y se caracteriza por la presencia de pústulas, fiebre, malestar general y cefalea. La presencia de importantes adenopatías regionales es una característica diferencial con los episodios de viruela clásica. La proctitis y la faringitis, con mínimas lesiones cutáneas, pueden ser otras formas de presentación. El diagnóstico puede confirmarse con una prueba de PCR en las lesiones o con la demostración de MPVX en otros fluidos o tejidos corporales, aunque en el contexto epidemiológico oportuno el cuadro clínico es altamente sugerente de la enfermedad. Hay tratamiento medicamentoso eficaz que ha sido desarrollado como parte de los programas de protección frente a potenciales agentes bioterroristas y se sabe que los vacunados de viruela tienen una protección elevada frente a H-MPVX. Se dispone de nuevas vacunas, pero ni los medicamentos ni las vacunas están todavía libremente disponibles en el mercado. El pronóstico de la enfermedad parece bueno, al menos en países desarrollados y en adultos, con cifras de mortalidad muy bajas y un comportamiento mucho menos agresivo que el descrito en la viruela clásica. Las medidas de aislamiento, imprescindibles para el control del brote, han sido publicadas por las autoridades sanitarias

Palabras clave: Monkeypox, MPVX, Viruela del mono, Poxvirus, brotes, vacunas, viruela

INTRODUCTION

Monkeypox (MP) is a zoonotic viral infection (transmitted to humans from animals) caused by a member of the genus Orthopoxvirus of the family Poxviridae.

Monkeypox virus (MPVX) was discovered in a Danish laboratory in 1958 [1] when it was identified as the causative agent of a disease in Cynomolgus monkeys similar to human smallpox.

Initially, man was considered to have little susceptibility to this new virus [2], but in 1970 its zoonotic nature was demonstrated by its confirmed transmission to a 9-month-old child (Human Monkeypox) in the Democratic Republic of Congo [3].

The disease in humans has remained endemic in Central African countries [4-9] and, outside Africa, a multistate outbreak of cases occurred in the United States of America [10] in 2003, among people who had contact with imported animals.

The disease has just jumped into the media with an outbreak in humans residing outside Africa starting in May 2022, with more than 3.000 cases reported from more than 50 countries in the first month after the beginning of the outbreak. The outbreak is, at present, linked primarily, but not exclusively, to groups of men who have sex with men (MSM).

The COVID and Transmissible Pathogens Committee of the Illustrious College of Physicians of Madrid (ICOMEM) has tried to bring together existing and rapidly changing information on this topic that could be of interest to members of ICOMEM and anyone else. Trying to stratify the data, we have formulated some questions that the members of the Committee have considered relevant. We have invited physicians from Madrid who are not usually part of the Standing Committee of the College to participate in the writing of this paper because of their experience in the diagnostic and therapeutic management of these cases [11] and from the Center for Molecular Biology Severo Ochoa of the Spanish National Research Council.

We will now discuss some of the questions raised.

WHAT IS A POXVIRUS AND WHAT CHARACTERIZES IT?

The Poxvirus family (Poxviridae) consists of a group of large, complex, double-stranded deoxyribonucleic acid (DNA) viruses that replicate in human cells and are defined by their genomic, structural, and antigenic characteristics.

The 8 vertebrate Poxvirus genera are: Orthopoxvirus, Parapoxvirus, Avipoxvirus, Capripoxvirus, Leporipoxvirus, Suipoxvirus, Molluscipoxvirus and Yatapoxvirus. They all share a similar DNA sequence with very similar antigens that have cross-reactivity [12].

Poxviruses include human smallpox virus (Variola), which was declared eradicated by the World Health Organization (WHO) in 1980. Other poxviruses that can affect humans include cowpox virus (Cowpox), the virus used in the smallpox vaccine (Vaccinia), Akhmeta virus and monkeypox virus (MPVX), as well as other zoonotic species with epidemic potential. Of these, MPVX is the most prevalent in humans.

Bovine smallpox is another zoonotic disease, with a wide reservoir in the animal kingdom, which can produce papulo-vesicular disease in humans, in a clear relationship of contact with cattle, of variable dissemination and severity, generally with very low mortality and weeks of evolution.

Another viral genus of the Poxvirus family are the Parapoxviruses, which include species of wide dissemination in sheep, goats, cattle, camelids and cervids. They are Orf viruses that produce different clinical manifestations in animals, in general with oral or pharyngeal affection (papular stomatitis) or cutaneous (papular dermatosis) that by contact affect humans (milker's nodule) and that induce nodular or macrovesicular lesions with granulomatous reaction, of medium duration. This genus induces less immunity than Orthopoxviruses and therefore reinfections are more frequent.

Molluscum Contagiosum is a frequent disease of humans, children and adults, produced by species of the genus Molluscipoxvirus, with special epidermal tropism and inducing papules that mimic soft tumors, generally small, of variable number depending on the sites of inoculation, preferentially distributed on the trunk and root of the extremities, self-lim-

ited in the immunocompetent. Molluscum Contagiosum is transmitted by direct contact, facilitated by sexual intercourse with genital proximity lesions and also transmitted by fomites.

Finally, the genus Yatapoxvirus, which includes the Tana River pox virus (Kenya), induces in humans a vesicular-papular disease in areas of exposed skin, with adenopathic reaction and systemic symptoms of up to 6 weeks of evolution.

Equivalent in African and Asian apes is the disease produced by the Yaba virus, which produces lesions that mimic histiocytomas and can be transmitted to humans by bites or direct inoculation.

Unlike other viruses, the ability of Poxviruses to mutate is much lower than that exhibited by RNA viruses, such as influenza virus or SARS-CoV-2. Nevertheless, these viruses have a large genetic endowment responsible for their virulence and ability to invade and evade the immune system [13,14].

Interest in these viruses has been sustained, among other reasons, because of the potential use of some of these agents, particularly Variola virus, as a biological weapon since Orthopoxviruses and Poxviruses can be created and modified in laboratories using molecular biology tools [15,16].

Two distinct genetic clades of MPVX have been identified, Congo Basin and West African. The former is more virulent and transmissible than the latter. The geographical division between the two clades is believed to be in Cameroon, as it is the only country where both clades of the virus have been detected simultaneously. The cases currently affecting Spain are caused by the less virulent West African clade [17].

WHAT DID SMALLPOX MEAN IN THE HISTORY OF MANKIND?

Smallpox was a disease with very high mortality, which fortunately was eradicated in 1980 thanks to vaccination and the fact that its reservoir was only human [18,19]. Smallpox lesions have already been identified in Egyptian mummies from 300 BC, and it has subsequently affected humanity periodically through large epidemic outbreaks [20-22]. Recent studies have sequenced complete smallpox virus genomes in human remains from the Viking era (9th-12th centuries), a Lithuanian mummy (18th century) and specimens from a 19th century Czech museum [23]. Human smallpox, after infection, is followed by an incubation period ranging from 7 to 14 days, after which skin lesions appear abruptly and intensely. A rash usually appears, either diffuse (scarlatiniform) or macular (macular or measles) and with variable involvement of the skin surface, mainly on the face, extremities, hands and feet and later on the trunk, evolving into papules.

Between the fifth and sixth day after their appearance, the papules become round vesicles, with a central umblication and on the eighth day they become clear pustules with a grayish and turgid content. It is very characteristic, from the semiological point of view, that all the cutaneous elements are in the same morphological stage, a fact that differentiates it from other vesicular-eruptive diseases, mainly with chickenpox.

In the crust formation phase, these may remain adhered to the skin for a long time and their fall will leave a scar.

Smallpox was, for centuries, one of the first causes of blindness in mankind and its mortality has ranged from 10-75%, It is estimated that in the 20th century approximately 300 million people worldwide still died from smallpox before its eradication [20-22,24-28].

Smallpox was the first disease against which a vaccine was available, and Spain played a key role in spreading smallpox vaccination throughout its extensive empire, by means of the famous Royal Philanthropic Vaccine Expedition, led by Francisco Javier Balmis [29-32].

WHAT ANIMALS ARE INFECTED BY MPVX?

There are multiple animals that are susceptible to infection by MPVX including non-human primates, rodents, squirrels and dormice. Some of these animals have been used as models on poxvirus acquisition and transmission and for testing vaccines and protective drugs after it was learned that the former Soviet Union had turned these viruses into potential biological weapons [33].

In non-human primates, MPVX usually produces a short-lived rash. Initial clinical signs are fever and cutaneous papules of 1-4 mm, which develop into pustules and then crust over. A typical lesion has a red, necrotic, depressed center surrounded by epidermal hyperplasia. These "smallpox pustules" can be located all over the body, but preferentially on the face and extremities. Most infected animals recover quickly; however, fatal cases can occur, especially in neonatal monkeys.

The disease caused by MPVX has also been observed naturally in some rodents, such as prairie dogs, and after inoculation in dormice and squirrels. In all of these cases, clinical manifestations are varied, but fever, weight loss, nasal discharge, sneezing and/or coughing, respiratory involvement, and nodular skin rash or mouth ulcers may occur [33-37].

WHAT CASES OF MPVX INFECTION HAVE BEEN DESCRIBED IN HUMANS?

Until recently, MPVX was an occasional, endemic disease in humans in contact with animals that spread mainly in rainforest areas of Central and West Africa, causing isolated cases or small outbreaks in 11 African countries: Benin, Cameroon, Central African Republic, Democratic Republic of Congo, Gabon, Ivory Coast, Liberia, Nigeria, Republic of Congo, Sierra Leone and South Sudan.

In 1996-1997, a large outbreak of MPVX in humans was described in the Democratic Republic of Congo with a low case fatality rate, but with a higher than usual attack rate [38-42]. In 2017 Nigeria experienced the largest documented outbreak, 40 years after the last confirmed case [43].

As of 2018, occasional cases have been reported in Israel [44] in September 2018, in the United Kingdom in December

2019 [45,46] and in Singapore in May 2019 [47] in travelers from Nigeria who became ill with monkeypox after arrival. One health care worker was infected and became ill.

WHEN AND HOW DOES THE CURRENT OUTBREAK BEGIN?

On May 7, 2022, a case of H-MPVX is confirmed in the United Kingdom in a patient with recent travel to Nigeria [48]. On May 14, 2022, two additional cases of H-MPVX were identified in London in two unrelated cohabitants of the previous case and new cases have been confirmed in the United Kingdom, in and outside of London. The current outbreak is multinational and includes more than 50 countries as far afield as Australia, North America and Europe [49-51].

The majority of cases have occurred in young men, many of whom were identified as MSM, with genital lesions suggesting that transmission likely occurred through close physical contact [52-58].

In Spain, the number of confirmed cases as of June 10 exceeds 200, mostly related to MSM parties.

WHAT ARE THE CLINICAL MANIFESTATIONS OF MONKEYPOX IN HUMANS?

A publication presents the clinical features and evolution of 282 patients with H-MPVX in the Congo during 1980-1985. The ages of the patients ranged from one month to 69 years; 90% were younger than 15 years. The clinical picture was similar to that of the ordinary and modified forms of smallpox. Lymphadenopathy, appearing early in the disease, was the most important sign differentiating monkeypox from smallpox and chickenpox in humans. Symptoms, signs, and disease course in patients who had been vaccinated against smallpox differed significantly from those of unvaccinated subjects. Varicella-like pleomorphism and cropping occurred in 31% of vaccinated and 18% of unvaccinated patients. Prognosis was largely dependent on the presence of severe complications, and no deaths occurred among vaccinated patients. In unvaccinated patients, the crude mortality rate was 11%, but was higher among younger children (15%) [59].

Clinical manifestations of human infection usually appear after incubation periods of 5 to 21 days. It usually presents clinically with fever, rash and swollen lymph nodes. It is usually a self-limiting disease with symptoms lasting 2 to 4 weeks. Severe cases are more frequent in children and are related to the degree of exposure to the virus, the patient's state of health and the nature of the complications.

Skin lesions are more frequent on the face and extremities than on the trunk. The lesions do not affect the palms of the hands and soles of the feet (in 75% of cases) or the oral, genital or rectal mucous membranes. The conjunctiva and cornea may be affected.

Complications of monkeypox may include deep abscesses and secondary infections.

Some patients, in this outbreak, manifest preferentially with proctitis or pharyngitis with minimal or absent skin lesions.

WHAT IS THE EPIDEMIOLOGY OF HUMAN MPVX DISEASE?

In 1980 the WHO [60] reported that since 1970 there have been 47 human cases of monkeypox in 5 countries in Central and West Africa; 38 of which have been reported in the Congo. Human MPVX disease had at that time a mortality rate of approximately 17% and children under 10 years of age accounted for 83% of the cases. All cases had occurred in tropical rainforest areas and clustering of cases had been observed in certain areas within countries and within families. Although the low rate of transmission and the low frequency of disease indicated that monkeypox was not then a public health problem, authorities cautioned that more data on this disease were needed.

Many animals near human cases of MPVX were shown to have antibodies to Orthopoxvirus, but the natural reservoirs and vectors of MPVX were unknown [61]. Even so, the disease was beginning to be accepted as a probable zoonosis [60,62-64].

Human-to-human transmission was progressively well demonstrated [65] by small family outbreaks after acquisition of the index case from a monkey [65].

A study of 2,510 contacts of 214 patients with H-MPVX was conducted in Zaire between 1980 and 1984 [66]. Among the contacts of 130 primary cases, an additional 22 co-primary and 62 secondary cases were detected, and fourteen other persons who had no evidence of clinical disease had positive serological results. Most of those clinical and subclinical cases of monkeypox occurred in children under 10 years of age. The overall attack rate of contacts without smallpox vaccination scar (7.2%) was significantly different from those with vaccination scar (0.9%) [66].

Secondary or person-to-person transmission would occur by close contact with infected respiratory tract secretions or skin lesions of an infected person, or with objects recently contaminated with the patient's fluids or lesion materials. Transmission occurred mainly by respiratory droplets, usually after prolonged face-to-face contact with the patient, exposing family members of active cases to an increased risk of infection [67].

Infection of index cases results from direct contact with blood, body fluids, or skin or mucosal lesions of infected animals.

In recently reported cases outside the African continent, the virus is transmitted from person to person by contact with skin lesions, body fluids, respiratory droplets, and contaminated materials such as bedding. This form of transmission is what is occurring in the present outbreak. Transmission via respiratory droplet particles usually requires prolonged face-to-face contact, which poses a greater risk to unprotected health care

workers and specially to close household members of active cases. Transmission can also occur through the placenta from mother to fetus (congenital monkeypox).

WHAT SHOULD BE DONE AT THIS TIME IN SPAIN IN THE EVENT OF A SUSPECTED CASE? HOW IS THE DIAGNOSIS CONFIRMED?

If H-MPVX is suspected, healthcare personnel should:

1) Attend patients with appropriate PPE: waterproof gown, gloves, FP2 mask and closed goggles.

2) Inform the Microbiology laboratory of the existence of a suspected case.

3) Prior to sampling, make sure to have a swab for sample collection, which should be sent in a dry sterile tube or in virus transport medium and kept cold. The sample should be accompanied by sufficient clinical information so that the microbiologist can fill in all the sections requested by the reference laboratory. At a minimum: age, sex, risk factors, date of onset of symptoms, date of onset of rash.

4) So far, a blood sample for serology and another in an EDTA tube can also be obtained, as well as a urine sample to be sent in the urine culture bottle.

5) It should be noted that the optimal samples for MPVX diagnosis come from skin lesions: the roof or fluid of vesicles and pustules, and dry crusts.

6) Once ready, the on-call microbiologist will be notified of their shipment, correctly identified.

7) The Microbiology laboratory staff will arrange for the samples to be sent to the corresponding reference laboratory where the polymerase chain reaction (PCR) will be performed, this being the laboratory test of choice due to its accuracy and sensitivity.

8) Clinical samples are considered category B samples. Similar precautions to those used for COVID with three successive containers are sufficient for specimen transport.

Mucosal specimens showing lesions, including respiratory, vaginal or rectal mucosa, may also be used. In cases of proctitis, it is recommended to send rectal specimens as well, since in current cases there is a strong association with sexual transmission.

In biopsy specimens, the histopathological lesions of MPVX are indistinguishable from those of human smallpox [68] and consist of necrosis affecting the stratum basale of the skin and adjacent areas of the dermal papillae. The necrosis also affects the stratum spinosum above the destroyed stratum basale. There are occasional multinuclear giant cells and some bodies resembling Guarnieri bodies.

Electron microscopy shows abundant orthopoxvirus particles in the cytoplasm of infected epidermal cells. In general, the features are indistinguishable from the papulonecrotic phase of smallpox [68].

Viral strains of MPVX isolated initially were readily differentiated from Variola and Vaccinia viruses. The isolated strains produced small necrotic hemorrhagic spots, grew well at 39.0 degrees, formed large plaques in Vero cell cultures and showed markedly greater virulence to chick embryos and mice than the Variola strains [6,7,69,70].

ARE THERE DRUGS EFFECTIVE AGAINST MPVX IN HUMANS?

Tecovirimat (ST-246) is a drug first reported in 2005. It is a low molecular weight compound with potent activity against multiple Orthopoxviruses, including smallpox, vaccinia, MPVX, camelpox, cowpox and mousepox viruses. It acts against the Orthopoxvirus V061 gene that encodes an important envelope protein (p37) required for extracellular virus production. In cell culture. ST-246 inhibited plaque formation and virus-induced cytopathic effects [71-76). Oral administration of ST-246 protected BALB/c mice from lethal infection following intranasal inoculation of the vaccinia virus strain. ST-246-treated mice that survived infection acquired protective immunity and were resistant to subsequent challenge with a new lethal dose (10x LD(50)) of vaccinia virus [71,76].

Tecovirimat is an antiviral drug tested in several animal species in which it has demonstrated efficacy against Orthopoxvirus infections. The drug has undergone clinical trials in non-human primates and human volunteers demonstrating good tolerance for up to 14 days of oral administration [77-80]. Also in the treatment of cases of bovine smallpox in accidentally infected or vaccinated immunodeficient patients [81, 82]. A British patient who received the drug in 2021 experienced very short duration of symptoms and viral excretion from the respiratory tract [83]

The approval of tecovirimat to treat smallpox represents a major milestone in biosafety preparedness. Incorporation of the drug into the CDC smallpox response plan, development of pediatric liquid and intravenous formulations, and approval for post-exposure prophylaxis would be an additional health security benefit. Although currently stockpiled by the U.S. Strategic National Stockpile, use of ST-246 may be administered under special circumstances (IND) [84-90].

Tecovirimat's efficacy is maintained even when administration is delayed a few days after inoculation in animal models of Poxvirus infection [73,75]. Its activity is synergistic with other drugs such as cidofovir or CMX001 [74].

No data are available on the efficacy of cidofovir and brincidofovir in the treatment of human cases of MPVX. However, both have demonstrated activity against poxviruses in in vitro and animal studies [91,92].

WHAT VACCINES ARE AVAILABLE AGAINST THIS DISEASE?

The first vaccines, without even knowing the viral nature of smallpox, were made from the material of bovine smallpox pustules. Subsequently, the vaccine virus replaced attenuated strains of smallpox for mass immunization of

populations prior to the eradication of smallpox. It was administered by scarification with a bifurcated needle, inducing a suffusion in the skin on which a papule appeared between the following two and five days, evolving into a vesicle and pustule from the eighth to the tenth day. The vaccinal pustule reached up to 1 cm in diameter, which finally dried and left a scab, which after falling off, between the 14th and 21st day, left a scar. The development of regional adenopathy was frequent.

The efficacy of these vaccines against smallpox is estimated to be complete in the first years, very important in the following twenty years and with protection against severe disease, of longer duration, probably for life.

These vaccines, however, had their complications. One of them was the local extension of the vaccine inoculation lesion to a necrotizing or gangrenous lesion, sometimes with little inflammatory component, with high mortality, and which has been described even in immunocompetent patients. Another more frequent complication (4.6 cases per million vaccinees) was the so-called Eczema Vaccinatum, or extension by inoculation on atopic skin or skin with other skin diseases, of the vaccinee himself or of accidental contacts. As in the previous complication, it was treated with hyperimmune serum.

The generalized extension of the vaccine lesion was another complication, difficult to justify. It was usually self-limited and generally did not require treatment and its incidence was estimated in large vaccination series at 242 cases per million vaccinated. Finally, encephalomyelitis could appear between day 11 to 15 of the first vaccination and was not described in the revaccinations. Its incidence was between 2.9 to 12.3 cases per million vaccinated. Focal lesions with aphasia or hemiplegia presenting closer to vaccination could occur in children under two years of age.

A vaccine produced with an attenuated, non-replicating strain approved for Smallpox and Monkeypox (AVA/ANKARA) could be considered in the immunocompromised and is licensed for use in emergency situations. It has different names in different regions (Invanex; Jynneos; Imvamune). It was approved by the EMA in 2013 for the prevention of human smallpox in adults aged 18 years and older (data sheet updated in April 2022 in Spanish). In the U.S., it received approval for the prevention of human and monkeypox in 2019.

The vast majority of H-MPVX, both in Africa and worldwide, have occurred in people who were not vaccinated against human smallpox [4,34,93-102]. Furthermore, in a study of confirmed and suspected cases of monkeypox in Central Africa, the disease attack rate was much lower in subjects with variola vaccination than among unvaccinated subjects (0.95/1000 versus 3.6/1000) [103].

The smallpox vaccine has so far not been available to the general public and was part of a strategic stockpile.

WHAT SHOULD BE THE PREVENTION MEASURES IN CONTACTS OF PATIENTS WITH H-MPVX?

In any case of H-MPVX under investigation or confirmed, the search for and identification of close contacts will be initiated both among healthcare personnel and among work or social cohabitants. The search for contacts will be interrupted if the case is ruled out after laboratory results.

Contacts of cases will be classified as close, direct or low-risk contacts.

Close contact (less than 1 meter in the same room is defined as contact with an investigational or confirmed case of monkeypox virus in its infectious period, without PPE (or with incidences in its use). Cohabitants, contacts at work, social activities and health personnel who have cared for the patient should be assessed.

Direct contact is defined as contact with clothing, bedding or fomites used by an investigational or confirmed case of monkeypox virus during the infectious period, without the appropriate PPE (or with incidences in its use).

Low-risk contacts are those that do not meet the above criteria.

All contacts will be informed of the symptoms of monkeypox and will be instructed to self-monitor their temperature twice daily for 21 days after exposure. Close contacts will not be quarantined, but should exercise extreme caution and reduce social interactions as much as possible by wearing a facemask at all times.

The responsible person/institution will contact high-risk contacts at least once daily to record temperature and inquire about the presence of any symptoms related to the disease. Contacts should be reachable throughout the follow-up period.

In any of the cases, if any of the contacts presents fever or any other symptom compatible with the clinical signs of the disease, they should immediately self-isolate at home, and urgently contact the person in charge of the follow-up. In this case, the contact will be considered as a case under investigation until laboratory results are available.

The recommended environmental control measures are as follows:

Bed linens, towels, etc., should be washed in a standard washing machine with hot water (60 degrees) and detergent. Bleach may be added, but is not necessary. Care should be taken when handling soiled linen to avoid direct contact with contaminated material. Soiled linen should not be shaken or handled in a manner that could disperse infectious particles.

Dishes and other eating utensils should not be shared with those of cases. Dirty dishes and eating utensils should be washed in a dishwasher or by hand with soap and hot water.

Contaminated surfaces and objects should be cleaned and disinfected with a hospital-grade disinfectant or a 1:100 dilution of household sodium hypochlorite (bleach) [104,105].

WHAT IS THE PROGNOSIS OF MONKEYPOX IN HUMANS?

The case fatality rate of monkeypox has ranged from 0 to 11% in the general population of African countries, and is highest among young children. In addition, persons younger than 40 or 50 years of age (depending on the country may be more susceptible to monkeypox as a result of the end of routine smallpox vaccination worldwide following the eradication of smallpox.

The clade currently circulating in Spain, in adult patients, is associated with very low mortality [93].

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest

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Review

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Unresolved issues in the epidemiology and diagnosis of bacteremia: an opinion paper

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ABSTRACT

Bacteremia is an important cause of morbidity and mortality worldwide and, despite the diagnostic and therapeutic advances of the last decades, the evidence supporting many diagnostic aspects of bacteremia is scarce. Information on the epidemiological evolution of this entity is limited and many methodological aspects of blood culture collection and analysis are under discussion. Furthermore, the recommendations of the main scientific societies on many of these aspects are variable and, in many cases, have not been updated recently.

In this scenario, we have arranged a series of questions on different aspects of bacteremia and reviewed the literature trying to find proper answers for them. We offer our opinion on the topics where the evidence was weak.

The topics covered include epidemiological aspects of bacteremia, indications for blood culture extraction, methods for obtaining and incubating samples, or ways of transmitting results from the microbiology laboratory.

We do not intend to summarize the current clinical practice guidelines, nor will we deal with the therapeutic management of this entity. The aim of this paper is to review the current perspective on the diagnosis of bacteremia with a critical approach, to point out the gaps in the literature, to offer the opinion of a team dedicated to infectious diseases and clinical microbiology, and to identify some areas of knowledge on which future studies should focus.

Keywords: Bacteremia; bloodstream infection; endovascular infection; blood cultures; microbiological reporting.

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Cuestiones no resueltas en la epidemiología y el diagnóstico de la bacteriemia: un documento de opinión

RESUMEN

La bacteriemia es una causa importante de morbilidad y mortalidad en todo el mundo y, a pesar de los avances diagnósticos y terapéuticos de las últimas décadas, la evidencia que apoya muchos aspectos diagnósticos suele ser escasa. La información sobre la evolución epidemiológica de esta entidad es limitada y muchos aspectos metodológicos sobre la obtención y análisis de hemocultivos están en discusión. Además, las recomendaciones de las principales sociedades científicas sobre muchos de estos aspectos son variables y, en muchos casos, no se han actualizado recientemente.

En este escenario, hemos preparado una serie de preguntas sobre diferentes aspectos de la bacteriemia y hemos revisado la literatura tratando de encontrar respuestas adecuadas para ellas. Ofrecemos nuestra opinión sobre los temas en los que la evidencia era débil.

Los temas tratados incluyen los aspectos epidemiológicos de la bacteriemia, las indicaciones para la extracción de hemocultivos, los métodos de obtención e incubación de muestras o las formas de transmisión de los resultados desde el laboratorio de microbiología.

No pretendemos resumir las guías de práctica clínica actuales, ni trataremos el manejo terapéutico de esta entidad. El objetivo de este trabajo es revisar la perspectiva actual sobre el diagnóstico de la bacteriemia con un enfoque crítico, señalar las carencias en la literatura, ofrecer la opinión de un equipo dedicado a las enfermedades infecciosas y a la microbiología clínica, e identificar algunas áreas de conocimiento en las que deberían centrarse futuros estudios.

Palabras clave: Bacteriemia, Infección del torrente circulatorio, Infección endovascular, , Hemocultivos, Transmisión de resultados de microbiología.

INTRODUCTION

Bloodstream infection (BSI) is an entity with a high morbi-mortality worldwide. A study in Finland during 2004–2018, using data from national registries, identified a total of 173,715 BSIs with an annual incidence that increased from 150 to 309 cases/100,000 population, and a 1-month all-cause mortality rate of patients with BSI that rose from 20 to 39 deaths/100,000 population [1]. In addition, the increase of some multi-drug resistant (MDR) microorganisms causing bacteremia in recent years has become a public health concern [2].

Despite the great advances in alternative diagnostic methods of BSI in recent decades [3], blood culture remains the fundamental piece in the diagnostic approach to this entity.

However, many epidemiological and diagnostic aspects of bacteremia remain controversial. The information on the evolution of its incidence and etiology over the years is highly heterogeneous, studies show conflictive results on some key issues, and clinical guidelines offer little or no advice in some aspects of blood culture analysis.

In this scenario, we have reviewed the available literature on the diagnosis of bacteremia from a critical point of view, formulating a series of 15 questions that often arise in the evaluation of these patients. First, we analyzed the evidence about the evolution of the incidence, mortality and etiology of bacteremia. Then, we reviewed methodological aspects of blood culture analysis, including blood culture indications and various laboratory techniques, and some aspects of catheter-related bacteremia. Finally, we reviewed the information on the different methods of reporting blood cultures results from the Microbiology laboratory.

The following pages summarize the discussion and opinion on each of these questions by a team dedicated to Clinical Microbiology and Infectious Diseases.

1. HAS THE INCIDENCE OF BACTEREMIA CHANGED IN RECENT YEARS? HAS THE COVID-19 PANDEMIC HAD ANY IMPACT ON IT?

The incidence of bacteremia has been progressively increasing over the last 50 years, but current data do not give a clear idea of its more recent evolution. Information on the epidemiology of bacteremia in the last decade is very heterogeneous in the few population-based studies available, even more so when analyzing data at the institutional level, with numbers ranging from 101 to 309 episodes per 100,000 inhabitants/year [1,4] and between 1.3 to 15.4 episodes per 1,000 hospital admissions [5,6] (Table 1). In Spain, data ranging from 14.7 to 31.2 episodes per 1,000 admissions have been published [7, 8], and in our own institution, the mean number of bacteremic episodes has barely changed, from an average of 30.17 to 31.45 episodes per 1,000 admissions between 2002-2011 and 2012-2021, respectively (unpublished data). These numbers have not changed substantially in the last 10 years [9] with respect to data published in the previous decade [7,8,10].

Table 1 collects some of the data we have discussed [1,4-6, 1-18]. Moreover, very few studies compare the evolution with respect to previous studies in the same region or hospital [13, 19]. Therefore, there is a lack of evidence to be able to delineate a clear temporal trend in the incidence of bacteremia over the last decade.

For all these reasons, there is a need to carry out population-based studies with more recent data, studying the same regions analyzed and including the years of the pandemic, as well as to continue with institutional surveillance systems.

Information on the impact of COVID-19 on the incidence of bacteremia comes from single or multicenter cohorts. In general, low rates of bacteremia are reported in these patients, although very heterogeneous numbers ranging from 3 to 68% have been described, depending on the selected cohort [20-29]. The rate of bacteremia appears to increase in patients who have more severe disease and require ICU admission [22,23,30]. COVID-19 had a particular impact on catheter-related BSI (CR-BSI), which, after steadily decreasing in the pre-pandemic years [31-33], suffered an alarming 24% (and up to 50% in ICU) increase in incidence during the pandemic [34,35]. In our institution we observed an increase in CR-BSI from 1.89 to 5.53 episodes per 1,000 hospitalizations between 2019 and 2020 [36].

Conclusion:

We cannot establish that there is a clear increase or change of trend in the incidence of bacteremia in the last ten years. The COVID-19 pandemic could have caused an increase in episodes of bacteremia, fundamentally those originating in intravascular catheters.

2. HAS THE MORTALITY OF BACTEREMIA CHANGED IN RECENT YEARS?

Most of the available information on mortality rates has been extrapolated from multicenter cohorts, and the available population-based studies provide very disparate results (Table 1). This variability depends on multiple factors, such as the design of the studies, the population selected, the incidence of bacteremia, the causative microorganism, or the different definitions used (sepsis vs. bacteremia).

It has been estimated that mortality of patients with bacteremia reaches 250,000 deaths annually in North America and Europe combined [37]. According to the results of population-based studies published since 2010 (Table 1), the current global mortality rate for bacteremic episodes is approximately 21-32 deaths per 100,000 population, although the data are very heterogeneous [1,6,11,13,16,19]. These numbers are not very different from previous estimates [38-41].

The data are highly variable depending on the site of acquisition, with numbers ranging from 10-19% for community-onset bacteremia, to 17-28% for nosocomial-acquired episodes [6,7,11,42,43]. It reaches up to 35-50% in patients with septic shock or admitted to intensive care units [44-46].

Table 1 **Estimated incidence of bacteremia and site of acquisition, according to population-based studies published since 2010.**

Reference	Period	Country	Overall incidence		Community acquired	Health-care related acquisition	Nosocomial acquisition	Mortality
			x100,000 inhabitants	x1,000 admissions				
Søgaard [11]	1992-2006	Denmark	114-166	-	45.1-53.3%	8.4-19.6%	35-38.4%	20.6-22.7%[b]
Wilson [12]	2004-2008	England	189	-	-	-	-	-
Skogberg [13]	2004-2007	Finland	159 (149-168)	-	-	-	-	13%[b] 20.8 (19.2-21.6)[c]
Laupland [4]	1998-2005	Canada	101.2	-	-	-	-	13%[a]
Nielsen [14]	2000-2008	Denmark	215.7 (198-254)	-	99 (x100,000 person-years)	50 (x100,000 person-years)	66.7 (x100,000 person-years)	-
Holmbom [6]	2000-2013	Sweden	169-265	9.4-15.4	67%	-	33%	12.8%[b] 10.6% CA-BSI[b] 17.2% HA-BSI[b] 142-205[d]
Laupland [15]	2010-2015	Canada	117.8	-	48.6 (x100,000)	69.2 (x100,000)		10.6%[a] 12.7% HCA-BSI[a] 7.6% CA-BSI[a]
Mehl [16]	2002-2013	Norway	215	-	102 (x100,000 person-years)	85 (x100,000 person-years)	30 (x100,000 person-years)	32[c]
Buetti [17]	2008-2014	Switzerland	220 (211-240)	-	-	-	-	-
Rhodes [5]	2007-2014	Thailand	110	1.3	89%	-	9.9%	-
Kontula [1]	2004-2018	Finland	216 (150-309)	-	29%	71%		13%[b] 28[c]
Verway [18]	2017	Canada	150	-	17.1%	1.1%	81.8%	17%[b]

CA: community associated bloodstream infection. HCA: healthcare associated bloodstream infection. HA: hospital-acquired bloodstream infection. [a]In-hospital case fatality rate, [b]30-day case fatality rate, [c]mortality per 100,000 person-years, [d]mortality per 100,000 admissions.

In the case of the elderly population, a 30-day mortality rate of 22% and an annual mortality of 133 per 100,000 inhabitants have been described [47]. A 19.5% mortality rate has been estimated in nursing-home populations in a study from Spain [43].

Conclusion:

Bacteremia-associated mortality remains significatively high, but there is no convincing evidence of an increase in the last ten years.

3. HAS THE ETIOLOGY OF BACTEREMIA CHANGED IN RECENT DECADES?

E. coli and *S. aureus* are the most frequent microorganisms causing bacteremia [48, 49], with the incidence of *E. coli* [9] probably being higher nowadays (Table 2). The etiology varies according to the site of acquisition, such that *S. aureus* and *P. aeruginosa* are associated with the healthcare setting, whereas *S. pneumoniae* and *E. coli* are usually associated with community onset [9,43,48].

Although, according to epidemiological surveillance programs, there seems to be an increase in bacteremias caused by Gram-negative bacilli (GNB) in general and *E. coli* in particular [48,50,51], the available information is, again, very heterogeneous [11,13,43,48,51-54]. At our institution, between 2019-2021, the incidence of Gram-positive bacteremia has been reported to range between 13.8-17.2 episodes per 1,000 admissions, compared to 18.4-19.1 episodes per 1,000 admissions for GNB (unpublished data).

Table 2 **Most relevant microorganisms in bacteremia according to population-based studies published since 2010**

Reference	Period	Country	Most frequent etiology (in order of frequency)	Comment
Søgaard [11]	1992-2006	Denmark	*E. coli* *S. aureus* *S. pneumoniae*	- Significant increase in urinary and intra-abdominal infection. - Rise in *E. coli* episodes. - No change in the prevalence of Gram-positive infections.
Wilson [12]	2004-2008	England	*E. coli* (23%) CNS (16.9%) *S. aureus* (11.4%)	- *E. coli* increased by 33% during this period. - Increase in bacteremia due to GNB. - Decrease of *S. aureus*.
Skogberg [13]	2004-2007	Finland	*E. coli* (27%) *S. aureus* (13%) *S. pneumoniae* (9%)	- No significant changes in trends in Gram-positive and Gram-negative infections. - Important gender-associated differences.
Laupland [4]	1998-2005	Canada	*E. coli* (32x105 patient-years) *S. aureus* (15.5x105 patient-years) *S. pneumoniae* (10.2x105 patient-years)	- Only evaluates community acquired bacteremia.
Nielsen [14]	2000-2008	Denmark	*E. coli* (28.3%) *S. aureus* (12.3%) CNS (10%) *S. pneumoniae* (9.1%)	- Decrease in CNS bacteremia. - Decrease in *E. coli* bacteremia - Increase of enterococcal bacteremia.
Holmbom [6]	2000-2013	Sweden	*E. coli* *S. aureus* CNS	- Increase of *E. coli* and *S.aureus* by 126% and 77%, respectively.
Laupland [15]	2010-2015	Canada	*E. coli* (37%) *S. aureus* (16%) *S. pneumoniae* (6%)	- Increase in bacteremias due to *Enterococcus*, *Pseudomonas* and enterobacteria other than *E. coli* - Decresase in episodes due to *S. viridans* in nosocomial acquired bacteremias.
Mehl [16]	2002-2013	Norway	*E. coli* *S. pneumoniae* *S. aureus*	- Increase in bacteremias due to GNB and *E. coli*. - Decrease in Gram-positive bacteremia.
Buetti [17]	2008-2014	Switzerland	*E. coli* *S. aureus* GNB (other than *E. coli*)	- Rise of bacteremias due to *E. coli*, GNB and enterococci. - Stable incidence of *S. aureus*.
Rhodes [5]	2007-2014	Thailand	Community-acquired: *E. coli* *K. pneumoniae* *Burkholderia pseudomallei* Hospital-acquired: *E. coli* *Acinetobacter* spp. *K. pneumoniae*	- Performed in rural areas - Frequent isolation of ESBLs in *E. coli* and *Acinetobacter* spp. - No clear trend in MRSA
Kontula [1]	2004-2018	Finland	*E. coli* (29%) *S. aureus* (13%) CNS (8%), *Streptococcus B-hemolyticus* (8%) *S. pneumoniae*	- Significant increase in the incidence of bacteremia due to *E. coli* - Low proportion of BMR bacteremia, but with an upward trend, especially due to an increase in ESBL *E. coli*.
Verway [18]	2017	Canada	*E. coli* (26.9%) *S. aureus* (15.9%) CNS (9.2%) *Klebsiella* spp.(8.2%)	- No data on antibiotic susceptibilities to differentiate the burden of susceptible from resistant pathogens.

CNS: Coagulase-negative streptococci; GNB: Gram-negative bacilli; MRSA: Methicillin-resistant *S. aureus*.

The reduction in the incidence of *S. aureus* and *S. pneumoniae* can be attributed to prevention campaigns in hospitals and to the application of pneumococcal vaccines, respectively [48].

The evidence is more robust in terms of the evolution of resistance phenotypes, highlighting a stabilization or decline in the proportion of episodes caused by resistant Gram-positive pathogens, mainly methicillin-resistant *S. aureus* (MRSA) [48, 52, 55-57], and an increase in those caused by multidrug-resistant GNB (MDR-GNB) [48]. The increase of bacteremias produced by MDR-GNB coincides with the global expansion of ESBLs [58] and carbapenemases [59], with *Klebsiella pneumoniae* being the most frequent carbapenem-resistant Gram-negative bacteria causing bacteremia [60,61], although there is considerable geographical heterogeneity in the prevalence of these enzymes.

Conclusion:

***E. coli* seems to be the main cause of bacteremia at present, but current data do not allow to define a clear generalized change in the trend of Gram-positive and Gram-negative episodes. There is evidence pointing to a decrease in MRSA cases and a progressive increase in MDR-GNB, with differences according to the geographical area.**

4. WHAT ARE THE FUNDAMENTAL INDICATIONS AND THE IDEAL TIME TO OBTAIN BLOOD CULTURES?

Although the diagnosis of bacteremia depends directly on the results of blood cultures, the information offered by guidelines regarding the indications for their extraction is very limited, with imprecise information that has not been reviewed recently [62] or without specific recommendations in this regard [63] (Table 3). Moreover, clinical variables that usually guide the indication of blood cultures, such as fever or the presence of leukocytosis, do not correctly predict the presence of bacteremia in immunocompetent patients [64,65].

Different models that attempt to predict the presence of bacteremia have been proposed [66,67] (although they are not implemented in clinical practice nor are there currently data to evaluate their safety or cost-effectiveness [68]), as well as algorithms that propose the extraction of blood cultures according to the pretest probability of bacteremia [64,69], in an effort to obtain the maximum cost-effectiveness of the test.

In our opinion, it is not advisable to make a very restrictive use of blood cultures, given the critical importance of the diagnosis of bacteremia. Blood culture is an inexpensive, very specific and practically harmless test, patient's treatment and prognosis depend on its result, and it has epidemiological importance. We believe that the greater probability of obtaining false positive results can be overcome with a good extraction methodology, and that the associated costs are offset by the importance of the information provided by a positive result. Thus, we agree with the broader recommendations to obtain blood cultures of some societies [62,70], which also include the presence of fever, chills, hypothermia or sudden decay in neonates and the elderly or a clinical deterioration that justifies a hospital admission not justified by other causes.

The time of blood culture collection does not seem to be a decisive factor in its cost-effectiveness, which does not depend on its coincidence with fever spikes, which can occur within 1-2 hours of bacteremia [71,72]. Therefore, their extraction should not be postponed, especially in situations of sepsis. Although it is common to draw blood cultures with an arbitrary time separation of 10-30 minutes, Li et al. [73] did not observe increased performance when drawing blood cultures simultaneously or at different intervals over a 24-hour period. Unless attempting to document ongoing bacteremia for suspected endovascular infection, cultures can be drawn simultaneously [74].

Table 3 **Specific indications for blood culture extraction in clinical guidelines.**

Reference	Fever or hypothermia	Leukocytosis or leukopenia	Neutropenia	Clinical deterioration	Extreme ages
SEIMC [70]	Yes	Yes	No	Yes	Yes
ASM Cumitech [62]	Yes	Yes	Yes	Yes	Yes
CLSI [74]	Yes	Yes	Yes	No	No
IDSA, ASM [63]	No specific recommendations				

SEIMC: Spanish Society of Infectious Diseases and Clinical Microbiology; ASM Cumitech: American Society for Microbiology Cumitech 1C, Blood Cultures IV; IDSA, ASM: Infectious Diseases Society of America, American Society for Microbiology; CLSI: Clinical and Laboratory Standards Institute.

Conclusion:

The indications for obtaining blood cultures should be re-discussed and clarified by the scientific societies most concerned. We are not in favor of a restrictive use of blood cultures, nor do we believe that they should be limited to febrile episodes. Current predictive models are still based on speculation and not in the complex reality of daily clinical practice.

5. HOW MANY BLOOD CULTURES SHOULD BE TAKEN ROUTINELY AND WHAT VOLUME OF BLOOD SHOULD BE OBTAINED?

Assuming that a blood culture set is usually composed of two bottles per venipuncture (one for aerobic microorganisms and one for anaerobes), it is generally recommended that two to four sets be drawn, with at least 40-80mL of blood in total (i.e. 20-30mL of blood per set, with 10mL per bottle, depending on the manufacturer). Unfortunately, current guidelines are often not specific as to the volume and number of bottles that should comprise each set (Table 4) [62,63,70,74]. There is less evidence on the ideal volume to extract in the pediatric age, which depends on the age and weight of the patient [74,75].

Drawing enough volume of blood is the most important factor in improving the performance of blood cultures [76,77]. Since episodes of bacteremia have been documented with low concentrations of microorganisms (from 1-10 colony-forming units per milliliter) [78,79], there is evidence that the larger the volume of blood cultured, the higher the yield of the test [80-86], whose sensitivity can increase on the order of 3% per milliliter of cultured blood [87].

Despite its importance, it has been published that, in daily practice, up to 48% of blood cultures may have insufficient blood volume inoculated [88,89]. To determine whether sufficient volume has been drawn, visual analysis or weighing of bottles (before and after inoculation) in the laboratory [85,90] have been used, but these are tedious procedures. Therefore, tools based on different technologies have been developed to estimate the volume of cultured blood while incubating (BACTEC™ FX system, BacT/ALERT®VIRTUO™) with apparent good results [91-93]. However, these tools are currently poorly implemented and their validity and clinical impact should be studied in depth [94].

Regarding the specific number of blood cultures, the extraction of a single set should be avoided in all cases because of its low sensitivity and potential difficulties in the interpretation of results. In a study analyzing the value of drawing three sets, the omission of the third set would result in missing up to 7.5% of bloodstream infections [95]. Therefore, in our institution, the standard of care is the extraction of three sets of blood cultures routinely. Drawing more than three sets of blood cultures is not usually necessary.

Conclusion:

In adults whose hemodynamic situation allows it, three sets of blood cultures should be drawn, ensuring the collection of at least 60mL of blood.

6. IN PATIENTS IN WHOM BLOOD CULTURES ARE TAKEN, IS THERE ANY EVIDENCE ON THE DIAGNOSTIC VALUE AND THE ABILITY TO ADVANCE THE DIAGNOSIS OF BACTEREMIA OF OTHER SAMPLES OBTAINED SIMULTANEOUSLY WITH BLOOD CULTURES?

There is practically no evidence on the simultaneous extraction of samples in parallel to blood cultures to try to predict a positive result, but it is very common to receive blood cultures and other samples in parallel in the laboratory. Since it is necessary to wait for the growth of microorganisms in blood cultures to guide antibiotic treatment, it is worth considering whether the information provided by those other biological samples could be used.

In our institution, rapid urine testing has been useful in patients with simultaneous referral of blood and urine samples to the laboratory [96]. Our data show that the presence in urine of microorganisms visible with a Gram stain doubles the possibility of having positive blood cultures in the next hours and could provide guidance on the etiology. In addition, there is evidence that in patients with bacteremic urinary tract infections in whom the same pathogen is isolated in both samples, urine culture susceptibility results correctly predict

Table 4 **Recommendations on the number of blood cultures and volume of blood to be drawn in blood cultures.**

Reference	Year	Recommended volume	Recommended number of blood cultures
ASM Cumitech [62]	2005	20-30 mL per set	2-4 sets
CLSI [74]	2022	20-30 mL per set	2-3 sets
SEIMC [70]	2017	10-20 mL per set	2-4 sets
IDSA [63]	2018	20-30 mL per set	2-4 sets

ASM Cumitech: American Society for Microbiology Cumitech 1C, Blood Cultures IV; CLSI: Clinical and Laboratory Standards Institute; SEIMC: Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica; IDSA: Infectious Diseases Society of America.

blood culture results [97], allowing fast targeted antibiotic treatment. We couldn't find any studies analyzing other types of samples, which could help to identify and treat bacteremic infections sooner.

Conclusion:

There is a need for studies evaluating the contribution of a rapid examination of samples simultaneously submitted with blood cultures to microbiology departments.

7. COMPARED TO CONVENTIONAL BLOOD CULTURE IDENTIFICATION, IS THERE A POSITIVE CLINICAL IMPACT ASSOCIATED WITH THE USE OF MALDI-TOF AND OTHER RAPID TECHNIQUES?

Since the introduction of molecular and proteomic bacterial diagnostic methods, there is increasing evidence of the usefulness of these techniques. Rapid techniques (RTs) include tests such as PCR (polymerase chain reaction), MALDI-TOF MS (matrix-assisted laser desorption/ionization-time off light mass spectrometry) or PNA-FISH (peptide nucleic acid fluorescent in situ hybridization), which provide results in less than 2 hours. These techniques allow shortening the time needed to identify microorganisms from sample receipt compared to conventional blood culture analysis [98]. In a meta-analysis of their clinical impact [99], RTs are associated with significant decreases in mortality in the presence of an antimicrobial stewardship (AMS) team, but not in its absence. In our opinion, although they pose an important benefit, their actual clinical impact and cost-effectiveness has not yet been analyzed in depth.

MALDI-TOF systems are one of the most widespread tools in recent years. Most evidence on the clinical impact of this procedure comes from retrospective observational studies, and few studies have a prospective design or use a comparator (Table 5) [100-107]. The potential benefit of this technique, including lower mortality [101,103], is associated with the existence of an AMS team in most cases. For complex patients, such as critically ill or immunosuppressed, the evidence of efficacy for these techniques is lower [108].

The use of molecular tests, such as those based on PCR panels, have also been shown to be useful in achieving a shorter time to appropriate treatment and to guide de-escalation strategies [109,110].

Table 5 | **Studies analyzing clinical impact of MALDI-TOF with prospective design or using a comparator.**

Reference	Year	Design	Result	Comment
Vlek [100]	February-April 2010	Prospective comparative study.	- Reduction of species identification time by 28.8 hours. - Increase of 11.3% in the proportion of patients with appropriate treatment.	- Does not evaluate mortality or cost-effectiveness
Huang [101]	September- November 2012	Pre-post quasi-experimental study.	- Integration of MALDI-TOF with AMS team reduces microorganism identification time and time to effective treatment. - Mortality, length of stay and recurrent bacteremia were lower in the intervention group.	- Integration with AMS team.
Clerc [102]	2010	Prospective, observational	- MALDI-TOF had an impact on 35% of Gram-negative bacteremia cases.	- Single arm. - Does not evaluate hospital stay, clinical impact or mortality.
Perez. [103]	2012-2013	Quasi-experimental study.	- Reduced time to optimal and effective treatment, shorter hospitalization time, lower mortality and estimated lower associated costs.	- Integration with AMS team.
Verroken [104]	2013-2014	Prospective comparative study with two sequential intervention periods.	- Reduced time to identification and time to optimal treatment	- Integration with AMS team.
Lockwood [105]	2014	Prospective comparative study.	- Reduced time to identification and time to optimal treatment	- Integration with AMS team.
Osthoff [106]	2014-2015	Prospective, open-label, controlled clinical trial	- Reduced treatment of contaminated blood cultures - Shorter time to active treatment and admission to ICU in intervention group	- Integration with AMS team.
O'Donnell [107]	2015	Pragmatic, controlled clinical trial	- Shorter time to definitive treatment, shorter antibiotic therapy and shorter hospital stay	- Integration with AMS team.

AMS: antimicrobial stewardship

Conclusion:

There is insufficient evidence on the clinical impact of routine use of MALDI-TOF and other RTs in patients with bacteremia. The benefit lies in the combination of the technique results and rapid expert information to the clinicians by a specialized team.

8. ARE THERE ANY AUTOMATED INCUBATION SYSTEMS FOR BLOOD CULTURES CLEARLY SUPERIOR TO OTHERS?

The introduction of automated incubation systems and continuous monitoring of blood cultures led to a significant improvement in the efficiency of these processes compared to manual methods. Currently, the most widely used systems are BacT/Alert® VIRTUO™, BD BACTEC™ FX and, to a lesser extent in Europe, VersaTREK, with some differences among them.

The only study that directly compares these three systems is by Yarbrough et al. [111], using simulations of blood cultures under standardized conditions with the same inoculum for all three systems, also comparing time to positivity (TTP) in different volumes and culture media. In this study, VIRTUO detected the main causes of bacteremia earlier, although it also showed a higher TTP for *B. fragilis* and failures in the detection of *K. kingae*.

Although most studies seem to reflect lower TTP with VIRTUO for most microorganisms [112-114], they are performed under standardized conditions, using simulations, and the results are not uniform [115].

Conclusion:

The clinical impact of the different automatic growth detection systems in blood cultures has not been adequately studied and their advantages and disadvantages are usually deduced from laboratory tests.

9. SHOULD BLOOD CULTURE INCUBATION BE MAINTAINED FOR FIVE DAYS BEFORE BEING DISCARDED?

With the evolution of automated blood culture systems, a five-day incubation period is now recommended for most commercial systems [62,74] and incubation for seven or more days is not necessary [116]. However, certain microorganisms, such as mycobacteria and dimorphic fungi, may require prolonging this period [63].

Although infective endocarditis guidelines [117,118] do not recommend a specific incubation time and suggest that detection of fastidious microorganisms, such as the HACEK group (*Haemophilus*, *Aggregatibacter*, *Cardiobacterium*, *Eikenella*, and *Kingella*) may require prolonging this period, there is evidence that these could be detected with a five-day period with current systems [119,120]. The information on incubation time for *Brucella* spp. is more heterogeneous [121-123], but it is currently assumed that the standard five-day period is sufficient.

The evidence is not favorable to extending the incubation period generally, although some authors propose lengthening this period in specific circumstances, such as men carrying prosthetic material at risk of *C. acnes* infection [124-126].

On the other hand, optimizing blood culture incubation time may be an important factor in avoiding unnecessary antimicrobial treatments, reducing laboratory workload, and improving antibiotic policies. In the study by Ransom et al. [127], a four-day period was sufficient to detect the vast majority of microorganisms, and only 0.11% of blood cultures were positive after four days of incubation. There is already some evidence in favor of reducing this period below five days [26,127-130], although it comes from retrospective studies using different culture systems and media, as reflected in Table 6.

Conclusion:

A five-day incubation period is sufficient to detect the vast majority of microorganisms, and only in individual cases should its extension be considered. In our opinion, there is insufficient data to recommend a reduction of the incubation time below five days at present.

10. CAN TIME TO POSITIVITY OF BLOOD CULTURES BE A PREDICTOR OF ETIOLOGY OR PROGNOSIS OF BACTEREMIA?

Time to positivity (TTP) is defined as the time from start of incubation to the detection of growth by an automated system, and it provides indirect information on the bacterial inoculum: theoretically, the higher the bacterial load, the higher the growth rate and the lower the TTP. Its main use at present is in the diagnosis of catheter-related bacteremia, and other potential uses of this determination are being investigated, as a marker of severity and predictor of the etiology of bacteremia, or to guide de-escalation treatments; but the evidence is currently contradictory and heterogeneous (Table 7).

A recent meta-analysis concludes that a short TTP is a prognostic marker associated with mortality and septic shock, applicable for most analyzed species except *Candida* spp., but it has substantial limitations [131]. Although there is evidence in favor of TTP being associated with worse prognosis in bacteremia due to *S. aureus* [132,133], *E. coli* [134], *S. pneumoniae* [135], *P. aeruginosa* [136], or *K. pneumoniae* [137], not all cases have been able to demonstrate this association between TTP and mortality [138]. Furthermore, a linear relationship is not always found, with a worse prognosis being described with both short and long TTP for *S. aureus* [139], and with long TTP for *C. albicans* [138,140].

A possible association between TTP and etiology has been described for *S. pneumoniae*, beta-hemolytic streptococci, *E. coli*, *Klebsiella* spp. and *S. aureus* [141], as well as for *P. aeruginosa* [142]. TTP has also been associated with the presence of endocarditis in cases of bacteremia by *S. aureus* [143], *E. faecalis* [144] and *A. baumanii* [145], but not by non-beta-hemolytic streptococci [146].

Table 6 Summary of a sample of studies proposing a reduction of the 5-day incubation time

Reference	Year	Country	Design	System	Number of samples	Results and comments
Ransom [127]	2018-2019	United States	Retrospective	- BacT/Alert Virtuo (FA Plus and FN plus bottles)	158,710	- No benefit is observed in prolonging incubation longer than 4 days, including simulation with HACEK group.
Sepulveda [26]	January-March 2020	United States	Retrospective	- Bactec FX - VersaTrek	88,201	- No benefit is observed in prolonging incubation for more than 4 days, detecting 98% of microorganisms. - Conducted with a large proportion of COVID-19 patients, with a possible low proportion of bacteremia.
Bourbeau [128]	N/A (30 months)	United States	Retrospective	- BacT/Alert (FA and FN bottles)	35,500	- 3 days may be sufficient for detection of bacteria and fungi. - Use a specific type of media only.
Bourbeau [129]	N/A (18 months)	United States	Retrospective	- BacT/Alert (FAN bottles)	17,887	- 3 days may be sufficient for detection of bacteria and fungi. - Use a specific type of media only.
Doern [130]	1994-1995	United States	Retrospective	- Difco ESP	7,362	- No decrease in the detection of microorganisms is observed when decreasing to 4 days, except for *K. pneumoniae*.

Table 7 Representation of the heterogeneity of results and methodology of a selection of recent studies on the usefulness of time to blood culture positivity as a predictor of severity.

Reference	Country	Type of study	Result	Comment
Hsieh [131]	Multinational	Meta-analysis	A short TTP was associated with higher mortality and septic shock in some bacterial species, but not in *Candida* spp.	- Notable biases, presence of heterogeneity, mixing of pediatric and adult populations, important confounding factors not assessed, meta-regression analysis not significant.
Hamilton [138]	United Kingdom	Prospective multicenter cohort study.	TTP not associated with mortality except in *Candida* spp. (elevated TTP) and possibly in streptococci.	- More methodological soundness than most studies (includes time to incubation). - Limitations: does not assess time to effective treatment, small samples in some groups.
Siméon [143],	France	Prospective multicenter cohort study.	A short TTP is related to mortality and to the presence of endocarditis in *S. aureus* bacteremia.	- Some limitations: small sample, blood culture systems used, does not analyze blood culture volume.
Kim [139]	Canada	Retrospective study	Elevated TTP is associated with mortality in *S. aureus* bacteremia.	- Some limitations: retrospective, does not have detailed clinical information, does not analyze foci of infection, does not analyze antibiotic treatment.
Oldberg [144]	Sweden	Retrospective observational study	No association was observed between TTP with mortality or the presence of endocarditis in *E. faecalis* bacteremia.	- Some limitations: retrospective study, transesophageal echocardiogram not performed in all patients, does not include patients under treatment, does not analyze blood culture volume.

TTP: time to positivity

The use of TTP has important limitations (such as different definitions of what is considered a short or long TTP) and is related to multiple confounding factors (such as the volume of blood drawn or the time between collection and start of incubation) that have not been analyzed in most studies. In addition, variability of TTP depending on blood culture incubation systems has also been described [111]. The heterogeneity of the literature, as well as the absence of evidence on its real clinical impact, limit the use of TTP in daily clinical practice, although it is likely that it may be useful in the future.

Conclusion:

Currently, the use of TTP to predict severity and etiology of bacteremia is controversial, and it requires a careful evaluation.

11. IN WHICH CASES ARE FOLLOW-UP BLOOD CULTURES INDICATED AFTER INITIATING APPROPRIATE TREATMENT?

Follow-up blood cultures (FUBC) are recommended in cases of infective endocarditis (IE) [117,118] or endovascular infection (such as pacemaker infection, catheter infection or septic thrombophlebitis) [147], as well as candidemia [148] or bacteremia due to *S. aureus* or *S. lugdunensis*. Their extraction is also reasonable in other clinical circumstances, such as patients at high risk of endovascular infection, suspected central nervous system infection or in areas difficult to access for antimicrobials, or in the event of poor evolution despite appropriate treatment, among others.

In the case of Gram-positive microorganisms, there is evidence that justifies the extraction of FUBC in the presence of *S. aureus* bacteremia [149] due to its high virulence and capacity to produce persistent bacteremia. The same recommendations are made for *S. lugdunensis* [150]. Evidence for the rest of Gram-positives is scarce. FUBCs have limited utility in streptococcal bacteremia, and their collection should be limited in patients at low risk for deep infections, persistent bacteremia or endovascular infection [151].

The usefulness of FUBC in Gram-negative bacilli bacteremia has been evaluated in multiple studies recently [152], with very heterogeneous results. There are several cases where FUBC would have little value due to the low probability of obtaining positive cultures, which was estimated to range between 5-10.9% [153-155]. However, these studies have important limitations, including small heterogeneous populations [153,154], or assessing only episodes produced by *K. pneumoniae* [156] or bacteremias with urinary tract focus [157]. In contrast, in other studies the cost-effectiveness of FUBC reached 38.5% [158] and their collection was associated with lower mortality [158,159]. Some tools have been proposed to identify those patients with GNB bacteremia at higher risk in whom FUBC should be performed [155,159,160].

In some cases such as *Pseudomonas* spp., FUBC are usually negative if adequate focus control is obtained, but these are small series [161], and there is little evidence about their usefulness in bacteremia due to other microorganisms such as *Stenotrophomonas* or *Acinetobacter* [69].

Conclusion:

Follow-up blood cultures are recommended in bacteremia due to *S. aureus*, *S. lugdunensis*, and candidemia, or in cases of uncontrolled infection. In all other cases, the evidence is controversial.

12. IN PATIENTS WITH AN ENDOVASCULAR CATHETER AND NO CLINICAL SUSPICION OF CATHETER-RELATED BLOODSTREAM INFECTION, CAN BLOOD CULTURES BE DRAWN FROM THE CATHETER?

When obtaining blood cultures, it is recommended that blood should be drawn by direct venipuncture and extraction from the catheter should be avoided [70,77,162], unless catheter-associated infection is suspected. However, in clinical practice it is common to draw blood from the catheter in certain clinical scenarios (such as patients with poor peripheral venous access or with multiple episodes of blood collection), or to draw one set of blood cultures from the catheter and another from venipuncture, because it is a less difficult and uncomfortable process for the patient.

The recommendation not to obtain blood cultures from the catheter is based on the results of studies that point to higher false positive rates in blood cultures obtained from the catheter. In a systematic review and meta-analysis [162], all nine studies analyzed offer lower contamination rates with extraction via venipuncture.

In a systematic review of six studies [163], blood cultures obtained from the catheter have higher sensitivity and negative predictive value than those obtained by venipuncture, but also have lower specificity and positive predictive value. According to this study, out of 1,000 patients whose blood cultures are obtained from a catheter, 8 more cases of bacteremia would be detected than if they were obtained by venipuncture (103 versus 96), but 59 cases would also be incorrectly diagnosed (84 versus 25). Its higher sensitivity makes some authors consider obtaining at least one set of blood cultures from the catheter [163,164].

Conclusion:

Blood cultures should not be drawn from an endovascular catheter unless catheter-associated infection is suspected. Their extraction from the catheter in certain circumstances requires a very careful interpretation of results.

13. WHAT IS THE DIAGNOSTIC APPROACH IN PATIENTS WITH ENDOVASCULAR CATHETERS AND SUSPECTED CATHETER-RELATED BLOODSTREAM INFECTIONS (CR-BSI)?

In case of suspected CR-BSI, the latest SEIMC guidelines recommend obtaining at least two sets of blood cultures, one from peripheral venipuncture and one from the catheter, drawing blood from all lumens in case of multi-lumen catheters [165], while other guidelines do not specify this recommendation [166]. There are several studies that support obtaining blood from all catheter lumens [167,168], being equally effective the extraction from several lumens for the same culture as the extraction of a culture from each lumen [169]. In one of these studies, performed at our insti-

tution [167], if two cultures for triple-lumen catheters were eliminated, up to 37.3% of CR-BSI episodes would have been missed.

Indirect markers, such as differential time to positivity (TTP), or quantitative methods can be used to diagnose CR-BSI. Differential TTP has been implemented as the main diagnostic tool, and positivity of blood cultures obtained from a catheter 120 minutes or more apart from a culture obtained from peripheral puncture is highly suggestive of CR-BSI. The use of this cutoff point has a sensitivity and specificity of 72-96% and 90-95%, respectively [170,171]. However, there is uncertainty about its usefulness in critically ill patients [172] and in the case of certain microorganisms, such as *S. aureus* [173,174] or *Candida* spp. [175]. Therefore, the status of the host and the microorganism causing the infection must be considered, and a negative result does not exclude the diagnosis.

The reference quantitative methods are based on lysis-centrifugation procedures, being suggestive of CR-BSI if a 3-fold higher colony count is observed in the sample obtained from the catheter. Although it offers good results, it is a relatively complex and laborious technique, and it requires the sample to be processed in 20-30 minutes from blood inoculation [165], so its use is infrequent.

Conclusion:

If CR-BSI is suspected, blood culture collection from all catheter lumens should be taken in parallel with blood from peripheral veins. A differential TTP ≥120 minutes in blood cultures taken through the catheter lumen and peripheral veins is highly suggestive of catheter-related infection of bacterial etiology.

14. SHOULD A BLOOD CULTURE REQUEST CONSTITUTE A SEPSIS ALERT?

Prompt recognition of sepsis and early use of appropriate antibiotic therapy have been shown to reduce mortality from sepsis [176]. Assuming that a request for blood cultures implies a suspicion of bacteremia and a potential septic episode, it is pertinent to ask whether the simple request for blood cultures should in itself constitute a sepsis alert in an institution.

It is noteworthy that the literature is practically non-existent regarding the potential implied value of a blood culture request in itself. Currently, both clinical guidelines and current recommendations on the implementation of the sepsis code only recommend early blood culture collection [176,177].

We are only aware of one study analyzing this aspect [178]. In this prospective study, conducted at our institution, a telephone interview from the Microbiology department after receipt of blood cultures was generally well received and was associated with better recognition of sepsis, optimization of antimicrobial treatment and lower associated costs. We observed that medical and nursing staff outside the intensive care unit tend to underestimate the presence of sepsis, even if blood cultures have been requested.

In our opinion, not only should attention be paid to a positive blood culture result, but its request alone should be considered an alert for sepsis. Further studies are needed on the appropriateness of implementing a sepsis alert from the Microbiology Department upon receipt of a simple blood culture request.

Conclusion:

A request for blood cultures should constitute a sepsis alert. A phone call from the Microbiology Department can contribute to the better recognition and clinical management of sepsis.

15. WHAT IS THE BEST METHOD OF TRANSMITTING INFORMATION FROM THE MICROBIOLOGIST TO THE PHYSICIAN IN CHARGE OF THE PATIENT IN THE EVENT OF A POSITIVE BLOOD CULTURE RESULT?

Obtaining a positive blood culture result can have a major clinical impact. There are studies on the usefulness of the preliminary information provided by the Gram stain [179]. However, it is surprising that the best way to deliver this information has not been analyzed in depth.

In a clinical trial [180], communication of results through written reports in the patient's medical record, and oral communication at the bedside along with clinical advice, were significantly associated with a higher proportion of appropriate treatment days and lower economic costs with respect to simply issuing a report, although no associated shorter hospital stay or mortality could be demonstrated.

Although controversial and scarce, there is evidence in favor of a presential assessment by the infectious diseases specialist over a telephone assessment [181,182]. In our opinion, the person and method of transmitting blood culture results is also relevant. Although the ideal method probably involves active presential communication by an infectious disease specialist providing clinical support, studies directly comparing the clinical impact and cost-effectiveness of different procedures of communicating this information are lacking.

Given that each day of delay until definitive blood culture information is available is associated with an increase in mortality of 1.2 times per day [180], analyzing the clinical impact of different methods of transmitting information to optimize this process would influence the management of patients with bacteremia and should be considered an issue in future research.

Conclusion:

The limited evidence available suggests that there is a clinical benefit associated with the active communication of results of a positive blood culture, either orally or in writing, compared to only issuing a conventional report.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Valoración de dos pruebas inmunocromatográficas para la detección de anticuerpos frente a SARS-CoV-2

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RESUMEN

Introducción. Las pruebas serológicas han resultado una herramienta de gran valor en el transcurso de la pandemia por SARS-CoV-2, tanto en la detección, apoyando a los métodos moleculares, como en el seguimiento de la respuesta inmune, provocada por la vacunación o por la infección natural. Dentro de todas estas técnicas, las pruebas rápidas resultan interesantes por su fácil uso, rápida respuesta y bajo coste económico.

Material y métodos. Se evaluaron dos técnicas inmunológicas diferentes: Realy Tech y Mikrogen Diagnostik *recom*Line SARS-CoV-2 IgG. Como técnicas de referencia se utilizaron pruebas automatizadas: SARS-CoV-2 IgG II Quant antibody test y SARS-CoV-IgG assay, ambos de Abbott Diagnostics.

Resultados. Mikrogen Diagnostik fue el que, en conjunto, ofreció mejores resultados (S=0,985; E=0,839). Las dos técnicas mostraron buenos valores predictivos positivos, pero los valores predictivos negativos de Realy Tech estuvieron lejos de lo deseable.

Conclusiones. Mikrogen Diagnostik *recom*Line SARS-CoV-2 IgG ofreció muy buenos resultados en la detección de anticuerpos frente a SARS-CoV-2 y podría ser utilizada como alternativa a las técnicas automatizadas.

Palabras clave: COVID. SARS-CoV-2. Serología. Prueba rápida.

Evaluation of two immunocromatographic tests for the detection of antibodies against SARS-CoV-2

ABSTRACT

Introduction. Serological tests have been a valuable tool during the SARS-CoV-2 pandemic, supporting molecular methods for detection, and monitoring the immune response, caused by vaccination or by natural infection. Within all these techniques, rapid tests are interesting due to their ease of use, rapid response and low cost.

Methods. Two different immunological techniques were evaluated: Realy Tech and Mikrogen Diagnostik *recom*Line SARS-CoV-2 IgG. SARS-CoV-2 IgG II Quant antibody test and SARS-CoV-IgG assay, both from Abbott Diagnostics, were used as reference techniques.

Results. Mikrogen Diagnostik *recom*Line SARS-CoV-2 IgG shows the best results (S=0.985; E=0.839). Three techniques offered good positive predictive values, but Realy Tech and Healgen negative predictive values left to be desired.

Conclusions. Mikrogen Diagnostik *recom*Line SARS-CoV-2 IgG showed good results in the detection of antibodies against SARS-CoV-2 and could be used as an alternative to automated techniques

Keywords: COVID. SARS-CoV-2. Serology. Rapid test.

INTRODUCCIÓN

A finales de diciembre de 2019 las autoridades sanitarias de la ciudad de Wuhan (Hubei, China), informaron de una serie de casos de neumonía atípica producida por un nuevo coronavirus. A este nuevo coronavirus se le dio el nombre de SARS-CoV-2, y a la enfermedad que produce se la denominó COVID-19.

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En muy poco tiempo la infección por este coronavirus, que se inició como a un brote aislado, pasó a constituir un problema sanitario de carácter mundial, debido a su rápida capacidad de transmisión a través de secreciones respiratorias [1].

En la mayor parte de casos, la infección por SARS-CoV-2 cursa de manera asintomática o provocando un cuadro pseudogripal. Sin embargo, este nuevo coronavirus también puede ocasionar cuadros de neumonía grave, síndrome de dificultad respiratoria aguda (SDRA), tromboembolismo pulmonar (TEP) y otras complicaciones que pueden conducir a un desenlace fatal para el paciente [2].

La detección de este virus se realiza mayoritariamente por técnicas moleculares y, entre ellas, el principal método diagnóstico, es la RT-PCR (reacción en cadena de la polimerasa con retrotranscripción) [3]. Esta técnica resulta muy útil para el diagnóstico de la infección aguda, y, por tanto, para el rastreo y control de brotes. Junto a las pruebas moleculares se han desarrollado distintas pruebas serológicas para la detección de anticuerpos específicos, que resultan interesantes tanto para el diagnóstico a posteriori de pacientes que han pasado la infección, como para evaluar el grado de protección frente a SARS-CoV-2 [4].

En la actualidad, la principal estrategia para romper la cadena de transmisión y terminar con la pandemia causada por el SARS-CoV-2, es la vacunación mundial. Es por ello que ha aumentado en gran medida la utilización de test serológicos, que son de gran utilidad para la determinación de anticuerpos vacunales. Los test serológicos son técnicas sencillas que ofrecen resultados con rapidez y bajo coste económico [5].

En este estudio evaluamos una prueba inmunocromatográfica de flujo lateral y un test de inmunoanálisis en línea, determinando su sensibilidad y especificidad en tres cohortes de pacientes: la primera integrada por individuos sin vacunar, que no habían pasado COVID; la segunda cohorte, por pacientes a los que se les había detectado la infección, bien por estudios serológicos, bien por PCR; y, el tercer y último grupo, integrado por individuos vacunados con distinto nivel de respuesta a la vacuna. Se evaluó, asimismo, la capacidad de estas técnicas como herramienta de diagnóstico en aquellos laboratorios en los que no se disponga de los medios ni de la tecnología para el diagnóstico molecular.

MATERIAL Y MÉTODOS

Muestras y diseño del estudio. Se realizó un estudio retrospectivo, analizando 98 muestras de suero de la colección del Hospital Clínico San Carlos. Las muestras se dividieron en tres grupos distintos: en el grupo A se incluyeron 25 sueros de pacientes que, a priori, no habían pasado enfermedad COVID-19; en el grupo B, 23 sueros de pacientes diagnosticados de infección por SARS-CoV-2 mediante serología o RT-PCR y en el grupo C, 50 sueros de individuos vacunados con distinta respuesta inmunológica. No se aplicaron criterios de exclusión.

La inclusión en cada uno de los grupos se realizó acorde a los resultados obtenidos mediante las técnicas de referencia utilizadas en la rutina del laboratorio de Microbiología.

La determinación cualitativa de IgG anti-NP se llevó a cabo mediante SARS-CoV-IgG assay de Abbott Diagnostics. Las muestras se consideraron positivas cuando el ensayo daba valores >1.4 tal y como recomienda el fabricante. Esta prueba posibilitó la distribución entre los grupos A y B.

Los niveles de anticuerpos frente a la región RBD del SARS-CoV-2 se determinaron mediante la técnica SARS-CoV-2 IgG Quant assay (Abbot Diagnostics) en un equipo ARCHITECT

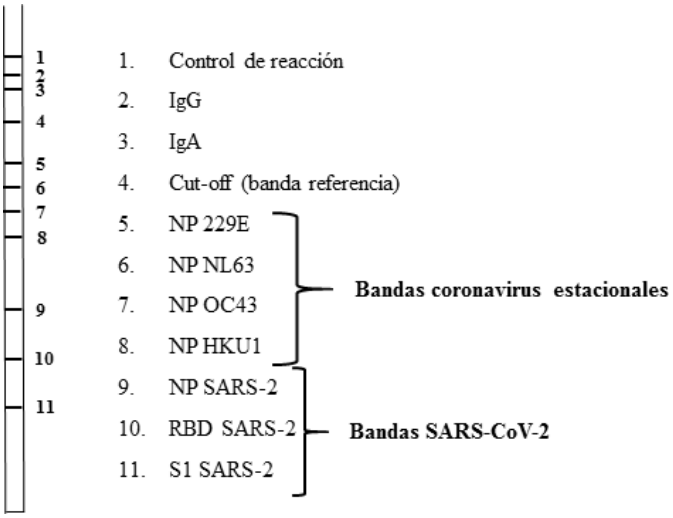

Figura 1 | **Esquema de bandas de Mikrogen Diagnostik recomLine SARS-CoV-2**

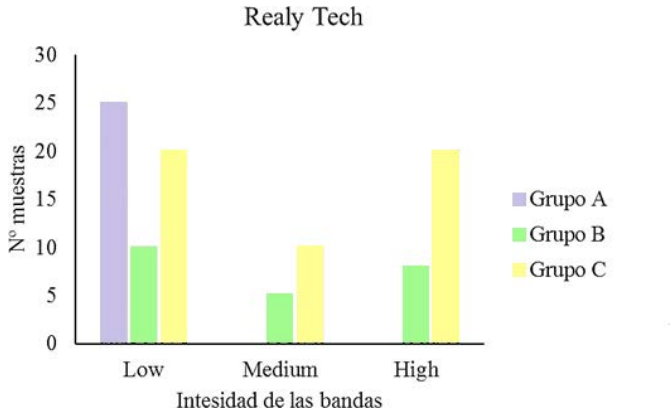

Figura 2 | **Distribución de los resultados obtenidos por Realy Tech en función del grupo seleccionado y de la intensidad de las bandas.**

Tabla 1 | **Distribución de los resultados obtenidos para las bandas anti N, anti RBD y anti S1en la técnica Mikrogen Diagnostik recomLine SARS-CoV-2.**

	Bandas SARS-CoV-2			
	0 bandas	1 banda	2 bandas	3 bandas
Grupo A	22	1	1	1
Grupo B	3	1	0	18+(1)*
Grupo C	0	0	31+(3)*	4+(12)*

*Positivo débil

i2000. Los resultados se expresaron como Unidades Arbitrarias por mililitro y, tal y como recomienda el fabricante, se consideraron positivos todos los valores superiores a 50 UA/mL. Esta técnica se utilizó para valorar el nivel de respuesta a la vacunación. Este último ensayo también se utilizó en aquellos casos en los que se observaron discrepancias atribuibles a la sensibilidad de la prueba cualitativa.

Técnicas inmunocromatográficas. Se evaluaron dos técnicas inmunocromatográficas diferentes. Realy Tech es una prueba inmunocromatográfica de flujo lateral que detecta anticuerpos frente a la proteína S de SARS-CoV-2. Permite evaluar de manera semicuantitativa el nivel de respuesta, distinguiendo entre respuesta alta (High), respuesta media (Medium) y respuesta baja (Low), en función de la intensidad de las bandas.

Mikrogen Diagnostik *recom*Line SARS-CoV-2 IgG, un inmunoanálisis de línea (LIA) que permite la detección de anticuerpos específicos frente a la nucleoproteína (NP), la región de unión al receptor de la proteína de la espícula (RBD) y la región S1 de esta misma proteína. Asimismo, la tira de detección cuenta con bandas que permiten la identificación de anticuerpos frente a coronavirus estacionales (HcCoV: 229E, NL63, OC43, HKU1) (Figura 1).

Ambas técnicas inmunocromatográficas se llevaron a cabo siguiendo las instrucciones de uso de los fabricantes. La lectura de los resultados se realizó mediante evaluación visual, siendo revisada al menos por dos personas diferentes.

Análisis estadístico. Se calculó la sensibilidad, especificidad, valor predictivo positivo (VPP) y valor predictivo negativo (VPN) siguiendo sus definiciones y utilizando como técnica de referencia el ensayo cuantitativo de Abbott. El análisis de los datos se realizó mediante el software estadístico IBM SPSS Statistics v.26

RESULTADOS

Realy Tech. Del total de muestras analizadas, 55 (56,1%) dieron señal baja (Low), 15 (15,3%) dieron bandas de intensidad media (Medium) y 28 (28,6%) dieron señal alta (High).

Se pueden observar diferencias entre los resultados en función del grupo que se considere (Figura 2). En el grupo A, las 25 (100%) muestras dieron bandas de intensidad baja. En el grupo B, 10 (43,5%) de las muestras dieron señal baja, 5 (21,7%) dieron bandas de intensidad media y 8 (34,8%) dieron bandas de intensidad alta. En el grupo C se obtuvieron 20

Tabla 2 **Valores de sensibilidad y especificidad de las técnicas estudiadas**

	Sensibilidad	Especificidad	VPP	VPN
Realy Tech	0,627	0,968	0,977	0,545
Mikrogen Diagnostik *recom*Line S	0,985	0,839	0,93	0,963

VPP: Valor predictivo positivo, VPN: Valor predictivo negativo

(40%) muestras con señal alta, 10 (20%) con señal media y las 20 (40%) restantes dieron intensidad baja. Se analizó la posible relación entre la intensidad de las bandas obtenidas y los niveles de anticuerpos anti-RBD para cada una de las muestras. Los resultados obtenidos indicaron que no existía ningún tipo de relación entre ambos análisis. Así, el rango de IgGs anti-RBD de las muestras con bandas de baja intensidad iba desde 46,3 hasta 61894,8 UA/mL; el de las muestras con bandas de intensidad media desde 325,5-1879,1 UA/mL y el de las muestras con bandas de intensidad alta desde 0 hasta 80000 UA/mL.

Mikrogen Diagnostik; recomLine SARS-CoV-2 IgG. Mediante esta técnica se evaluaron tanto la respuesta frente a SARS-CoV-2 como la respuesta frente a la nucleoproteína de cuatro coronavirus estacionales (HcCoV: 229E, NL63, OC43, HKU1),

De las 98 muestras analizadas solo 2 (2,04%) fueron negativas para los cuatro coronavirus estudiados. Las 96 muestras restantes (97,96%), tuvieron señal con, al menos una de las cuatro bandas asociadas a los coronavirus estacionales. La distribución de los resultados fue la siguiente: 2 muestras (2,04%) negativas para todos los coronavirus estacionales, 1 muestra (1,02%) con una banda positiva; 5 muestras (5,1%) con dos bandas positivas; 7 muestras (7,14%) con tres bandas y 83 muestras (84,7%) con señal en las cuatro bandas. La cepa HcCoV-NL63 fue la que se detectó en un mayor número de muestras (95,92%) seguida de HcCoV-OC43 (93,88%), 229E (92,86%) y HKU1 (89,8%).

No se observaron diferencias significativas entre los distintos grupos estudiados.

La respuesta frente a SARS-CoV-2, sin embargo, sí mostró mayor variabilidad en cada grupo.

En el grupo A, 22 sueros (88%) no tuvieron reacción en ninguna de las bandas del SARS-CoV-2. Las 3 muestras restantes (12%) presentaron señal en alguna de las bandas correspondientes a las proteínas específicas de SARS-CoV-2: una de ellas dio señal frente a las tres proteínas (NP, RBD, S1); otra solo frente a NP y la muestra restante dio positivo frente a S1 y RBD. Estos tres sueros habían resultado negativos en el análisis cualitativo de SARS-CoV-2 IgG de Abbott (IgG anti-NP). Con objeto de resolver estas discrepancias se analizaron las tres muestras con el test cuantitativo de Abbott, para determinar la posible presencia de anticuerpos frente a la proteína S. La muestra positiva para las tres proteínas de SARS-CoV-2 incluidas en el ensayo Mikrogen Diagnostik; *recom*Line SARS-CoV-2 IgG, tuvo un valor claramente positivo con el ensayo de Abbott, no así las dos muestras restantes.

En el grupo B, 19 muestras (84%) fueron positivas para las tres proteínas de SARS-CoV-2 estudiadas en el test. Una muestra (4%) mostró reacción en la banda NP pero no con las correspondientes a las proteínas S1 y RBD; esta muestra no se tuvo en cuenta para los estudios estadísticos posteriores. Las 3 muestras restantes (12%) fueron negativas para las tres bandas de proteínas. Estas 3 muestras fueron también negativas por el ensayo cualitativo de Abbott. La infección de estos pacientes se había confirmado por RT-PCR en el momento en el que acudieron al hospital con clínica compatible con COVID. Para comprobar los resultados negativos del test inmunocromatográfico, las tres muestras se analizaron por el ensayo cuantitativo de Abbott, dando resultado negativo, lo que parece indicar un resultado falso positivo en el momento del diagnóstico. La muestra positiva para NP y negativa para las otras dos proteínas incluidas en las tiras del inmunoanálisis de línea fue claramente positiva por el test cualitativo de Abbott.

En el grupo C, todas las muestras procedentes de individuos vacunados (100%), mostraron bandas positivas de mayor o menor intensidad frente a la proteína S1 y la RBD, incluso aquellas en los que el ensayo cuantitativo de Abbott había dado valores por debajo del punto de corte establecido por la casa comercial.

La banda correspondiente a la proteína NP, que solo debería positivizar en aquellos individuos que hubiesen pasado la infección, dio valores positivos (de intensidad variable según la muestra) en 16 (32%) de las 50 muestras incluidas en este grupo. En cuatro de estas 16 muestras, la banda NP-SARS-2 mostró una intensidad claramente superior a la de la banda control. El análisis por SARS-CoV-IgG assay de Abbott Diagnostics reveló que estas cuatro muestras eran claramente positivas, indicando que correspondían a individuos que habían pasado la infección por COVID. De las 12 muestras restantes, 4 dieron valores claramente negativos (<0.1) por la técnica de referencia, los otros 8 dieron valores negativos pero cercanos al punto de corte.

La tabla 1 es un resumen de los resultados obtenidos mediante está técnica.

Sensibilidad y especificidad de las técnicas evaluadas. Los datos estadísticos de las pruebas evaluadas se recogen en la tabla 2.

Realy Tech mostró el peor resultado de sensibilidad de las técnicas a estudio; por el contrario, los datos de especificidad fueron los más favorables. La sensibilidad más alta se obtuvo con Mikrogen Diagnostik *recom*Line (con las distintas bandas de la proteína S). En general, las dos técnicas obtuvieron buenos valores predictivos positivos, si bien, salvo en el caso de Mikrogen Diagnostik *recom*Line, los valores predictivos negativos fueron peores de lo esperado.

DISCUSIÓN

La necesidad de control de la pandemia de COVID-19 ha llevado al desarrollo de numerosas técnicas de diagnóstico y seguimiento, con el fin de facilitar el manejo de la infección por este nuevo coronavirus. Dentro de estas técnicas, las pruebas rápidas han jugado un papel esencial debido a su sencillez y fácil manejo, así como su menor coste económico y la rapidez de resultados. Aunque actualmente, en nuestro entorno, la mayor parte de la población ha sido vacuna, infectada o ambas, no podemos olvidar que existen otros lugares en las que el grado de vacunación es mucho menor y en los que no disponen de los recursos o la tecnología necesarias para implementar técnicas automatizadas. Por ello, contar con una técnica de las características de las que se evalúan, puede seguir siendo de gran interés.

Los principales usos de las pruebas serológicas en la infección por SARS-CoV-2 serían la detección de la infección pasada, seguimiento de la respuesta inmune tras la vacunación y como complemento a las técnicas moleculares en el diagnóstico de la infección.

La capacidad de las pruebas serológicas de detectar infecciones pasadas tiene mucha utilidad tanto a nivel epidemiológico (West *et al.* [6]) como para el diseño de vacunas (Galipeau *et al.* [7]). Este estudio es un ejemplo del papel que puede desempeñar la serología para la detección de pacientes asintomáticos.

Son muchas las plataformas tecnológicas que han desarrollado test rápidos para la detección de anticuerpos frente a SARS-CoV-2. Esto hace que sean necesarios estudios que evalúen la utilidad de los mismos en la práctica diaria, por comparación con una técnica de referencia estandarizada y validada. Varios autores (Nicol *et al.* [8] Montesinos *et al.* [9]*)* han realizado trabajos de este tipo, valorando distintos inmunoensayos por comparación con técnicas de referencia. En la mayoría de los casos de han obtenido muy buenos resultados en la detección de anticuerpos frente al SARS-CoV-2 a los 14 días del inicio de los síntomas de COVID-19. En nuestro caso, las técnicas que se han evaluado son más simples, pero, salvo con las bandas frente a la espícula de Mikrogen diagnostik *recom*Line, los resultados obtenidos son menos prometedores.

Aunque existen diferencias en función del grupo de pacientes considerado, las dos pruebas de flujo lateral han mostrado una sensibilidad y especificidad mejorable. En el caso de Realy Tech debe resaltarse, además, la imposibilidad de discernir visualmente entre el resultado negativo y el "low".

En cuanto a su papel en la respuesta a la vacunación, todos los individuos del grupo de vacunados de nuestro estudio mostraron reacción en las bandas de la proteína S con la técnica de Mikrogen diagnostik *recom*Line. Incluso dos pacientes con valores inferiores al punto de corte establecido con la técnica de referencia (50 UA/mL) mostraron una clara señal en las bandas correspondientes a RBD-SARS-2 y la S1-SARS-2. Sin embargo, no ha sido posible establecer una correlación clara entre los valores obtenidos por la técnica de referencia y la intensidad de las bandas del test de inmunoanálisis en línea.

Diversos autores han resaltado el papel que este tipo de técnicas tiene como complemento de los ensayos moleculares. Así Turbett *et al.* [10] pusieron de manifiesto que la serología es una herramienta que complementa la detección del ARN viral. La detección de ARN es muy sensible en los primeros días después del inicio de la sintomatología, pero esta efectividad cae por debajo del 50% después de 1 semana; por lo contrario, los anticuerpos totales son detectables en el 50% de los pacientes después de la primera semana, y la sensibilidad supera el 90% después de 2 semanas. Por ello las pruebas serológicas resultan adecuadas para respaldar el diagnóstico de infección por SARS-CoV-2.

Los resultados de este estudio concuerdan con estas afirmaciones ya que mediante el análisis serológico se ha podido detectar a un individuo sin constancia previa de infección que, inicialmente, se había incluido en el grupo de pacientes negativos y que, tras las determinaciones llevadas a cabo, se confirmó que había pasado infección de manera asintomática. Cabe destacar también que con la técnica de referencia fueron los anticuerpos frente a la espícula los que revelaron la infección previa y no los anticuerpos frente a la nucleocápside. Este hecho ya había sido constatado por Fedele *et al.* [11], que comprobaron que los anticuerpos anti-S son más duraderos y estables y que, por tanto, son mejores para el diagnóstico de infección pasada.

Sin embargo, debe tenerse en cuenta que aunque los anticuerpos anti-N tienen una vida media corta, son los únicos que sirven para detectar la infección en individuos vacunados y que, además, y gracias a la rapidez con la que se negativizan, pueden ser de gran utilidad para detectar reinfecciones. Dado que las manifestaciones clínicas de la enfermedad no suelen ser tan evidentes en los individuos vacunados o que ya han pasado una infección, la detección de IgG frente a la nucleocápside podría ser un buen recurso para detectarlas, aunque solo sea para fines epidemiológicos. En estos casos, los valores de IgGs anti-RBD con una duración mucho mayor tendrían una utilidad menor.

Como resumen, de las dos pruebas evaluadas, Mikrogen diagnostik *recom*Line es la que ofrece unos resultados más satisfactorios, mostrando los otros dos valores de sensibilidad por debajo de lo que sería deseable. No obstante, dado que los valores de predictivo positivo son aceptables en ambos casos, estas dos técnicas podrían ser de utilidad para detectar positivos en ausencia de otros sistemas.

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CONFLICTO DE INTERESES

Los autores no presentan ningún conflicto de intereses.

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Características clínicas, pronóstico y factores asociados de la bacteriemia por *Staphylococcus aureus* en la actualidad

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RESUMEN

Introducción. Describimos las características de los pacientes con bacteriemia por *Staphylococcus aureus* en un hospital de tercer nivel y analizamos sus complicaciones, la mortalidad y los factores asociados a las mismas.

Métodos. Se analizaron de manera retrospectiva los datos de los pacientes ingresados con bacteriemia por *S. aureus* entre marzo de 2020 y febrero de 2021 en el hospital universitario Miguel Servet de Zaragoza.

Resultados. La mortalidad a los 14 días fue del 24,2% y la mortalidad a los 30 días del 40%. La aparición de complicaciones [HR 3,1 (1,2-8,05)] y la edad >65 años [HR 3,1 (IC95% 1,4-6,6)] disminuyeron la supervivencia global de manera significativa. En la regresión logística se asociaron a mayor mortalidad a los 30 días la edad >65 años [OR 6,3 (IC95% 1,7-23,1)], la presencia de sepsis [OR 19,3 (IC95% 5,4-68,7)] y solo con cierta tendencia, el número de frascos de HC (+) ≥3 [OR 5,4 (IC95% 0,8-34,1)]. Se asoció a mayor mortalidad a los 14 días el haber presentado sepsis [OR 58,2 (IC95% 5,7-592,9)], el número frascos de HC (+) ≥3 [OR 14,1 (IC95% 1,1-173,7)] y una edad >65 años [OR 1,1 (IC95% 1,03-1,1) años]. Cuando analizamos juntos aquellos con un TP ≤12 horas y un número frascos de HC (+) ≥3, la sepsis fue más frecuente [30 pacientes (66,6%) vs 15 pacientes (33,3%); OR 3,4 (IC95% 1,5-8)].

Conclusiones. La mortalidad a los 14 y a los 30 días fue elevada, observándose una peor evolución en los pacientes con mayor edad, presencia de sepsis, un mayor número de frascos de hemocultivos positivos y un tiempo hasta hemocultivos positivos ≤12 h.

Palabras clave: *Staphylococcus aureus*, Bacteriemia, Pronóstico, Mortalidad

Clinical characteristics and prognosis of *Staphylococcus aureus* bacteremia

ABSTRACT

Introduction. *Staphylococcus aureus* bacteremia patients characteristics at a tertiary hospital are described, and complications, mortality and associated factors are analyzed.

Methods. Data from patients with *S. aureus* bacteremia admitted between March 2020 and February2021 at Miguel Servet university hospital in Zaragoza were retrospectively analyzed.

Results. Results showed a 14 days mortality of 24.2% and an 30 days mortality of 40%. Overall survival decreased with complications appearance [HR 3.1 (1.2-8.05)] and age over 65 years [HR 3.1 (1.4-6.6)]. The adjusted analysis showed correlation between a higher mortality at 14 and 30 days with age over 65 years [OR 6.3 (1.7-23.1)], sepsis presence [OR 19.3 (5.4-68.7)] and number of positive (+) blood cultures ≥3 [OR 5.4 (0.8-34.1)]. Mortality at 14 days was associated with sepsis presence [OR 58.2 (5.7-592.9)], number of positive (+) blood cultures ≥3 [OR 14.1 (1.1-173.7)] and an older age [OR 1.1 (1.03-1.1)]. Analyzing time to positive blood cultures ≤12 hours and number of positive blood cultures ≥ 3 at the same time, frequency of sepsis increased [30 patients (66.6%) vs 15 patients (33.3%); OR 3.4 (IC95% 1.5-8)].

Conclusions. High 14- and 30-days mortality were found, as well as a worse evolution in older age patients, with sepsis presence, and with greater number of positive blood cultures and times to positive blood cultures ≤12 h.

Keywords: *Staphylococcus aureus*, Bacteremia, Prognosis, Mortality.

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INTRODUCCIÓN

Staphylococcus aureus (SA) es un microorganismo que puede invadir cualquier órgano y tiene una gran capacidad, debido a su mayor virulencia, de originar metástasis por vía hematógena, que con frecuencia son graves e implican mal pronóstico a corto plazo. Aproximadamente el 30% de la población está colonizada por SA [1].

La bacteriemia por SA (BSA) es una de las más frecuentes en nuestro medio, tanto de origen comunitario como la relacionada con la atención sanitaria o nosocomial. Su incidencia varía entre los 20-50 casos/100.000 personas al año en función de series, siendo más prevalente en pacientes con determinados factores de riesgo [1,2].

El foco de infección más común son los catéteres intravasculares, las infecciones de piel y partes blandas, pulmonares, osteoarticulares y la endocarditis infecciosa [1].

El riesgo de aparición de complicaciones y mortalidad es elevado, del 30,3% (3), habiéndose sugerido que un inicio precoz y adecuado del tratamiento podría reducirlo [4,5].

Describimos las características de los pacientes ingresados con BSA en un centro hospitalario de tercer nivel y analizamos su evolución en cuanto a supervivencia, mortalidad y complicaciones, así como los factores que pudieran estar asociados con ellas.

MATERIAL Y MÉTODOS

Se trata de un estudio retrospectivo y observacional. Se analizaron los datos de los pacientes mayores de 18 años ingresados entre el 1 de marzo de 2020 y el 28 de febrero de 2021 en el Hospital universitario Miguel Servet de Zaragoza, por bacteriemia por SA, considerada como tal, si al menos se objetivaba un hemocultivo positivo al inicio o durante el ingreso. Los pacientes se seleccionaron a través del programa informático del servicio de microbiología que registra el resultado de todos los hemocultivos tomados en el hospital.

Se recogieron los datos demográficos y sus comorbilidades, incluida la presencia de prótesis articulares o endovasculares, y diversas variables clínicas, entre ellas el foco de la bacteriemia, la presencia de complicaciones como embolismos sépticos o el desarrollo de sepsis durante el ingreso. Se recogió también si el origen de la bacteriemia fue nosocomial o comunitario, considerándose nosocomial en aquellos pacientes que presentaron fiebre llevando más de 48h ingresados, extrayéndose a partir de ese momento los hemocultivos que resultaron positivos.

Se recogieron los datos sobre el tipo de tratamiento antibiótico empírico pautado, el tratamiento antibiótico dirigido y su duración. Se consideró que el tratamiento antibiótico empírico era totalmente adecuado si se ajustaba a las recomendaciones de las guías nacionales de tratamiento actuales [6] y se administraba por la vía adecuada y con una posología correcta, parcialmente adecuado si cubría SA pero no era el tratamiento de elección o no se administraba con una posología adecuada según las guías de tratamiento, e inadecuado si SA quedaba fuera del espectro de cobertura antibiótica. Se consideró también el tratamiento antibiótico empírico inadecuado si en los pacientes que presentaban factores de riesgo para SARM no se cubrió.

En cuanto a las variables microbiológicas, se recogieron los casos de resistencia a meticilina (SARM), la realización o no y el resultado de hemocultivos (HC) de seguimiento, el tiempo que tardaron en positivizarse los HC (TP) y el número de frascos en los que se aisló SA, como medida indirecta de su patogenicidad y concentración bacteriana en sangre.

Para la detección microbiológica, los HC se incubaron en BD BACTEC ™ FX durante 5 días. Cuando se detectó un HC positivo, se realizó una tinción de Gram e identificación por espectrometría de masas (MALDI-TOF MS) (MaldiBiotyper® Bruker Daltonics) del subcultivo. La sensibilidad antibiótica se determinó mediante MicroScan WalkAway (Beckman Coulter) siguiendo los criterios vigentes de EUCAST.

Las variables principales empleadas para valorar el impacto de la bacteriemia fueron la mortalidad hospitalaria a los 14 y a los 30 días del primer hemocultivo positivo, considerándose una complicación la presencia de embolismos sépticos, la aparición de sepsis o la persistencia de la bacteriemia, definida por la presencia de hemocultivos positivos a las 72 horas del inicio de un tratamiento antibiótico adecuado. Se estudió la supervivencia durante el ingreso, así como los factores que pudieran estar asociados a la misma.

En el análisis estadístico, las variables cualitativas se expresan en porcentaje (%) y las cuantitativas con media y desviación típica (DT). Para las asociaciones entre variables categóricas utilizamos chi cuadrado (X2), para variables cuantitativas la t de Student, y curvas de supervivencia de Kaplan-Meyer y regresión logística para el análisis multivariante. Se considera un nivel de significación de $p<0,05$. El programa estadístico utilizado es G-STAT 2.0.

RESULTADOS

Características clínicas, microbiológicas y terapéuticas. Se recogieron datos de 95 pacientes, 72 eran hombres (75,7%), la media de edad fue 68,2 años (límites: 19-95; DT: 16,1), siendo el 60% mayores de 65 años. Las comorbilidades más frecuentes fueron: hipertensión arterial (HTA), dislipemia (DLP), enfermedad cardiovascular (ECV), diabetes mellitus (DM), obesidad y el antecedente de neoplasias. Un 27,3% de los pacientes presentaban coinfección por SARS-CoV-2, considerada como la BSA en pacientes ingresados por COVID o con PCR de SARS-CoV-2 positiva en el momento de extraerse los primeros hemocultivos positivos (Tabla 1).

Del total de la muestra, 13 pacientes (13,7%) eran portadores de algún tipo de prótesis intravascular (marcapasos, prótesis valvular o endoprótesis vascular) y 5 pacientes (5,3%) tenían prótesis articulares, con 1 paciente que portaba dispositivos de ambos tipos.

Tabla 1 **Características demográficas, clínicas, microbiológicas y de tratamiento.**

	Total
Edad	68,2 años (16,1 – 19-95)
Sexo	
Varón	72 (75,7%)
Comorbilidades	
Hipertensión arterial	61 (64,2%)
Enfermedad cardiovascular	46 (48,4%)
Dislipemia	41 (43,1%)
Diabetes Mellitus	35 (36,8%)
Obesidad	32 (33,6%)
Neoplasias	30 (31,5%)
Enfermedad renal crónica	27 (28,4%)
Broncopatía	17 (17,8%)
Hepatopatía	9 (9,4%)
Enfermedad reumática	7 (7,3%)
Trasplante	5 (5,2%)
VIH	0
COVID-19	26 (27,3%)
Presencia de dispositivos intravasculares o protésicos	17 (17,9%)
Prótesis intravasculares	13 (13,6%)
Prótesis articulares	5 (5,2%)
Prótesis de ambos tipos	1
Origen de la bacteriemia	
Nosocomial o relacionada con la asistencia sanitaria	52 (54,7%)
Adquirida en la comunidad	43 (45,3%)
Foco de la bacteriemia	
Catéter vascular	27 (28,4%)
Piel y partes blandas	20 (21%)
Urinario	15 (15,7%)
Respiratorio	10 (10,5%)
Desconocido	23 (24,2%)
Se realizó ecocardiograma	64 (67,3%)
Complicaciones	
Sepsis	49 (51,5%)
Endocarditis	5 (5,3%)
Espondilodiscitis	8 (8,4%)
Artritis	6 (6,3%)
Aortitis	4 (4,2%)
Abscesos cutáneos o de órgano sólido	5 (5,3%)
Embolismos periféricos	5 (5,2%)
Ingreso en UCI	23 (24,2%)
Necesidad de VMI	16 (16,8%)
Hemocultivos de control	69 (72,6%)
Bacteriemia persistente	27 (39%)
Aislamiento de SARM	15 (15,8%)
Bacteriemia polimicrobiana	8 (8,4%)
Frascos de HC (+) ≥3	76 (80%)
Tiempo hasta HC positivo (horas) (TP) ≤12 horas	16 (17%)
Tratamiento adecuado	49 (51,1%)
Tratamiento parcialmente adecuado	37 (38,9%)
Tratamiento inadecuado	9 (9,4%)
Tiempo de tratamiento	15 días (20,6)
Biterapia	30 (31,5%)
Tiempo de biterapia	14,8 días (14,4)
Mortalidad antes de 14 días	23 (24,2%)
Mortalidad antes de 30 días	38 (40%)

El 54,7% (52 pacientes) de las BSA fueron de origen nosocomial o relacionado con la asistencia sanitaria, siendo el resto de origen comunitario. En la mayoría la fuente fue el catéter vascular en 27 casos (28,4%), piel y partes blandas en 20 casos (21%), urinaria en 15 casos (15,7%) y respiratoria en 10 casos (10,5%); el foco fue desconocido en 23 pacientes (24,2%).

En 15 casos (15,8%) se aisló SARM y en 8 la bacteriemia fue polimicrobiana, con crecimiento en 3 casos de *Enterobacter cloacae*, siendo *Streptococcus oralis, Candida albicans, Streptococcus salivarius, Klebsiellas pneumoniae, Streptococcus gallolyticus, Staphylococcus epidermidis, Pseudomonas aeruginosa y Bacteroide fragilis* el resto de los aislamientos.

La media de tiempo de incubación de los HC hasta ser positivos fue de 16 horas (DT 17), en 76 casos (80%) *SA* creció en los 4 frascos extraídos y se realizaron HC de control en 69 pacientes (72,6%), encontrando un total de 27 pacientes (39%) con bacteriemia persistente.

En cuanto al tratamiento antibiótico empírico, en 49 casos (51,5%) se consideró adecuado para SA, mientras que en 37 casos (38,9%) lo era solo parcialmente y en 9 casos (9.4%) era totalmente inadecuado. La media de duración del tratamiento antibiótico fue de 15 días (DT 20,6). El 31,5% de los pacientes (30 casos) iniciaron el tratamiento con biterapia, que se mantuvo una media de 14,8 días (DT 14,4).

Características evolutivas. Complicaciones y mortalidad. Análisis de la supervivencia. La estancia media en supervivientes fue de 35,6 días (DT 30,1). Se realizó ecocardiograma para descartar endocarditis en el 67,3% de los casos (64 pacientes), diagnosticando un total de 5 episodios (5,3%) de endocarditis infecciosa. Otras complicaciones fueron espondilodiscitis en 8 casos (8,4%), aortitis infecciosa en 4 casos (4,2%), artritis séptica en 6 casos (6,3%) y abscesos de órganos sólidos o cutáneos en 5 casos (5,3%). Presentaron criterios de sepsis 49 pacientes (51,5%) y en total 23 pacientes (24,2%) precisaron ingreso en UCI.

La mortalidad a los 14 días fue del 24,2% (23 pacientes) y la mortalidad a los 30 días del 40% (38 pacientes). La probabilidad de morir antes de 14 días fue del 20% (Figura 1).

La supervivencia global no se asoció con la utilización de biterapia, la coinfección por SARS-CoV-2, el número de frascos de HC (+) ≥3 o el tratamiento adecuado, Sin embargo, la aparición de complicaciones [HR 3,1 (1,2-8,05)] y la edad >65 años [HR 3,1 (IC95% 1,4-6,6)] disminuyeron la supervivencia global de manera significativa (Figuras 2 y 3).

Los resultados más relevantes del análisis univariante y multivariante se presentan en la tabla 2. Se asociaron a una mayor mortalidad a los 30 días la presencia de HTA como comorbilidad [29 pacientes

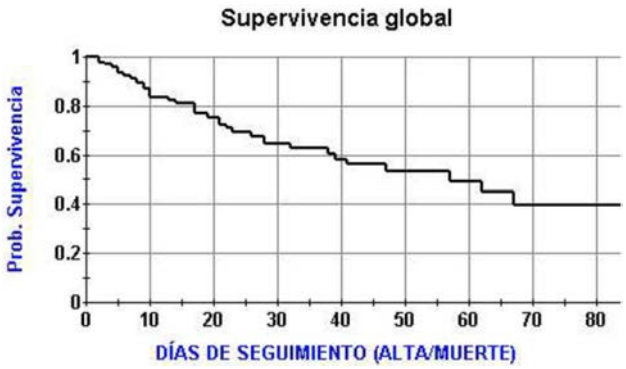

Figura 1 Supervivencia global: días de seguimiento hasta alta o fallecimiento.

Figura 2 Supervivencia en pacientes con o sin complicaciones.

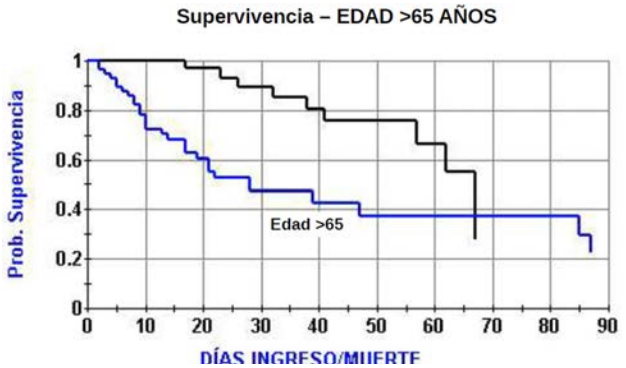

Figura 3 Supervivencia según edad mayor o menor de 65 años.

(47,5%) vs 9 pacientes (26,4%); OR 2,5 (IC95% 1,01-6,2)], el uso de ventilación mecánica invasiva (VMI) [10 pacientes (62,5%) vs 28 pacientes (35,4%); OR 3,03 (IC95% 1-9)], la aparición de sepsis como complicación [33 pacientes (67,3%) vs 5 pacientes (10,8%); OR 16,9 (IC95% 5,6-51)], los pacientes de mayor edad [74,5 años (DT 13,6) vs 64,1 años (DT 16,4); p<0,01] y los casos con un n.º de frascos de HC (+) ≥3 [34 pacientes (44,7%) vs 4 pacientes (21%); OR 1,2 (IC95% 1-1,4)]. En la regresión logística lo hicieron la edad >65 años [OR 6,3 (IC95% 1,7-23,1)], la presencia de sepsis [OR 19,3 (IC95% 5,4-68,7)] y mostró cierta tendencia el número de frascos de HC (+) ≥3 [OR 5,4 (IC95% 0,8-34,1)].

Presentaron mayor mortalidad a los 14 días los pacientes con un número de frascos de HC (+) ≥3 [22 pacientes (28.9%) vs 1 paciente (5.2%); OR 7.3 (IC95% 1-58)], los que presentaron sepsis como complicación [24 pacientes (96%) vs 9 pacientes (69.2); OR 2 (IC95% 1-5.5)], que el foco de la bacteriemia no fuera conocido [9 pacientes (39.1%) vs 14 pacientes (19.4%); OR 2.9 (IC95% 1-8)] y una mayor edad [78.2 años (DT 11) vs 67.2 años (DT 15.6) años; p<0.01]. Al ajustar el análisis, solo el haber presentado sepsis [OR 58,2 (IC95% 5,7-592,9)], el número de frascos de HC (+) ≥3 [OR 14,1 (IC95% 1,1-173,7)] y una edad >65 años [OR 1,1 (IC95% 1,03-1,1)] se asociaron a mayor mortalidad a los 14 días. Por otro lado, se observó una menor mortalidad a los 14 días, pero no a los 30 días, en los casos que se utilizó biterapia [HR 0,05 (IC95% 0,008-0,4)], lo que implica 16,9 veces menos probabilidades de morir antes de 14 días.

Se asociaron a más complicaciones la HTA [42 casos (73,6%) vs 19 casos (50%); OR 2,8 (IC95% 1,1-6,6)], la fuente de bacteriemia no conocida [18 casos (31,5%) vs 5 casos (13,1%); OR 2,9 (IC95% 1,1-8,8)], la bacteriemia persistente [22 casos (62,8%) vs 5 casos (14,7%); OR 9,8 (IC95% 3,4-31,6)] y el número de frascos de HC (+) ≥3 [51 casos (89,4%) vs 25 casos (65,7%); OR 4,4 (IC95% 1,5-13)]. Se observó una menor tasa de complicaciones cuando el foco fue el catéter vascular [OR 0,2 (IC95% 0,08-0,6)], lo que implica 4.3 veces menos posibilidades de tener complicaciones con respecto a otros focos. En el análisis ajustado, el número de frascos de HC (+) ≥3 [OR 4,3 (IC95% 1,3-13,7)] y la HTA [OR 3,4 (IC95% 1,3-9,2)] fueron los factores que se asociaron a más complicaciones.

Se observó una tendencia a un TP menor en aquellos que murieron antes de los 14 días [2,50 horas (DT 50,8) vs 10 horas (DT 39); p=0,07], y en los que presentaron sepsis [10 horas (DT 44,2) vs 14 horas (DT 54,1); p<0,05], siendo más frecuente la sepsis cuando el TP fue ≤12 horas [32 casos (62,7%) vs 17 casos (38,6%); OR 2,6 (IC95% 1,1-6,2)]. En este sentido, el TP fue menor en aquellos con un número de frascos de HC (+) ≥3 [13,3 horas (DT 10,9) vs 27,3 horas (DT 29,7); p<0,01]. Cuando analizamos juntos aquellos con un TP ≤12 horas y un número de frascos de HC (+) ≥3, la sepsis fue más frecuente en aquellos con mayor número de de frascos de HC (+) [30 pacientes (66.6%) vs 15 pacientes (33.3%); OR 3.4 (IC95% 1.5-8)].

Tabla 2 **Análisis univariante y multivariante de asociaciones.**

	Mortalidad antes de 30 días (M30)	Mortalidad antes de 14 días (M14)	Complicaciones (C)	Tiempo hasta hemocultivos positivos (TP)
Edad	[74,5 (DT 13,6) Vs 64,1 (DT 16,4) años; p<0,01]	[78,2 (DT 11) Vs 67,2 (DT 15,6) años; p<0,01]		
Edad >65*	HR 3,1 (IC95% 1,4-6,6)			
HTA**	OR 2,5 (IC95% 1,01-6,2)		OR 2,8 (IC95% 1,1-6,6)	
Complicaciones	HR 3,1 (1,2-8,05)			
Sepsis***	OR 16,9 (IC95% 5,6-51)	OR 2 (IC95% 1-5,5)		[10 (DT 44,2) Vs 14 (DT 54,1) horas; p<0,05]
VMI	OR 3,03 (IC95% 1-9)			
Foco no conocido		OR 2,9 (IC95% 1-8)	OR 2,9 (IC95% 1,1-8,8)	
Foco catéter vascular			OR 0,2 (IC95% 0,08-0,6)	
Número de frascos de HC(+)≥3****	OR 1,2 (IC95% 1-1,4)	OR 7,3 (IC95% 1-58)	OR 4,4 (IC95% 1,5-13)	[13,3 (DT 10,9) Vs 27,3 (DT 29,7) horas; p<0,01]
Bacteriemia persistente			OR 9,8 (IC95% 3,4-31,6)	
Biterapia*****		Regresión logística		
Tiempo hasta hemocultivos positivos (TP)		[2,50 (DT 50,8) Vs 10 (DT 39) horas; p=0,07]		

***Regresión logística: M30 [OR 6,3 (IC95% 1,7-23,1)]; M14 [OR 1,1 (IC95% 1,03-1,1)],**
****Regresión logística: C [OR 3,4 (IC95% 1,3-9,2)],**
*****Regresión logística: M30 [OR 19,3 (IC95% 5,4-68,7); M14 [OR 58,2 (IC95% 5,7-592,9)]**
******Regresión logística: M30 [OR 14,1 (IC95% 1,1-173,7); C [OR 4,3 (IC95% 1,3-13,7)]**
*******Regresión logística: M14 [OR 1,1 (IC95% 1,03-1,1)]**

DISCUSIÓN

En concordancia con otros estudios [7], observamos que la BSA afecta de manera predominante a hombres con edad elevada. La asociación con la edad parece estar relacionada con los factores propios del envejecimiento, como el aumento de las comorbilidades o el mayor contacto con el sistema sanitario [2].

El 27,3% de nuestros pacientes presentaban infección por SARS-CoV-2 en el momento de la bacteriemia. En los pacientes con infección por SARS-CoV-2 la tasa de BSA se ha descrito hasta en un 1,2% más que en aquellos sin infección por SARS-CoV-2, siendo ésta principalmente de origen nosocomial y asociada a catéter vascular [3].

Más de la mitad de las bacteriemias fueron de origen nosocomial o relacionado con la asistencia sanitaria, inferior a lo descrito por otros autores, aunque se observa una tendencia a disminuir debido a unas mejores medidas de control y vigilancia en los hospitales [1].

El catéter vascular fue el foco más frecuente, aunque cabe resaltar el origen respiratorio (10,5%), probablemente relacionado con el elevado número de coinfeccciones por SARS-CoV-2 en nuestros datos, en los que SA se ha descrito como uno de los causantes de sobreinfección bacteriana [3], mientras que hasta en el 24,2% fue desconocido, inferior a lo observado en otras series [1].

El 15,8% de SARM aislado está por debajo de lo registrado en nuestro hospital en otras series previas, y es ligeramente inferior a lo observado en otros estudios similares [8] y en nuestro propio centro (21%), lo que podría deberse a un mayor control de las medidas de aislamiento de contacto durante la pandemia de SARS-CoV-2.

La presencia de SARM no se relacionó con una mayor mortalidad ni tasa de complicaciones, encontrando en la literatura datos discordantes al respecto [9, 10]. La presencia de prótesis en nuestro estudio no se asoció con más bacteriemias persistentes ni con una mayor mortalidad o tasa de complicaciones, al contrario de lo descrito en estudios recientes [11].

Un tratamiento inicial inadecuado, en el 9.4% de nuestros casos, supone un riesgo elevado de mala evolución [12], mientras que la biterapia antiestafilocócica, recogida por nosotros en el 31,5% de los casos y similar a la reportada en la literatura

[2], podría reducir el riesgo de complicaciones, hecho no demostrado ni compartido por todos los autores [13].

La estancia de 35 días en supervivientes fue relativamente prolongada, quizás relacionada con el elevado número de pacientes que presentaron sepsis, otras complicaciones o ingreso en UCI. La mortalidad del 40% es superior a lo descrito en nuestro centro para el global de bacteriemias (30%) [3], y aproximadamente la mitad lo hicieron antes de 14 días, lo que supone una posibilidad del 20% de morir en los primeros 14 días, lo que nos da una idea de la gravedad e importancia de la BSA.

La presencia de sepsis, la edad elevada y un número de frascos de HC (+) ≥3 se asociaron a una mayor mortalidad a los 14 y 30 días, mientras que el foco de la bacteriemia desconocido y un TP, sobre todo ≤12 horas, lo hicieron a mayor mortalidad a los 14 días. La HTA como comorbilidad y el número de HC (+) ≥3 también se asociaron a más complicaciones; y los cuadros de sepsis y complicaciones sépticas fueron entre 3 y 5 veces más frecuentes en los pacientes con bacteriemia persistente.

Por el contrario, se observó una menor tasa de complicaciones cuando el foco fue el catéter vascular, probablemente debido a que resulta más fácil el control del foco [10], y una menor mortalidad a los 14 días, pero no a los 30 días, en los casos que utilizaron biterapia, teniendo 17 veces menos probabilidades de morir antes de 14 días si se utilizan dos fármacos antiestafilocócicos, como algunos autores sugieren [13], ya que el sinergismo podría aclarar la bacteriemia de manera más precoz y reducir el inóculo con mayor rapidez.

Un menor TP parece expresar una mayor concentración bacteriana circulante y se asocia a un peor pronóstico de la BSA [14,15], tal y como nuestros hallazgos parecen también sugerir. Esto unido a un mayor número de frascos de HC positivos, la HTA como comorbilidad, una mayor edad, el desconocimiento del foco de origen y la presencia de criterios de sepsis, nos puede orientar hacia una peor evolución en las primeras semanas. La biterapia en casos seleccionados y en etapas iniciales quizás podría mejorar este pronóstico.

Consideramos que el estudio tiene ciertas limitaciones. En primer lugar, se trata de un estudio retrospectivo, unicéntrico y observacional con un reducido tamaño muestral. Además, el período de recogida de los datos coincide con la pandemia por SARS-CoV-2, donde se objetivó un menor número de hemocultivos extraídos [3], atribuido a un menor número de consultas en urgencias por otras causas y de cirugías programadas y a un cambio generalizado de las condiciones de trabajo en el hospital. Por otro lado, también consideramos una limitación la dificultad de establecer si la mortalidad, sobre todo la ocurrida después de los 14 días, podría estar relacionada con otros factores además de la propia BSA.

La BSA es un problema grave, que consume una importante cantidad de recursos y en la que es preciso instaurar una terapia eficaz lo más precozmente posible. En nuestro estudio más de la mitad de la BSA fue de origen nosocomial o relacionada con la asistencia sanitaria, con una tasa de SARM del 15.8%. La mortalidad antes de 14 y 30 días fue elevada y se asoció a la presencia de sepsis, observándose una peor evolución en los pacientes con mayor edad, un mayor número de frascos de HC positivos y un TP ≤12h, medida indirecta de la intensidad o el inóculo de la BSA. Esto podría permitir una identificación precoz de los casos con una mayor gravedad potencial que quizás pudieran beneficiarse, a corto plazo al menos, de una biterapia inicial. Son necesarios más estudios para poder afirmarlo.

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CONFLICTO DE INTERESES

Los autores no presentan ningún conflicto de intereses.

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Impact of the use of antibiotics on the clinical response to immune checkpoint inhibitors in patients with non-small cell lung cancer

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ABSTRACT

Objectives. Recent research suggests that the use of antibiotics could reduce the efficacy of checkpoint inhibitors, in addition to other well-known factors. It could be due to gut microbiota modification, which impact over the immune system response. However, the information available so far is contradictory. The objective of this research was to clarify whether antibiotic use influences efficacy of checkpoint inhibitors treatments in non-small cell lung cancer patients in clinical practice.

Methods. Therefore, a retrospective observational study was designed. Use of antibiotics among patients treated with atezolizumab, pembrolizumab or nivolumab was assessed within 2 months of checkpoint inhibitors treatments initiation.

Results. A total of 140 patients were included, mostly men, with good performance status (ECOG 0-1), all of them previously treated with chemotherapy. An antibiotic prescription was identified in 31% of these patients, mainly fluoroquinolones or beta-lactams. The most frequent indication was respiratory infection. Both progression-free survival and overall survival were lower for patients treated with anti-infective drugs, although this difference was not statistically significant.

Conclusion. More studies are needed to draw conclusions about the impact of antibiotics on the efficacy of immunotherapy.

Keywords: Antineoplastic agents, Immunotherapy, antibiotics, efficacy

Impacto del uso de antibióticos en la respuesta clínica de los inhibidores del punto de control en pacientes con carcinoma de pulmón no microcítico.

Objetivos. Investigaciones recientes sugieren que el uso de antibióticos podría reducir la eficacia de los inhibidores del punto de control inmunológico, además de otros factores ya conocidos. Podría deberse a la modificación de la microbiota, por su impacto en la respuesta del sistema inmune. En cualquier caso, la información disponible hasta el momento es contradictoria. El objetivo de esta investigación es esclarecer si el uso de antibióticos influye en la eficacia de los inhibidores del punto de control para el tratamiento de pacientes con carcinoma de pulmón no microcítico en la práctica clínica.

Métodos. Se diseñó un estudio observacional, retrospectivo. Se investigó el uso de antibióticos entre aquellos pacientes a tratamiento con atezolizumab, pembrolizumab o nivolumab en los 2 meses previos o posteriores a su inicio.

Resultados. Se incluyeron 140 pacientes, principalmente hombres con aceptable estado general (ECOG 0-1), todos previamente tratados con quimioterapia. Se identificó una prescripción antibiótica en el 31% de la población, principalmente fluoroquinolonas o betalactámicos. La indicación más frecuente para dicha prescripción era la infección respiratoria. Tanto la supervivencia libre de progresión con la supervivencia global fue inferior en el grupo tratado con antiinfecciosos, aunque no se alcanzó significación estadística.

Conclusiones. Son necesario más estudios para concluir acerca del impacto de los antibióticos en la eficacia de la inmunoterapia.

Palabras clave: agentes antineoplásicos, Inmunoterapia, antibióticos, eficacia

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INTRODUCTION

Lung cancer is the leading cause of cancer deaths worldwide, being tobacco the main risk factor [1]. It is the third most frequent type of tumour in Spain, after colorectal and prostate cancer [2]. Although lung cancer has been more prevalent in men, there has been an increased incidence in women due to the change in the prevalence of tobacco use [1].

Lung cancer can be divided in two main histological groups: small cell lung carcinoma (SCLC, 15% of all lung cancers) and non-SCLC (NSCLC, 85% of all lung cancers). Among non-SCLC, the most prevalent type is adenocarcinoma (40%) followed by squamous cell carcinoma (25%) [3].

Several key factors determine the choice of initial treatment for advanced or metastatic NSCLC: tumour-related factors (histology, molecular testing), patient-related factors (age, performance status, comorbidities, patient preferences) [4]. Platinum-based regimens have been the main treatment while using conventional chemotherapy, but they are no longer in the front-line setting due to its low overall survival (less than 50% after one year of treatment) [5,6]. The incorporation of immunotherapy into clinical practice has revolutionized the management of this pathology, specially with monoclonal antibodies directed against programmed death receptor 1 (PD-1) or its ligands (PD-L1) in patients without driver mutations; although in these patients the treatment will also depend on the general condition performance status (PS) and tumor PD-L1 expression [7].

The intestinal microbiota has recently been postulated as a potential predictor or modulator of ICI response such as the expression of PD-L1 or tumor mutational burden (TMB) [8].

Routy *et al.* [9] showed that the gut microbiome influenced the outcome of PD-1 blockade in both mice and humans: they designed a trial in which they transplanted fecal microbiota from cancer patients who responded to immune checkpoint inhibitors (ICI) into germ-free or antibiotic-treated mice, and they observed a better PD-1 blockade, while no effect was seen when the recipient was a non-respondent patient.

This idea was reinforced by series of published cases: fecal microbiota transplantation improved colitis associated with ICI through a relative increase in the proportion of regulatory T cells in the colon mucosa [10]. Some authors suggest that it could be due to the relative abundance of different bacteria such as *Akkermansia muciniphila*, *BIfidobacterium* or *Bacteroides fragilis* [11].

Antibiotics are widely used in clinical practice, and it is well known that their administration produces changes in the intestinal microbiota. Based on this line of research, several studies published last years have associated poorer overall survival and an increased risk of refractory disease in cancer patients treated with ICI therapy associated with antibiotic treatment [8;12-25], when administered within 60 days prior to the start of or during ICI therapy.

However, there is controversy regarding these findings, since other authors have not observed such association in their cohorts [8].

Patients with advanced or metastatic NSCLC are especially candidates for receiving antibiotic treatment during the course of their disease or prior to their diagnosis, based on 2 reasons [8]:

- Smoking: NSCLC is widely linked to smoking (80-90% of cases). Smoking favors lung infection as it weakens local epithelial immunity and cilio-induced mucus clearance [17]. In addition, tobacco induces a pulmonary obstruction that leads to more frequent respiratory infections, with cough and chronic expectoration, which requires repeated courses of antibiotics.

- Age: the median age for the diagnosis of patients with advanced or metastatic NSCLC is between 65 and 70 years, so they could be more sensitive to infections.

This study therefore aims to analyze the influence of

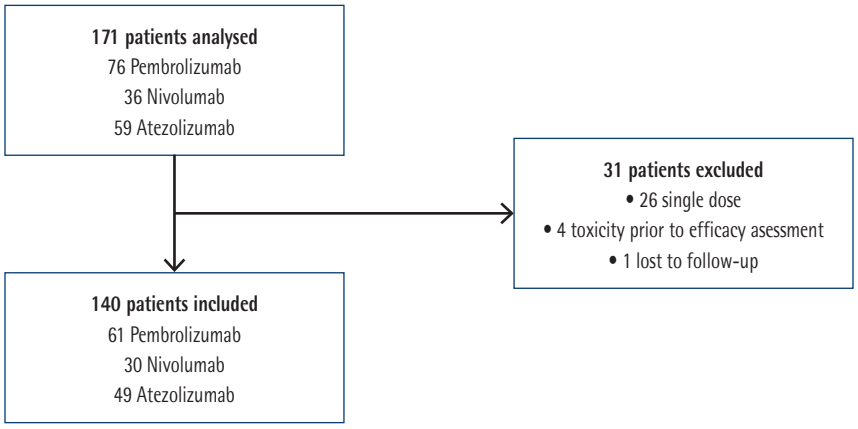

Figure 1 Flow diagram for inclusion and exclusion of studies.

Table 1 Patients' characteristics

	Non antibiotic exposure (n=97)	Antibiotic exposure (n=43)	P-value	Total (N=140)
Age				
Mean (SD)	65.5 (7.35)	65.9 (9.79)	0.61	65.7 (8.15)
Median [Q1, Q3]	65.8 [60.0, 71.0]	65.1 [60.9, 73.5]		65.5 [60.2, 71.8]
Sex				
Men	70 (72.2%)	27 (62.8%)	0.322	97 (69.3%)
Women	27 (27.8%)	16 (37.2%)		43 (30.7%)
Histology				
Adenocarcinoma	71 (73.2%)	23 (53.5%)	0.013	94 (67.1%)
Squamous	10 (10.3%)	13 (30.2%)		23 (16.4%)
Others	16 (16.5%)	7 (16.3%)		23 (16.4%)
Stage				
IIIa	1 (1.03%)	1 (2.33%)	0.622	2 (1.43%)
IIIb	7 (7.22%)	2 (4.65%)		9 (6.43%)
IV	89 (91.8%)	40 (93.0%)		129 (92.1%)
Drug				
Atezolizumab	38 (39.2%)	11 (25.6%)	0.238	49 (35.0%)
Nivolumab	18 (18.6%)	12 (27.9%)		30 (21.4%)
Pembrolizumab	41 (42.3%)	20 (46.5%)		61 (43.6%)
ECOG				
0	30 (30.9%)	8 (18.6%)	0.11	38 (27.1%)
1	67 (69.1%)	34 (79.1%)		101 (72.1%)
2	0 (0%)	1 (2.33%)		1 (0.714%)
Smoker				
Previous	55 (56.7%)	21 (48.8%)	0.658	76 (54.3%)
Never	5 (5.15%)	3 (6.98%)		8 (5.71%)
Current	37 (38.1%)	19 (44.2%)		56 (40.0%)
PDL1 expression				
PDL1 ≥ 50	38 (39.1%)	21 (48.8%)	0.146	59 (42.1%)
PDL1 < 50	43 (44.3%)	13 (30.2%)		56 (40.0%)

ECOG: Eastern Cooperative Oncology Group

antibiotics over the effectiveness of immunotherapy when used for advanced or metastatic NSCLC, using progression-free survival (PFS) end overall survival (OS) of atezolizumab, nivolumab, pembrolizumab based on the exposure to antibiotics.

MATERIAL AND METHODS

Retrospective cohort study from May 2016 to May 2021 in a third-level hospital, including every patient diagnosed of metastatic NSCLC and treated with at least two doses of atezolizumab, nivolumab or pembrolizumab, regardless of its use alone or in combination with other antineoplastic agents.

Exclusion criteria included non-available primary-care medical records, single dose ICI administration, treatment interruption due to adverse effects prior to response assessment, incomplete follow-up for any reason.

Table 2 Characteristics of the antibiotic treatment

		n	%
Pacients on antibiotics (n; %)		43	30.71%
Atezolizumab		12	27.90%
Nivolumab		12	27.90%
Pembrolizumab		19	44.18%
Antibiotic type (n; %)		51*	
Fluoroquinolone		24	47.06%
	Ciprofloxacin	4	
	Levofloxacin	18	
	Moxifloxacin	2	
Beta-Lactam		20	39.22%
	Penicillin	13	
	Cephalosporin	7	
Aminoglycoside	Tobramycin	1	1.96%
Glycopeptide	Vancomycin	1	1.96%
Lincosamide	Clindamycin	1	1.96%
Macrolide	Azithromycin	3	5.88%
Others	Fosfomycin	1	1.96%
Indication (n; %)			
Respiratory infection		41	80.39%
Urinary tract infection		4	7.84%
Bacteriemia		2	3.92%
Others		4	7.84%
Duration			
Mean - range (days)		8,25	1-15

A sample of 150-170 patients diagnosed with stage III-IV NSCLC was estimated, who had received a PD1 / PD-L1 antagonist. Antibiotic use within 2 months of checkpoint inhibitors treatments initiation (2 months before or 2 months after CPI initiation) was assessed.

The primary endpoint was progression-free survival (PFS) evaluated by iRECIST criteria. Secondary endpoints included overall survival (OS).

Patients` characteristics and treatment outcomes were analyzed based on exposure or non-exposure to antibiotics, and the data were compared using the Chi-square test for categorical variables and the Student's t test for continuous variables. A Cox model was used to estimate the hazard ratio (HR) of each endpoint associated with potential risk factors.

Ethics approval. Comité de Investigación Clínica de Cabueñes approved the study. (reference number 25052021).

RESULTS

A total of 171 patients were included in the study, 140 of whom were finally analysed. Flow diagram for patients`inclusion and exclusion is showed in Figure 1.

Patients' characteristics are described on table 1. Groups were well balanced, including predominantly stage IV NSCLC, mainly men (70%), in good general condition (ECOG 0-1) with a median age of 65.5 years at the start of treatment. ICI were used mainly in monotherapy, both in the first (46.4%) and second (47.1%) lines. The most frequently observed histology was adenocarcinoma (67.1%), with the PD-L1 marker being positive in 77% of the cases.

Table 2 lists the characteristics of antibiotic treatment. Antibiotic use within 2 months previous or after the start of ICI was observed in 43 patients (30.7%). The main indication was respiratory infections (80.4%), for which fluoroquinolones

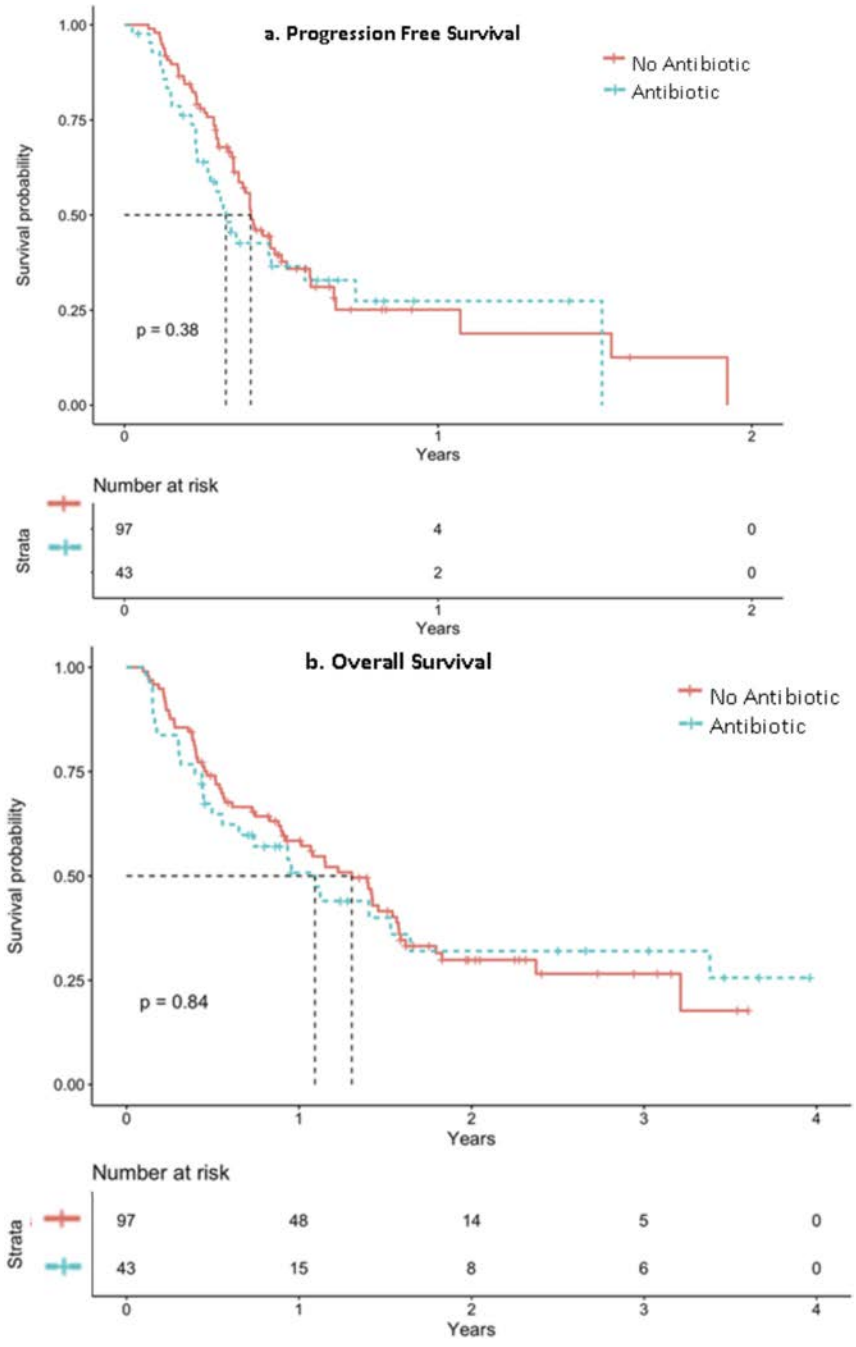

Figure 2 | **Progression free survival (figure 2a) and overall survival (figure 2b) based on their exposure to antibiotics.**

(47,1%) or beta-lactams (39.2%) were indicated.

Regarding the effectivity of ICIs, figure 2 shows PFS (figure 2a) and OS (figure 2b) based on their exposure to antibiotics. Not statistically differences were observed. The median PFS was 123 days (95% IC 126.84-170.82), being higher in patients without antibiotic treatment (170 days, 95% CI 116.041-223.96) compared to those who did received (124 days, 95% CI 52.79-195.21). Regarding OS, the median was also more favorable in patients without antibiotic treatment (median 477 days 95% CI 361.07-592.93 vs 399.00 days 95% CI 227.62-570.38), without reaching statistical significance in this case either (p 0.8).

a. Progression Free Survival

Variable		N	Hazard ratio	p
Age		140	0.97 (0.94, 0.99)	0.019
ECOG	0	38	Reference	
	1	102	1.37 (0.79, 2.36)	0.257
Antibiotic	Quinolones	21	Reference	
	No antibiotic	97	0.38 (0.22, 0.66)	<0.001
	Beta-lactams	16	0.23 (0.10, 0.57)	0.001
	Others	6	0.16 (0.04, 0.61)	0.007

b. Overall Survival

Variable		N	Hazard ratio	p
ECOG	0	38	Reference	
	1	102	1.67 (1.02, 2.75)	0.04
Antibiotic	Quinolones	21	Reference	
	Beta-lactams	16	0.34 (0.14, 0.83)	0.02
	No antibiotic	97	0.58 (0.33, 1.02)	0.06
	Others	6	0.33 (0.07, 1.48)	0.15
Drug	ATEZOLIZUMAB	49	Reference	
	NIVOLUMAB	30	0.81 (0.46, 1.43)	0.47
	PEMBROLIZUMAB	61	0.54 (0.33, 0.89)	0.02

Figure 3 | **Progression free survival (figure 3a) and overall survival (figure 3b): subgroup analysis.**

According to the subgroup analysis, both PFS and OS were worse among patients who received fluoroquinolones (figure 3). The OS was significantly better in patients receiving beta-lactams compared to those treated with fluoroquinolones (figure 3b). PFS was slightly better among patients exposed to IV antibitiotics (median PFS 167.9 days; 95% IC 54.0-NA) rather than oral antibiotics (median PFS 112 days; 95% IC 83.95-209.8). Better PFS were reached among patients on nivolumab (167.9 days; 95% IC 123.73-NA), followed by pembrolizumab (146.7 days; 95% IC 129.9-NA) and atezolizumab (140.9 days; 95% IC 108.77-182.86). The trend of better outcomes for patients without antibiotic treatment was observed regardless of ECOG value, sex or atezolizumab/pembrolizumab treatment. However, this trend was reversed in the group of patients who received nivolumab, among whom PFS was better in those exposed to antibiotics (167.90 days vs 137.97 days).

DISCUSSION

The demographic characteristics are similar to those of other studies analysing the same effect: in the meta-analysis carried out by Lurienne *et al.* [8], male patients predominated (58.3%; 40-82%), with non-squamous histology (71.2%) and ECOG 0-1 (92.6%). Patients included in our study were slightly younger than the referenced.

Most patients were receiving second or third line treatment, which is consistent with the fact that ICIs were used mainly in monotherapy, since the authorized use in monotherapy is reserved for patients with progression on or after platinum-based chemotherapy, with the exception of pembrolizumab, which can also be used as monotherapy as the first-line treatment of patients with metastatic NSCLC whose tumors express PD-L1 Tumor Proportion Score ≥50% [27-29].

The prevalence of antibiotic use was around 30%, similar to that previously reported in the literature [9; 16]; although it could range between 14 and 44% depending on the selected cohort [23-24]. Antibiotic types were also similar to the other authors, predominating fluoroquinolones and beta-lactams due to their wide coverage of pathogens that cause respiratory infections. However, no study or meta-analysis has been able to analyze the influence of the type of antibiotic on the effectiveness of the treatment. Nor the effect of its duration, which appears heterogeneously collected depending on the cohort.

When analyzing the effect of antibiotics on survival, there are two key aspects to consider. The window of exposure to

the antibiotic, which was set at 60 days before or after the start of treatment in our study. This period was selected based on the systematic review by Lurienne *et al.* [8], in which they subdivided the studies into 4 groups based on the window selected to perform the analysis, concluding that the effect of antibiotics on ICIs was greater when these were used within 60 days of the start of immunotherapy. However, despite being a critical factor that would explain the difference observed in terms of efficacy, there is no consensus on the time to analyze, varying from 30-90 days before [22-29] to more than 365 days later in some cases [25].

While analysing the influence of antibiotic exposure in survival, we obtained a difference of 1.5 months in PFS and 2.6 months in OS, without finding significant differences. These values differ from those of any other published study, probably due to their heterogeneity in terms of patients included and window of exposure to antibiotics.

This difference in survival could also be related to the limitations of the study: the bias inherent to this type of design due to its retrospective nature, the lack of controlled variables, introducing confounding factors that could influence the interpretation of the results. Other aspects to take into account are the limited number of patients, and the lack of information on other intestinal modulators such as diet, concomitant medications that can alter the bacterial flora or self-medication with over-the-counter prescriptions. Corticosteroid use, that has not been analysed, could also influence treatment response.

The use of antibiotics was associated with a reduction in PFS and OS in patients who received antibiotic treatment, not statistically different. More studies would be necessary to determine its real influence on the effectiveness of ICIs.

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None to declare.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Limitaciones del ensayo Xpert-MTB/RIF® en el diagnóstico inicial de tuberculosis en el contexto de un hospital rural en Etiopia

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RESUMEN

Introducción. Evaluar la implantación del Xpert-MTB/RIF®, como técnica de diagnóstico precoz, en una zona rural de Etiopía.

Material y métodos. Se recogieron retrospectivamente los datos de aquellos pacientes mayores de 13 años a los que se solicitó la prueba Xpert MTB/RIF® en un hospital rural situado a 45 km del laboratorio de referencia, durante los 3 primeros años de su implantación (abril 2015-abril 2018).

Resultados. Se evaluaron 306 pacientes mayores de 13 años, en 85 (27,8%) hubo un error en el procesamiento de la prueba y no se obtuvo el resultado. De las 221 muestras con resultados, la mediana de tiempo entre la obtención de la muestra y la recepción del resultado fue de 21 días y 42 de ellas fueron positivas (19%, IC 95%: 14,2-24,9%). La muestra de mayor rentabilidad diagnóstica fue la adenopatía (88,8%; [8/9];p<0.001).

Conclusiones. Hay más diagnósticos bacteriológicos con Xpert-MTB/RIF®, pero con una tardanza en obtener el resultado y no se logra su principal objetivo que es diagnóstico precoz.

Palabras clave: Xpert MTB/RIF; Etiopía; vida real; Tuberculosis

Limitations of the Xpert-MTB/RIF® assay in the initial diagnosis of tuberculosis in the context of a rural hospital in Ethiopia

ABSTRACT

Introduction. To evaluate the implementation of Xpert-MTB/RIF®, as an early diagnosis technique, in a rural area of Ethiopia.

Material and methods. Data were retrospectively collected from those patients over 13 years of age who were requested to take the Xpert MTB/RIF® test in a rural hospital located 45 km from the reference laboratory, during the first 3 years of its implementation (2015, April -2018, April).

Results. A total of 306 patients older than 13 years were evaluated, in 85 (27.8%) there was an error in the processing of the test and the result was not obtained. Of the 221 samples with results, the median time between obtaining the sample and receiving the result was 21 days and 42 of them were positive (19%, 95% CI: 14.2-24.9%). The sample with the highest diagnostic yield was adenopathy (88.8%; [8/9]; p<0.001).

Conclusions. There are more bacteriological diagnoses with Xpert-MTB/RIF®, but with a delay in obtaining the result and its main objective, which is early diagnosis, is not achieved.

Keywords: Xpert MTB/RIF; Ethiopia; real life; Tuberculosis

INTRODUCCIÓN

Etiopía es uno de los 30 países con mayores tasa de incidencia de tuberculosis (en 2020 se estimó en 132/100000 habitantes/año) y tasa VIH-tuberculosis y, hasta 2020, también uno de los que tenía mayor tasa de tuberculosis multirresistente (0,4% en 2020) [1]. La Organización Mundial de la Salud (OMS) recomienda desde 2010 el uso del Xpert-MTB/RIF® [2], prueba de diagnóstico rápido del *Complejo Mycobacterium tuberculosis* basada en biología molecular que también detecta resistencia a rifampicina. Inicialmente se recomendó para el diagnóstico de tuberculosis pulmonar en pacientes con sospecha de resistencia a la rifampicina o VIH positivos y, posteriormente, se amplió a tuberculosis extrapulmonares [3]. En Etiopía se puso en marcha en determinados laboratorios por todo el país, a partir de 2012 [4]. Numerosos estudios demuestran las ventajas diagnósticas que aporta Xpert MTB/RIF® [3,5,6], al facilitar que más pacientes comiencen un tratamiento adecuado lo antes posible.

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El impacto de una prueba depende no sólo de la rentabilidad diagnóstica de la misma, si no del contexto de la estrategia de implementación [7]. La experiencia en áreas de bajos recursos es escasa. El objetivo de este estudio fue valorar cómo Xpert-MTB/RIF® ha afectado al diagnóstico de tuberculosis en una zona rural de Etiopía.

MATERIAL Y MÉTODOS

Diseño del estudio. Se realizó un estudio observacional descriptivo retrospectivo. Se recogieron los datos de pacientes mayores de 13 años a los que se solicitó la prueba Xpert MTB/RIF® en el Hospital General de Gambo (HGG) durante los 3 primeros años de su implantación (desde abril de 2015 a abril de 2018).

El HGG es un hospital general rural situado en el sur de Etiopía que se encuentra aislado debido a las malas comunicaciones por vía terrestre y atiende a más de 200 pacientes adultos con tuberculosis al año [8].

Allí, el diagnóstico de tuberculosis se realizaba hasta 2015 según los protocolos del país mediante microscopía (convencional o fluorescencia (MF)) [9]. A partir de abril de 2015 se instauró en el Hospital de Kuyera (a 45 km de distancia, aproximadamente 2 horas en coche) un dispositivo para la realización de Xpert MTB/RIF® según los protocolos nacionales y las instrucciones del fabricante (Cepheid, Sunnyvale, CA, USA) [10]. Allí se remitió al menos una muestra (aspirado gástrico, esputo, aspirado de adenopatías, pus, líquido cefalorraquídeo, líquido ascítico) de cada paciente con sospecha clínica de TB del HGG que cumpliera alguno de los siguientes requisitos: VIH, tuberculosis extrapulmonar o sospecha de resistencia. Los resultados se reportaban desde el Hospital de Kuyera en papel y no de forma telemática.

Las variables que se recogieron fueron edad, sexo, fecha de envío de la muestra, tipo de muestra remitida, fecha de remisión de resultados, resultado de la muestra y presencia de resistencia a rifampicina.

Análisis de datos. Las variables continuas se expresaron como mediana y rango intercuartilico (RIC) y las cualitativas como su valor y porcentaje. Se calculó el intervalo de confianza del 95% (IC 95%) de la prevalencia de los resultados de Xpert MTB/RIF®. A continuación, se procedió a analizar los resultados de Xpert MTB/RIF®. En el análisis de las variables categóricas se empleó la chi-cuadrado y el test exacto de Fisher y las variables continuas mediante el test U-Man_Whitney. Además, se utilizó asociación de la magnitud mediante la odds ratio (ORs) (con IC 95%). Se consideró significativo un $p<0,05$. Se utilizó SPSS para el análisis estadístico.

Aspectos éticos. Los Comités de Ética en Investigación del Hospital Rural General Gambo, Etiopía (Código GGRH-241-18) y de la Fundación Hospital Universitario Jiménez Díaz, España (Código EO187-18_FJD) aprobaron el estudio.

Tabla 1 Características de la población

	Total
Edad, mediana (RIC)	35 (25-45)
Sexo	
Hombre	172 (56,2%)
Mujer	134 (43,8%)
Tipo de muestra, n (%)	
Esputo	213 (69,6)
Líquido pleural	42 (13,7)
Líquido ascítico	29 (9,5)
Adenopatía	9 (2,9)
Muestra purulenta	7 (2,3)
Aspirado gástrico	4 (1,3)
LCR	2 (0,7)
Días*, mediana (RIC) (n=116)	21 (13-33)
Resultado XPERT-MTB/RIF®	
Positivo	42 (13,7)
Negativo	179 (58,5)
Error en el procesamiento	85 (27,8)

***Días entre la toma de muestras y facilitación del resultado al paciente. LCR: líquido cefalorraquídeo. RIC: recorrido interquartilico.**

RESULTADOS

Durante el periodo de estudio se evaluaron 306 pacientes mayores de 13 años a los que se les había solicitado una prueba Xpert MTB/RIF desde el HGG. La mayoría de las muestras eran de esputo (69,6%). En la tabla 1 se recogen las principales características de los pacientes.

En 85 (27%, IC 95%: 22,8-32,8%) de 306 muestras procesadas hubo un error en el procesamiento de la prueba (pérdidas de muestras, pérdidas de los documentos de resultados, cortes de electricidad, faltas de reactivos...). De las 221 muestras con resultados, 42 fueron positivas (19%, IC 95%: 14,2-24,9%). La mediana de tiempo entre la obtención de la muestra y la recepción del resultado fue de 21 días, en los 116 casos en los que se dispuso de este dato (Tabla 1).

La muestra con mayor proporción de positivos fueron las adenopatías 8/9 (88,9%), en cambio los esputos lo fueron en un 23/146 (15,8%) (Tabla 2). Cuando se comparó el porcentaje de resultados positivos según el tipo de muestra, teniendo como referencia el esputo, destacaba un OR 10,69 de tener un resultado positivo en las adenopatías respecto al esputo ($p=0,001$). El OR de resultado positivos en aspirado gástrico y pus fue de 10,69 y 4,01 pero sin significación estadística (Tabla 2). De las 221 muestras, sólo una muestra de esputo fue resistente a rifampicina (0,45%).

Tabla 2 **Resultados del XPERT-MTB/RIF®**

	Positivo [n/N (%)]	Negativo [n/N (%)]	OR (IC del 95%)	Valor P
Esputo	23/146 (15,8)	123/146 (84,2)	Ref	-
Líquido pleural	3/29 (10,3)	26/29 (11,8)	0,67 (0,17-2,20)	0,458
Líquido ascítico	3/25 (12,0)	22/25 (88,0)	0,79 (0,20-2,63)	0,630
Adenopatías	8/9 (88,9)	1/9 (11,1)	42,78 (5,10-358)	0,001
Pus	3/7 (42,9)	4/7 (57,1)	4,01 (0,84-19,12)	0,081
Aspirado gástrico	2/3 (66,7)	1/3 (33.3)	10,69 (0,93-1,22)	0,057
LCR	0/2 (0.0)	2/2 (100)	-	-

LCR: líquido cefalorraquídeo, OR: odds ratio; IC: intervalo de confianza del 95%.

DISCUSIÓN

Según nuestros datos, el Xpert-MTB/RIF® permite llegar al diagnóstico en cerca del 20% de las muestras procesadas. Previamente se utilizaba la MF en el HGG y se diagnosticaban en torno al 8,8% de las muestras [9], por lo que sería una ventaja utilizar el Xpert-MTB/RIF®. Esto es especialmente relevante en las adenopatías (88,9% de resultados positivos), como refieren otros estudios [3] [11], siendo menor en otras muestras como pus (42,9%), líquido ascítico (12%) o líquido pleural (10,3%) y llamativamente menor en esputo (15,8%), probablemente debido a que se envían muestras respiratorias solo cuando existe sospecha de tuberculosis extrapulmonar y no se tiene otra muestra.

El impacto de una prueba diagnóstica no sólo depende de la sensibilidad y especificidad de ésta, sino de otras variables como la correcta obtención y procesamiento de las muestras, el envío en tiempo y forma y la accesibilidad a la misma. En el caso de zonas remotas como el HGG, la accesibilidad a dicha prueba es escasa por la dificultad de acceso terrestre, lo que lleva a demoras en el transporte de la muestra tanto hacia el hospital de referencia como de remisión de los resultados. A esa dificultad hay que añadir las propias asociadas a centros de países de baja renta que incluyen la falta puntual de kits, los cortes de electricidad...[12].

En nuestra serie hay un marcado retraso en los resultados (21 días de mediana) y un elevado error en el procesamiento (en casi un 28% de las muestras no se pudieron obtener resultados), en comparación con estudios previos (con un error en torno al 15%) [12]. Esto se podría explicar porque en el caso del HGG además de las dificultades por falta de medios, habría que sumarle el transporte a un centro de referencia con más posibilidades de pérdidas tanto de las muestras como de los resultados.

La OMS, para la implementación del Xpert MTB/RIF, señala que es necesario desarrollar sistemas para comunicar los resultados el mismo día en que se obtengan [13]. En nuestro caso, eso no se ha logrado durante los 3 primeros años de implementación de la técnica.

Las limitaciones fundamentales del estudio recaen sobre la dificultad de obtener datos de los registros no digitalizados de hospitales de países de baja renta. Sin embargo, este estudio es singular dado que pone de manifiesto lo que sucede en la vida real: la disponibilidad teórica de una prueba sensible y específica como Xpert-MTB/RIF® resulta de escasa utilidad clínica cuando los circuitos precisos para su empleo no funcionan adecuadamente.

Por ello podemos concluir que el Xpert-MTB/RIF® mejora el rendimiento diagnóstico de la tuberculosis, si bien la efectividad clínica se ve reducida por circunstancias relacionadas con el manejo de muestras y resultados. Por tanto, para para aprovechar todo el potencial diagnóstico de esta técnica, es preciso adaptar todos los procesos que requiere y eso incluye la correcta toma y envío de muestras y la rápida y eficiente comunicación de los resultados.

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CONFLICTO DE INTERESES

Los autores declaran no tener conflicto de interés

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Carta al Director

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Impacto de la COVID-19 en el diagnóstico tardío de VIH: a propósito de un caso

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Estimado Editor:

Durante la pandemia por COVID-19, han disminuido las consultas en los servicios de urgencias hospitalarias (SUH) no relacionadas con COVID-19. Este retraso en la búsqueda de atención médica puede conducir a un aumento de la morbilidad y la mortalidad. Este caso es un ejemplo de "daños colaterales" causados por la pandemia COVID-19.

Se presenta el caso de una mujer de 47 años, de origen latinoamericano y residente en España desde hacía tres meses, sin antecedentes patológicos de interés, y que consultó el 25 de noviembre de 2020 –segunda ola de pandemia COVID 19- en urgencias por un cuadro de un mes de evolución consistente en fiebre, odinofagia y astenia. Había realizado múltiples consultas previas por este mismo cuadro clínico y recibido tratamiento empírico con amoxicilina-clavulánico 875/125 mg durante 10 días sin respuesta. En la última semana se añadió epigastralgia intensa que dificultaba la ingesta oral y disnea a moderados esfuerzos. Al examen físico estaba hemodinamicamente estable, eupneica, y se observó la presencia de placas blanquecinas en lengua y paladar compatibles con candidiasis orofaríngea. En analítica tenía unos leucocitos totales de 1740 cel/mm^3 y unos linfocitos totales de 180 cel/mm^3. En la RX tórax se observaron infiltrados tenues bilaterales. La PCR para SARSCoV-2 fue negativa. Se ingresó en planta para estudio y tratamiento. La serología para virus de inmunodeficiencia humana (VIH), fue positiva y los linfocitos CD4 de 24 cel/mm^3. El TAC torácico fue compatible con neumonia por *Pneumocystis jirovecii* (Figura 1A), el diagnosticó se confirmó mediante lavado bronco-alveolar, se practicó una fibrogastroscopia que fue sugestiva de esofagitis por citomegalovirus (Figura 1B), la biopsia confirmó el diagnóstico. Se realizó tratamiento específico de las infecciones oportunistas diagnosticadas con buena respuesta y se inició tratamiento antirretroviral bictegravir, emtricitabina, tenofovir, con correcta tolerancia. Actualmente, presenta un recuento de CD4 366 cel/mm^3 con carga viral indetectable, y buena calidad de vida.

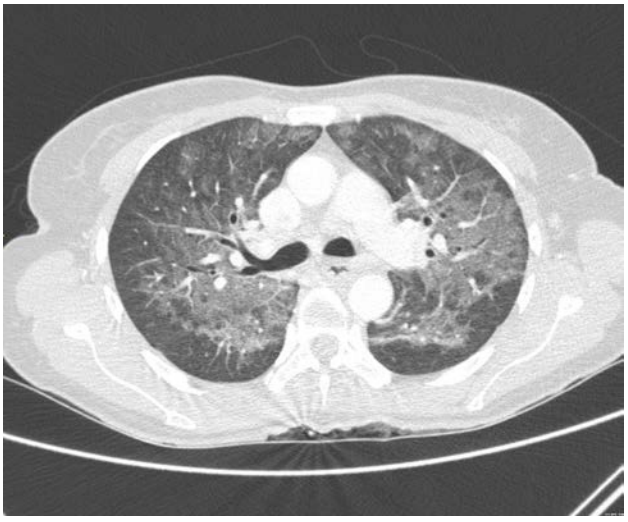

Figura 1A | **TAC Tórax. Imágenes en vidrio deslustrado de predominio central, de distribución bilateral y panlobar.**

En España la tasa de personas que desconocen estar infectados por el VIH es del 14% y se estima que en el 47,6% de los pacientes se diagnostican en estadios avanzados (cifras de linfocitos T CD4 inferiores a 350 células/µl en la analítica inicial) [1]. La mayoría de estos pacientes han tenido contactos previos con el sistema sanitario por lo que existen oportunidades diagnósticas perdidas [2]. Aproximadamente el 50% de los pacientes han tenido alguna visita en un SUH en los 3 años previos al diagnóstico [3]. Desde la Sociedad Española de Medicina de Urgencias y Emergencias (SEMES)

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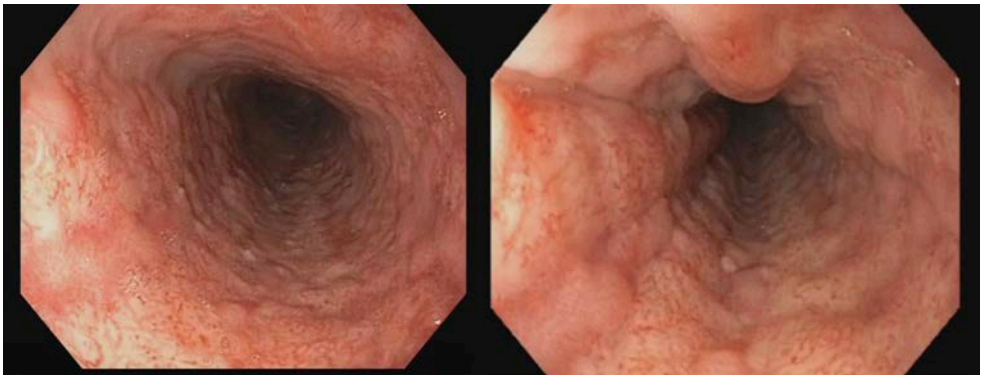

Figura 1B | **Gastroscopia. Afectación mucosa de toda la circunferencia, con presencia de úlceras superficiales, no profundas, de centro pálido, siendo compatibles con esofagitis en tercio medio.**

se han propuesto una serie de actuaciones en los SUH con el objetivo de promover el cribado del VIH en las consultas por infecciones de transmisión sexual, profilaxis post exposición, herpes zoster, práctica del chemsex, neumonía adquirida en la comunidad, y síndrome mononucleósico [4] . De forma similar a lo sucedido durante la primera ola pandémica de COVID-19 [5], se han objetivado diferencias en el acceso a los test diagnósticos de VIH en los SUH entre diferentes comunidades autónomas y según el tipo de hospital. Los centros terciarios o de mayor actividad asistencial disponen de circuitos más sólidos que permiten la solicitud y la obtención de resultados de la serología de VIH además proporcionan un seguimiento y tratamiento precoz por parte de los médicos especialistas en VIH [6].

Por otro lado, la pandemia por COVID-19, ha provocado cambios profundos a nivel social y en la organización del sistema sanitario [7]. En el caso de los servicios de urgencias hospitalarios, la asistencia se desdobla en el momento del *triage* en dos circuitos diferenciados en función de si el paciente es sospechoso de tener COVID-19 o no [8,9]. La paciente se valoró inicialmente en el circuito COVID ya que presentaba síntomas compatibles con dicha patología (fiebre, disnea y odinofagia), sin embargo, una vez descartado COVID-19 es necesario seguir el proceso diagnóstico ya que puede presentar otras patologías potencialmente graves.

Finalmente, destacar que el caso presentado, a pesar de llevar poco tiempo en nuestro país, ya había tenido varios contactos con el sistema sanitario, estas oportunidades perdidas condicionan diagnósticos tardíos, posiblemente contagios del virus a otras personas y, con seguridad, incremento de los costos sanitarios. La pandemia por COVID-19 podría favorecer esta situación, debido a los retrasos en la búsqueda de atención médica y a los cambios en la organización del sistema sanitario. En este sentido, queremos enfatizar la importancia de aplicar las recomendaciones dirigidas a los servicios de urgencias para el diagnóstico de infección por VIH [4].

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CONFLICTO DE INTERESES

Los autores declaran no tener conflicto de interés

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Carta el Editor

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Eumicetomas por *Fusarium oxysporum* y *Madurella mycetomatis*. Descripción de dos casos y revisión de la bibliografía

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Estimado Editor: El micetoma es una infección crónica de tipo inflamatorio con afectación de piel, tejido subcutáneo, fascia y hueso caracterizado por deformidad de la zona con aparición de lesiones de aspecto nodular y posible fistulización con drenaje de exudado en ocasiones en forma de granos. Una de sus localizaciones habituales, asociada al mecanismo de transmisión del agente causal, es la extremidad inferior, causando el denominado pie de Madura. Se considera una infección subcutánea clasificada en dos tipos según el agente etiológico [1]: el eumicetoma (por hongos filamentosos) y el actinomicetoma (por bacterias del Orden Actinomycetales entre las que destacan tres géneros [2]: *Nocardia*, *Streptomyces* y *Actinomadura*).

Los eumicetomas están producidos a su vez por dos tipos de hongo filamentoso tabicado: hongos negros (feohifomicetos del tipo *Madurella* spp.) y hongos blancos hialinos (hialohifomicetos del tipo *Pseudallescheria* spp, *Acremonium* spp., *Fusarium* spp. o incluso *Aspergillus* spp. o dermatofitos). Si bien se incluyen al menos 41 especies de hongos implicadas en estos procesos [3], a nivel mundial más del 90% de los mismos se asocian a cuatro agentes etiológicos: *Madurella mycetomatis, Madurella (Trematosphaeria) grisea, Pseudallescheria (Scedosporium) boydii y Leptophaeria (Falciformispora) senegalensis* [2]. Otras múltiples especies, entre las que destacan las de los géneros *Phialemonium* [4] y *Fusarium* [2] han sido descritas como causas de esta entidad, aunque con mucha menor frecuencia.

Esta infección tiene mayor incidencia en el área conocida como "cinturón o franja del micetoma". Algunos autores definen esta zona geográfica según países afectados ("Venezuela, Chad, Etiopía, India, Mauritania, México, Senegal, Somalia, Sudan y Yemen"), otros por la latitud geográfica dada entre 30ºN y 15ºS (próxima al Trópico de Cáncer) [5] y otros por el amplio término de "regiones tropicales y subtropicales".

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Sin embargo, la creciente migración y la facilidad para los desplazamientos poblacionales han hecho que esta patología pueda presentarse en prácticamente todo el mundo. Se han descrito infecciones de micetoma en 102 países, muchos de ellos europeos [6].

El objetivo del trabajo es describir 2 casos de eumicetoma con afectación de piel, partes blandas y/o hueso en pie producidos por *Fusarium oxysporum* y *Madurella mycetomatis* recogidos en el Hospital Universitario Doctor Peset de Valencia entre los años 2017 y 2020, analizando sus características clínico- radiológicas, diagnóstico etiológico y opciones terapéuticas médico- quirúrgicas adoptadas.

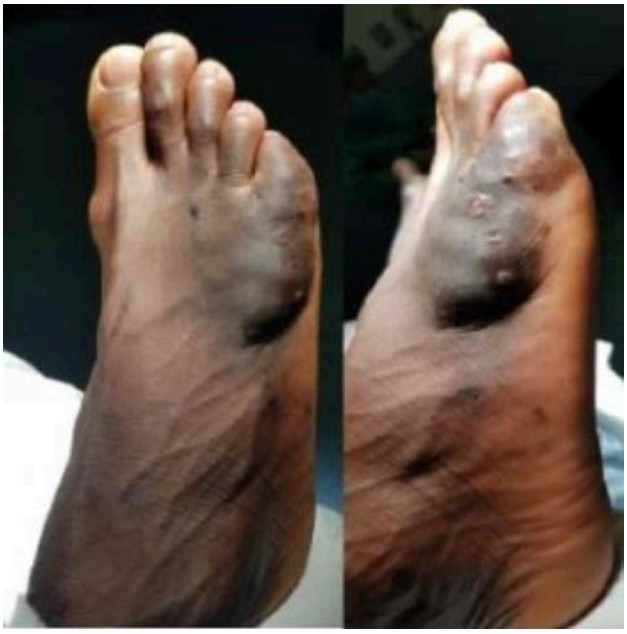

Figura 1 | **Lesión que provoca deformidad y tumoración en dorso de pie derecho.**

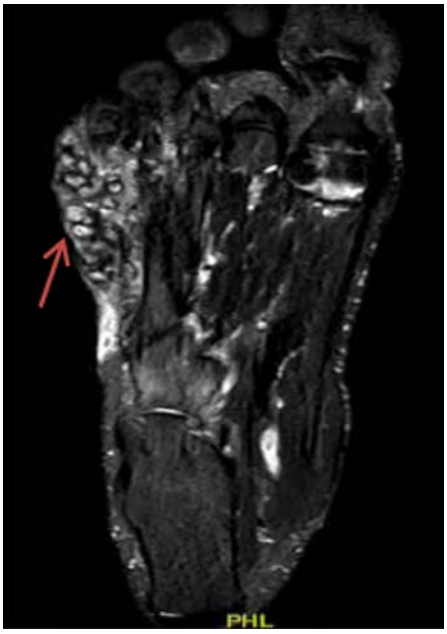

Figura 2 | **RM del pie derecho en secuencia STIR en la que se observa lesión heterogénea con el signo del punto central, sugestivo de micetoma.**

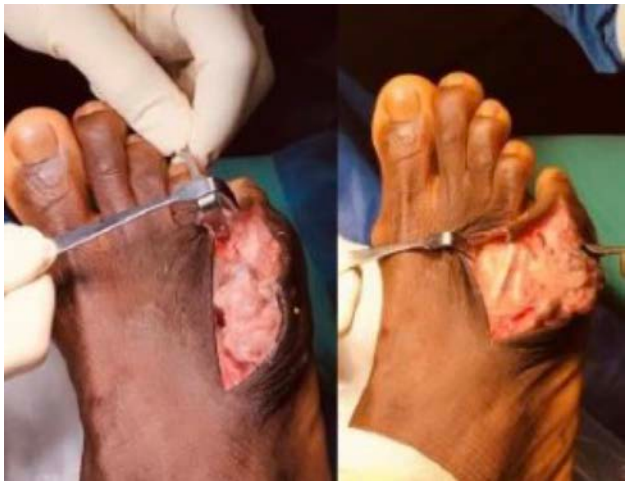

Figura 3 | **Imagen del desbridamiento de la lesión en la que se observa la infiltración del micetoma y la presencia de trayectos fistulosos.**

Caso 1. Varón de 40 años, procedente de Mali, que tras pinchazo con astilla de madera hace 6 meses en su país de origen, presentó tumoración con signos inflamatorios y fístulas en dorso de antepie derecho (Figura 1). En la radiografía simple no se apreció afectación ósea. Se solicita una ecografía en la que se observa una tumoración de partes blandas, de naturaleza predominantemente sólida, isoecoica, de aspecto polilobulado, con flujo vascular Doppler y presencia de zonas con marcada hipertrofia de los vasos asociada. Se realiza resonancia magnética (RM) en la que se aprecia una lesión heterogénea con hallazgos compatibles con infección por micetoma (actinomicetoma/eumicetoma) como es el signo del punto central (Figura 2)

Se realizó biopsia cutánea que fue remitida para estudio bacteriológico y micológico. En el examen microscópico tras procesamiento con hidróxido potásico (KOH) al 10% y tinción con blanco calcoflúor (en microscopio de fluorescencia) se informó de la presencia de formas compatibles con hongo filamentoso tabicado. La muestra fue cultivada en los medios de rutina, incluyendo siembra en medio de Sabouraud dextrosa agar. Se observó crecimiento a los 3-5 días de un hongo filamentoso hialino identificado mediante MALDI-TOF como *F. oxysporum,* que mostró sensibilidad *in vitro* a anfotericina B y voriconazol, siendo resistente a fluconazol.

Como tratamiento se realizó desbridamiento quirúrgico con exéresis completa de la lesión y piel afecta (Figura 3). Además, el tratamiento se completó con antifúngico (voriconazol oral 200mg cada 12 horas) durante 5 meses.

El paciente fue revisado periódicamente en consulta mostrando mejoría de la herida. A los 6 meses de la intervención el paciente se encontraba asintomático, realizándose una RM de control en la que se evidenció la ausencia de la tumoración.

Caso 2. Paciente de 23 años, procedente de Mali, sin antecedentes de interés, que tras pinchazo accidental con cuerpo extraño desconocido acude a urgencias, presentando tumoración en borde externo del pie y antepié derecho.

Se realiza radiografía simple de pie derecho en la que se aprecia una lesión lítica de 25 mm en hueso cuboides, con bordes bien definidos, sin observar reacción perióstica ni otros hallazgos que sugieran malignidad. También se realiza una ecografía, en la que se identifica una lesión sólida de 7 cm, de estructura heterogénea muy vascularizada que afecta a musculatura de planta del pie. En el dorso del pie se identifica material ecogénico, no fluctuante y sin vascularización con componente sólido adyacente muy vascularizado y con extensión a musculatura del dorso.

Se realiza RM con contraste (Figuras 4 y 5) en la que se aprecia una amplia afectación del pie tanto en la vertiente plantar como dorsal. La estructura de la lesión es heterogénea, identificando en secuencia STIR signos radiológicos de afectación por micetoma (signo del punto central) en toda la infiltración muscular.

Tras los hallazgos radiológicos, se realiza biopsia cutánea, en la que se aprecia macroscópicamente minúsculos fragmentos de coloración negruzca. Se remite la muestra a Microbiología que confirmas la presencia de granos negros eumicóticos al observar en el examen microscópico directo la presencia de estructura miceliares compatibles con hongo filamentoso y vesículas oscuras. El cultivo en medio de BHI agar y medio de Sabouraud positivizó a partir de los 10 días, siendo identificado *M. mycetomatis* sensible *in vitro* a anfotericina B y voriconazol.

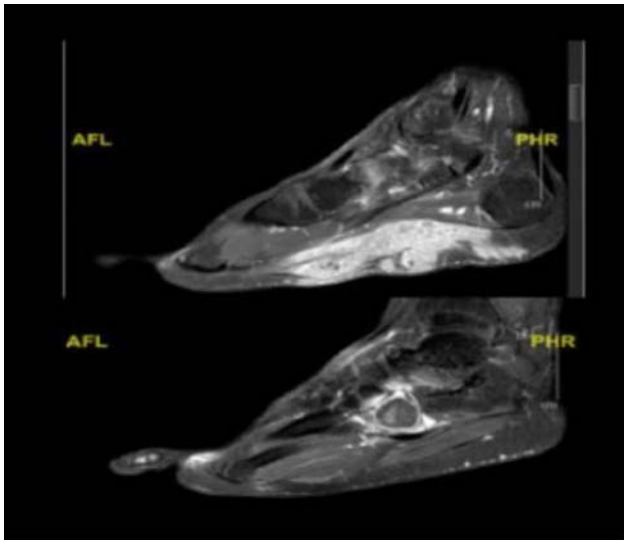

Figura 4 **RM T1. A nivel dorsal, ocupación completa de hueso cuboides por componente hipointenso y anillo periférico hiperintenso que infiltra M.extensor de los dedos. A nivel plantar, amplia infiltración desde dermis a M. Flexor de los dedos.**

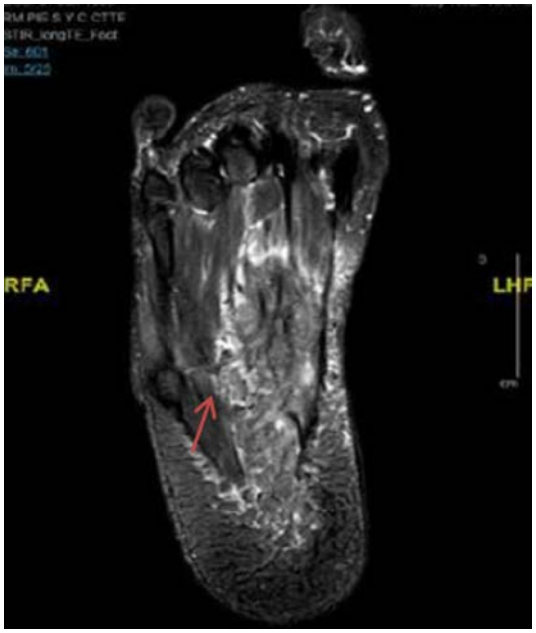

Figura 5 **En secuencia STIR, signo del punto central en infiltración muscular de región plantar.**

Como tratamiento se realizó desbridamiento quirúrgico con exéresis completa de la lesión y piel afecta. Para el tratamiento de la lesión ósea del cuboides se usó un sustituto de sulfato de calcio absorbible (Stimulan®) impregnado con voriconazol.

La herida quirúrgica tuvo una evolución tórpida, necesitando dos nuevas intervenciones y tratamiento antifúngico endovenoso (voriconazol iv a dosis de 4mg/kg/12 horas), dilatando el ingreso hospitalario hasta los 60 días.

Tras dicho periodo el paciente fue dado de alta con voriconazol 200mg/12 horas durante 5 meses. A los 6 meses del alta hospitalaria, el paciente se encontraba sin clínica y se le realizo una nueva RM en la que se evidenció la ausencia de tumoración.

En los casos presentados, tanto el origen de los pacientes y la lesión desencadenante, así como la tríada tumor, tractos sinusales y gránulos macroscópicos son útiles para establecer un diagnóstico clínico de micetoma. Además, la presencia en la lesión de gránulos negros se considera indicativa una lesión de etiología fúngica (eumicetoma) por feohifomicetos [7].

En relación al estudio radiológico de este tipo de lesiones diversos autores [8-11] subrayan la importancia de la resonancia magnética para visualizar la afectación de tejidos blandos y destrucción ósea asociados a micetoma. En la RM se aprecian múltiples pequeñas lesiones hiperintensas esféricas separadas por tejidos de baja intensidad de señal. Algunas de estas lesiones muestran un pequeño foco hipointenso central. Estos focos hipointensos se denominan "dot-in-circle" y se observan aproximadamente en el 80% de los pacientes, definido como patognomónico de los micetomas, siendo útil en el diagnóstico diferencial de esta identidad con otras infecciones y tumores [10].

Sin embargo, si bien la clínica y la radiología nos ayudan a definir la presencia de micetoma, éstas no resultan suficientes para establecer el diagnóstico definitivo. Resulta necesario el procesamiento de las muestras de biopsia de la lesión para estudio microbiológico, incluyendo visión directa y cultivo micológico, y poder así llegar al diagnóstico etiológico del proceso y al estudio *in vitro* de sensibilidad de la cepa implicada. Para ajustar en su caso el tratamiento antifúngico asociado. En nuestros casos ambos pacientes presentaron hifas en la visión directa tras tratamiento con KOH al 10%, apoyando de forma rápida el diagnóstico inicial de lesión fúngica. El cultivo micológico y aislamiento de *M. mycetomatis* y de *F. oxysporum* confirmó el diagnóstico de eumicetoma.

La mayoría de los estudios publicados [6,8,12,13] indican, en relación al abordaje terapéutico de estas lesiones, la necesidad de una combinación médica (antifúngico) y quirúrgica (exéresis de la lesión) para lograr el éxito en el tratamiento del eumicetoma,

Las lesiones pequeñas tienen una buena respuesta clínica al itraconazol, siendo el antibiótico de elección [9]. Otra buena opción de primera línea también es el voriconazol 400–600 mg/día [9,12,14], menos empleado en la literatura por su escasa

accesibilidad en los países en desarrollo, pero con muy buenos resultados [9].

Sin embargo, en las lesiones medianas a grandes, el tratamiento quirúrgico cobra más importancia. En la mayoría de los estudios la escisión quirúrgica amplia del micetoma y cierre directo es la intervención más frecuentemente realizada en comparación con la amputación [14,15,17]. Las lesiones mayores de 10 cm pueden requerir cobertura mediante un colgajo [17]. La amputación está indicada cuando se identifica amplia destrucción ósea, múltiples recurrencias que ponga en riesgo la vida del paciente o pacientes ya discapacitados [17].

Como adyuvante al tratamiento quirúrgico, el itraconazol a dosis de 200 a 400 mg al día hasta que se logre la curación parece ser la estrategia terapéutica más indicada [9] La duración óptima del tratamiento antifúngico para el eumicetoma es incierta y depende de las circunstancias de cada caso. En general, se considera adecuado mantener al menos 6-12 meses de terapia, aunque el tratamiento requerido puede alargarse hasta por dos años o más [16].

En los casos presentado el tratamiento se completó con antifúngico (voriconazol oral 200mg cada 12 horas) durante 5 meses

La tasa de recurrencia posoperatoria varía entre el 25 y el 50% [15] y puede ser local o a distancia, tanto en los ganglios linfáticos regionales o en órganos distales como el pulmón, el abdomen cavidad o médula espinal.

Para concluir, la mayoría de los casos de eumicetomas se producen en pacientes procedentes de países con alta incidencia. Sin embargo, la migración y/o la posible producción de casos autóctonos fuera del entorno geográfico habitualmente asociado a esta patología, nos obliga a su detección activa ante sospecha en nuestro medio.

La presencia de tumoraciones con tumefacción, fistulas y gránulos que responden mal a tratamiento antibiótico bacteriano en pacientes con antecedente epidemiológico sugerente, deben ser valorados como sospecha de infección eumicótica para mejorar la demora diagnóstica y el correcto tratamiento de estos pacientes.

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CONFLICTO DE INTERESES

Los autores no presentan ningún conflicto de intereses.

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Carta al Director

Revista Española de Quimioterapia
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Pacientes con inmunización frente a SARS-CoV-2 que han requerido ingreso hospitalario por neumonía COVID-19 en un hospital comarcal (Sierrallana-Cantabria)

Medicina Interna. Hospital Sierrallana. Barrio Ganzo s/n. Torrelavega. Cantabria

Article history
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Estimado Editor: Tras la instauración de la vacuna para SARS-CoV-2 en la población general, hemos asistido a un descenso en el número de ingresos por neumonía causada por este virus, impresionando de un curso más indolente con una menor mortalidad por el mismo [1]. Sin embargo, dado que la eficacia de la vacunación ha sido mundialmente debatida [2], pretendemos analizar con este estudio las características de los pacientes que a pesar de la vacunación han presentado neumonía y han requerido de ingreso hospitalario y cuáles han sido los factores de mal pronóstico.

Se trata de un estudio observacional retrospectivo, en el que recopilamos variables demográficas, clínicas y analíticas de todos los pacientes vacunados frente a SARS-CoV-2 (pauta completa para esa fecha: 2 dosis; "pauta incompleta": 1 dosis) que ingresaron a lo largo del año 2021 en nuestro hospital (hospital comarcal) con diagnóstico clínico/radiológico de neumonía COVID-19. Realizamos el análisis estadístico de las variables estudiadas utilizando el programa SPSS, empleando los estadísticos Chi cuadrado y t de Student, con un nivel de significación del 0.05.

Ingresaron un total de 183 pacientes. Con una edad media de 71,05 ± 15,28 años. El 40.4% fueron mujeres. 29 pacientes estaban institucionalizados (15,8%). El tiempo medio desde el inicio de síntomas al ingreso fue de 6,92 ± 4,28 días. La estancia media hospitalaria fue de 9,31 ± 8,33 días.

Al ingreso 39 pacientes (21,3%) presentaba neumonía con criterios de gravedad (PAFI inferior a 300). La PAFI media al ingreso fue de 372,58 ± 211,50. El 10,9% presentó clínica digestiva asociada. El 20,8% estaban obesos, el 14,2% padecía enfermedad respiratoria previa, el 16,4% eran fumadores, el 26,8% eran diabéticos, el 19,1% tenían enfermedad renal crónica, el 13,1% eran pacientes oncológicos, el 3,8% presentaban hepatopatía, el 6% eran inmunodeprimidos (uso crónico de corticoterapia, o fármacos biológicos, o VIH).

En cuanto a parámetros inflamatorios analíticos, observamos que el valor medio de PCR fue de 10,52 ± 17,05 mg/dl, la cifra media de Dímero D fue de 1740,11 ± 3889,842 mcg/ml y de ferritina 1142,81 ± 5488,496 ng/mL. La media de linfocitos fue 2490,45 ± 5095,87.

En cuanto al tratamiento instaurado al ingreso se pautaron broncodilatadores inhalados a un 13,7%, broncodilatadores nebulizados al 12%, remdesivir al 5,5%, tocilizumab al 8,2% y antibioterapia a un 49,7%.

En la tabla 1 adjuntamos los datos que relacionan la vacunación con la evolución clínica.

Las variables que relacionaron de forma estadísticamente significativa con traslado a UCI fueron la edad (p=0,03), la PaFi (p< 0,001), y niveles altos de dímero D, (p= 0,04), PCR (p= 0,001) y ferritina (p= 0,011).

Falleció en el Hospital Sierrallana el 4,9% del total de pacientes de la muestra (9 pacientes) (todos con una pauta "incompleta" de vacunación), siendo la edad, la PaFi, estar institucionalizado previamente y los días desde la instauración de síntomas hasta el ingreso, las variables que implicaron más mortalidad (significación de p<0,0001 -relación directa-, p=0,001 – relación inversa-, p=0,009 y p=0,001 – relación directa - respectivamente). No encontramos relación estadística entre mayor mortalidad y el sexo, el tipo de vacuna recibida, padecer síntomas digestivos o los valores de PCR, dímero D y ferritina.

En cuanto a las comorbilidades, observamos un aumento de la mortalidad en pacientes oncológicos (p=0,04) y con enfermedad cardiovascular (p=0,03). Sin embargo, no obtuvimos relación estadísticamente significativa entre pacientes con DM, patología respiratoria, ERC, hepatopatía, obesidad o hábito tabáquico. Tampoco encontramos mayor mortalidad en pacientes inmunosuprimidos no oncológicos.

De los 183 pacientes, 135 habían recibido la vacuna de

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Tabla 1 **Tasas de vacunación y evolución clínica**

	Vacunación completa	No vacunación completa	Total
No traslado a UCI	107 (58,5%)	40 (21,8%)	147 (80,3%)
Traslado a UCI	15 (8,2%)	12 (6,6%)	27 (14,8%)
Éxitus	0 (0%)	9 (4,9%)	9 (4,9%)
Total	122 (66,6%)	61 (33,4%)	183 (100%)

Del grupo de los pacientes con pauta de vacunación completa no falleció ningún paciente (p=0,03).

Pfizer (0,015% de neumonía en vacunados con Pfizer), 14 Moderna (0,008% de neumonía en vacunados con Moderna), 22 Astrazeneca (0,018% de neumonía en vacunados con Astrazeneca) y 12 Janssen (0,054% de neumonía en vacunados con Janssen) (los porcentajes se han realizado en relación a la vacunación global en la comunidad de Cantabria).

En el año 2020, previamente a la campaña de vacunación contra la COVID-19, el número de pacientes que ingresaron por neumonía por SARS-CoV-2 en nuestro hospital fue de 315 pacientes.

La mayoría de los pacientes vacunados que ingresaron con diagnóstico de neumonía COVID no presentaban criterios de gravedad, con una estancia media hospitalaria no excesivamente prolongada y una baja mortalidad.

En cuanto a los tratamientos que recibieron nuestros pacientes al ingreso, lo más utilizado fueron los broncodilatadores inhalados.

La pauta de vacunación más utilizada en los ingresados fue la de Pfizer. Sin embargo, el grupo de Moderna es en el que menos neumonías hemos registrado

Con los resultados de nuestro estudio podemos concluir que el hecho de presentar cifras elevadas de PCR, dímero D y ferritina arroja mayor gravedad a la infección por SARS-CoV-2 y mayor necesidad de traslado a unidades de cuidados críticos, sin embargo, no implica mayor mortalidad.

Presentan mayor mortalidad los pacientes de más edad, que presentaron menor PaFi, que estaban institucionalizados previamente o que habían ingresado tarde respecto al día de aparición de los síntomas. Además, ser paciente oncológico o padecer enfermedad cardiovascular también se relacionó con mayor mortalidad, no así otras comorbilidades habituales de la población general.

En el año anterior, en 2020, cuando todavía no se había completado la campaña de vacunación, el número de pacientes que ingresaron por neumonía por SARS-CoV-2 en nuestro hospital fue de 315 pacientes. Podemos afirmar que coincidiendo con el inicio de la vacunación el número de ingresos se vio reducido en aproximadamente un 40% pero que son necesarias más revisiones para descartar otra serie de factores que hayan podido influir en la reducción de la incidencia.

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CONFLICTO DE INTERESES

Los autores no presentan ningún conflicto de intereses

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Letter to the Editor

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Nosocomial meningitis caused by ESBL- and OXA-48-producing *Klebsiella pneumoniae* and treated with ceftazidime-avibactam. Report of one case and review of the literature

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Sir,

The infections caused by carbapenemase-producing *Klebsiella pneumoniae* (CPKP) have limited treatment options, [1-3]; this implies an even worse problem if the infection is located in the central nervous system (CNS), due to the limited blood-brain barrier (BBB) penetration of the antibiotics and, therefore, the need for higher doses. There is scarce evidence regarding the optimal dose and the cerebrospinal fluid (CSF) concentration of most new antibiotics used for the treatment of CPKP, including OXA-48-producing *K. pneumoniae*.

CAZ-AVI is a new beta-lactam combined with extended-spectrum beta-lactamase (ESBL) inhibitor against Gram-negative bacilli (GNB), both fermenters and non-fermenters [2,4], whose action spectrum include Ambler class A (KPC), class D (OXA-48), and many class C (Amp-C) CPKP [5-9]. Thus, it constitutes the first therapeutic option in CPKP-triggered infections that are not located in the urinary tract [10].

We report the case of a patient with nosocomial meningitis caused by ESBL- (CTX-M-15) and OXA-48-producing *K. pneumoniae* related to an external lumbar drainage (ELD) that was successfully treated intravenous CAZ-AVI in combination with intrathecal amikacin. In addition, we also perform a review of the literature of CPKP-triggered nosocomial meningitis.

Our patient was a 50-year-old male who was admitted to our hospital due to a craniocerebral trauma (CCT). A CT-scan was performed and showed multiple skull fractures, bilateral subdural hematoma, generalized subarachnoid hemorrhage, frontal lobe seizures, and cerebral edema. He required an urgent neurosurgery with a decompressive craniectomy, fracture repair, and edema removal. After the surgery, he was admitted to the Intensive Care Unit (ICU).

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During his stay in the ICU, he underwent a rectal culture that resulted positive for ESBL- and OXA-48-producing *K. pneumoniae*, which was isolated in selective chromogenic media (CHROMID ESBL and CHROMID CARBA SMART (bioMérieux)). Consequently, the appropriate isolation measures were implemented. Afterwards, the patient developed a mechanical ventilation-associated pneumonia due to such pathogen and, therefore, treatment with CAZ-AVI was decided.

At the 20th day of admission, the patient was moved to the neurosurgery ward in a situation of unresponsive wakefulness syndrome with a percutaneous tracheostomy. Nine days after, an ELD was required as a consequence of cognitive impairment. It was not possible to perform an external ventricular drainage (EVD) due to the lack of bone support at the cranial vault.

At the 39th day of admission, he developed a severe sepsis without any other apparent focal point of infection. Blood and CSF samples were obtained for culture, and treatment with meropenem, in expanded perfusion, and vancomycin was started. The CSF culture showed biochemical and cytological results consistent with bacterial meningitis (Leukocytes 1016 leuk/ul, 75% PMN, GLU 41 mg/dl, (blood glucose 93 mg/dl) proteins 129.3mg/dl, lactate 5.4 mmol/L). An ESBL- and OXA-48-producing *K. pneumoniae* was isolated, using mass spectrometry for the identification (MALDI Biotyper system®, Bruker Daltonics, Germany), and fast colorimetric (β-lactatest®, BIO-RAD y β-carbatest®, BIO-RAD); in addition, immunochromatographic (NG-Test CARBA5®, NG-BIOTECH) assays were employed for the evaluation of the susceptibility and mechanisms of resistance. The ESBL- (CTX-M-15) and OXA-48-producing *K. pneumoniae* was resistant to meropenem (MIC > 8 mg/L) and susceptible to CAZ-AVI, with a MIC of 0.5 mg/L (Vitek2 bioMérieux) [11].

Thus, CAZ-AVI treatment was decided and doses were optimized, increasing the frequency of administration to 2/0.5g every 6 hours, and intrathecal amikacin was added (following our hospital recommendations) during 21 days. After 48 hours

of treatment, the ELD shunt was changed and CSF cultures were repeated: at the moment the shunt was changed, 3 days after, and then every 5 days, with all of them giving negative results.

The hospital stay was long, there were no changes in the patient's status and, at the day +150, he was discharged to a long-stay hospital.

Globally, nosocomial infections have a high prevalence in patients who have undergone a craniectomy. Indeed, up to 40% of these patients suffer a nosocomial infection, with mechanical ventilation-associated pneumonia being the most common one (it accounts for 22% of cases). Drainage (both EVD and ELD) constitutes one of the most important risk factors for developing nosocomial meningitis, with an estimated prevalence of 5-16% for EVD and 7% for ELD.

Traditionally, the pathogens most frequently involved in nosocomial meningitis associated with drainage were Gram-positive bacteria, mainly *Staphylococcus* spp. However, over recent years there has been an increase in GNB, including multi-resistant GNB [12-14].

The BBB penetration of the antibiotics towards the CSF is poorly-defined or unknown, and many antibiotics cannot be used at the systemic doses that would be necessary due to the associated toxicity. Therefore, the combination of systemic and intrathecal treatments is frequent, especially in case of multi-resistant bacteria [15].

When ceftazidime is administered parenterally, it reaches a good concentration (as it occurs generally with all beta-lactams): above the minimal inhibitory concentration in CSF. However, its intrathecal administration is not recommended due to neurotoxicity, mainly because it can favor epileptic seizures. The optimal dose for the CNS is 2g in intervals of 6 or 8 h [14].

The IDSA Clinical Practice Guidelines state that, although the intrathecal use of antimicrobials is not approved by the FDA, it has to be considered as an option for nosocomial meningitis/ventriculitis that do not respond to systemic treatment. Aminoglycosides such as amikacin have a good safety profile, as well as better pharmacokinetic/pharmacodynamic (PK/PD) properties and effectiveness, compared to when they are administered systemically [16].

CAZ-AVI is a novel combination of beta-lactam + beta-lactamase inhibitor that has been approved by the FDA and EMA for intraabdominal and urinary tract infections, as well as for mechanical ventilation-associated pneumonia and in cases of multi-resistant *Enterobacteriaceae* and *Pseudomonas* [17-22].

Data on the use of CAZ-AVI in CSF infections are still scarce, but it has emerged as a therapeutic option in CNS infections caused by CPKP due to its BBB penetration, which has been shown in animal models to be 38% [11], and to its PK/PD properties; as almost 90% of the protein non-bound free fraction of avibactam is responsible for its pharmacodynamic effect [23].

In our study, we report the results of using CAZ-AVI in nosocomial meningitis and review the literature about CSF-drainage-associated meningitis due to CPKP and their treatment with CAZ-AVI. For that purpose, we used PubMed, Mesh, and Cochrane as search engines, and nosocomial meningitis, ceftazidime-avibactam, central nervous system infection; as well as EVD- and ELD-associated meningitis, as terms.

We included all reported cases and case series of CNS infections due to CPKP. We found a total of 11 original papers (9 clinical cases and 2 case series reporting episodes of CNS infections caused by CPKP) published between 2016 and 2021. Among them, 8 received treatment with CAZ-AVI and only one described that the infection was caused by ESBL- and OXA-48-producing *K. pneumoniae*. The characteristics of all cases are shown in Table 1.

In 2016, Mermer *et al.* described the first case of meningitis due to CPKP and associated with ventriculo-peritoneal drainage (VPD). It was successfully treated with polymyxin and changing the shunt [24]. Other authors have reported cases of postsurgical meningitis due to carbapenem-resistant *Enterobacteriaceae* (CRE) that have been successfully treated combining tigecycline or polymyxin with intrathecal amikacin or colistin [25-26].

Evidence regarding the treatment of nosocomial meningitis with CAZ-AVI is still limited; however, it seems to show good results as monotherapy and/or combined therapy in infections induced by CRE *P. aeruginosa* [27-29]. Regarding the retrospective case series, Shields *et al.* and Temkin *et al.* reported 37 and 38 cases (respectively) of CPKP infections that were treated with CAZ-AVI, with 76% and 73% of cure (respectively). Each one of these authors also included a case of ventriculitis due to CPKP [8,30].

Samuel *et al.* described for the first time a case of nosocomial meningitis due to KPC treated with CAZ-AVI every 6 hours during 14 days. This case, which resulted in clinical and microbiological cure [31], constitutes the unique reported case, apart from ours, in which an increase in CAZ-AVI frequency of administration from 8 to 6 hours has been performed.

Notwithstanding, Yasmin *et al.* [32] also published a case of KPC meningitis treated with standard doses of CAZ-AVI, every 8 hours during 14 days, and intrathecal amikacin. They evaluated CAZ-AVI levels in CSF and confirmed that the usual dose of 2g/0.5g every 8 hours achieved an adequate concentration.

Treatment duration in these infections is still under discussion. The IDSA guidelines recommend that treatments should last 10-14 days in meningitis caused by GNB, although some experts recommend 21 days [16]. Nonetheless, there are no recommendations neither on CPKP nor on OXA-48. However, the literature shows great variabilities regarding treatment duration, with good results being reported for treatments lasting 14 days, 21 days, and even more than 6 weeks [27,28,30,31] and treatment options are discussed. Summary. Few antibiotics to treat carbapenem-resistant Enterobacteriaceae (CRE. We decided to prescribe a 21-day treatment, which was effective to cure the infection

Table 1 **Main characteristics of the cases reported in the literature.**

Author, year [reference]	Material and methods	Microbiological isolation	Previous treatment	Systemic treatment, dose and duration	Intrathecal treatment	Evolution
Mermer S, 2016 [24]	1 case (infarction and VPD)	CPE *K. pneumoniae*	Not available	IV colistin	Colistin	Not available
Samuel S, 2016 [31]	1 case of nosocomial meningitis after a hemicraniectomy. No CSF drainage	KPC *K. pneumoniae*	No	CAZ-AVI 2g/0.5g/6h, 14 days	No	Clinical and microbiological cure
Shield R 2016 [30]	Retrospective series of 37 cases treated with CAZ-AVI. One case of ventriculitis and subdural empyema	KPC *K. pneumoniae* (84%) *E. coli* (8%) *E. clocace* (5%) *E. aerogenes* (3%)	No	CAZ-AVI 2g/0.5g/8h, 14 days (mean)	Colistin	30-day survival of 76%, 90-day survival of 69%, not specified for the CNS infection
Temkin E, 2017 [8]	Series of 38 cases: one case of ventriculitis/subdural abscess	*K. pneumoniae* 34: 12 OXA 48 *K. oxytoca* (1) OXA-48 *E coli* (1) *P aeruginosa* (2)	Colistin, tigecycline, carbapenems	CAZ-AVI 2g/0.5g/8h, 16 days (mean)	Not available	Clinical and microbiological cure of 73.7% of the series, not specified for the CNS infection
Gofman N, 2018 [27]	1 case, CCT, craniectomy, EVD, VPD +10 sepsis +30 VPD	*Carbapenemase K. pneumoniae* and *P. aeruginosa*	Vancomycin, ceftriaxone, cefepime, meropenem	CAZ-AVI 2g/0.5g/8h, 6 weeks	Amikacin 30mg, 4 weeks	Clinical and microbiological cure
Holyk A, 2018 [28]	1 case, elderly, subarachnoid hemorrhage, EVD, UIP day +23, CNS infect. +31	*Carbapenemase K. pneumoniae* (MIC meropenem>8)	Cefazolin, piperacillin tazobactam, CAZ-AVI, inhaled colistin, doxycycline	CAZ-AVI 2g/0-5g/8h, 21 days	Gentamicin, 15 days	Clinical and microbiological cure
Dacco V, 2019 [29]	1 case, multiple brain abscesses and bacteremia after lung transplantation, due to Burkholderia multivorans	PenA Class A *B. multivorans carbapenemase*	Meropenem, trimethoprim, sulfamethoxazole, levofloxacin	CAZ-AVI 2g/0.5g/ added post-hemodialysis, 102 days	No	Clinical and microbiological cure
Chen Y, 2019 [25]	1 case of meningitis with brain abscess after surgery, 29-year-old male. EVD	KPC *K. pneumoniae*	No	Tigecycline + IV amikacin, 23 days and TMP-SMX + oral minocycline, 32 days	Amikacin	Clinical and microbiological cure
Patrial Y, 2019 [26]	Case 1: 17-year-old male, CCT, EVD + decompressive craniectomy. Meningitis day +11 Case 2: 50-year-old female, CCT after subarachnoid hemorrhage, EVD. Meningitis day +16	KPC *K. pneumoniae* KPC *K. pneumoniae*	Case 1: Meropenem Case 2: Piperacillin tazobactam 4.5g/6h, 7 days, Linezolid 600mg/12h, 10 days	C1: polymyxin E 150mg/12h +Meropenem 2g/8h, 19 days C2: polymyxin E 150mg/12h, 7days +Meropenem 1g/8h	Polymyxin E 5mg/24h, 7 days Polymyxin E 5mg/24h, 7 days	Clinical and microbiological cure
Yasmin M, 2020 [32]	1 case, CCT, intrathecal pump. Pump removal. Sepsis with blood culture,CSF and ASB. CAZ-AVI blood and CSF levels were evaluated 184 min after administration: 19/4 in CSF and 61/13 in blood.	KPC-3 *K. pneumoniae*	Meropenem, vancomycin, meropenem vaborbactam, ciprofloxacin	CAZ-AVI 2g/0.5g/8h, 14 days	Amikacin 30mg, 14 days	Yes; CSF/plasma levels confirmed adequate concentration reached through usual dose 2,5g/8h * CAZ-AVI mouse concentration, BBB penetration of 40%
Pektezel M.Y, 2021 [33]	1 case, 62-year-old male, cerebellar hematoma with EVD, day +18 bacteremia due to CPE K pneumonia. Meningitis day +24 and brain abscess	OXA-48 *K. pneumoniae*	Meropenem, colistin, ertapenem, fosfomycin	Meropenem 2g/8h, 45 days CAZ-AVI 2g/0.5g/8h, 30 days + TMP-SMX, 15 days	Colistimethate sodium 10mg, 36 days Amikacin 15mg, from day +45	Clinical and microbiological cure

On another front, whether the use of CAZ-AVI for the treatment of CNS infections should be done as monotherapy or in combination with an intrathecally administered drug is also controversial. In the literature, it is frequently combined with intrathecal gentamicin, amikacin, or colistin, although good results have also been reported for CAZ-AVI as monotherapy [29-33].

In spite of the systematic use of CAZ-AVI as combined therapy not being recommended, there are no studies demonstrating that CAZ-AVI displays a worse performance in monotherapy, compared to a combined therapy. Thus, as our patient with nosocomial meningitis caused by ESBL- and OXA-48-producing *K. pneumoniae* had already received a previous treatment with CAZ-AVI (which could predispose towards treatment failure), we decided to use CAZ-AVI in combination with intrathecal amikacin, with good results [10]

As far as we are concerned, we report the second case of meningitis associated with external drainage due to ESBL- and OXA-48-producing *K. pneumoniae* successfully treated 2 g/0.5g/6h of CAZ-AVI and intrathecal amikacin.

In spite of more studies being required, CAZ-AVI has been shown to be a safe, effective, and well-tolerated treatment for CNS infections due to CPKP, either mono- or poly-microbial. Its PK/PD properties allow it penetrate the BBB and reach an adequate concentration in the CSF. CAZ-AVI in combination with intrathecal aminoglycosides seems to be a good option in cases of meningitis associated with drainage. Thus, CAZ-AVI stands as the first alternative for systemic treatment of CPKP-triggered nosocomial meningitis (a pathology whose incidence is increasing), being the first option for KPC- and OXA-48-producing CPKP.

CONFLICTS OF INTEREST

All authors declare no conflict of interest.

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Letter to the Editor

Revista Española de Quimioterapia
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Myroides odoratus as an unusual urinary tract infection pathogen in immunosuppressed patient

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Sir,

Myroides odoratus is an aerobic, oxidase-positive, gram-negative rod commonly found in humid environments. This microorganism was previously recognized as *Flavobacterium odoratum*, until 1996 when it was reclassified into the new genus *Myroides* [1]. It is a rare pathogen that affects mainly to patients with indwelling devices [2] or immunocompromised [3,4], and mostly produces urinary tract infections and bacteremia[5].

A 66-year-old male, resident in Granada, Spain, with a history of prostate cancer and bone metastasis came to the emergency department due to urinary symptoms, without episodes of fever. The urine reagent strip indicated the presence of nitrites, leukocytes and red blood cells. The indwelling catheter was replaced, a urine culture was ordered and was prescribed fosfomycin 500 mg /8h for 8 days.

Urine sample was inoculated in CPSO chromogenic medium (Biomerieux®, Marcy L´Etoile, France) and blood agar incubated for 24 hours in a 37ºC atmosphere. After the incubation time, a pure culture count of >100,000 CFU/ml of a yellowish colony was observed (Figure 1). Identification of the microorganism was performed by mass spectrometry, MALDI-TOF (Bruker®, Bremen, Germany). *M. odoratus* was obtained with a score of 2,29. The identification was confirmed by the partial sequencing of 16S rRNA gene. This isolate shared 100% sequence similarity with the reference sequence of *M. odoratus* available in GenBank (MT367748.1).

Antibiotic susceptibility test was carried out by automated microdilution broth test (Vitek2, Biomerieux) and interpreted following CLSI M100 guidelines (2021), using the minimum inhibitory concentration (MIC) breakpoints of other non-*Enterobacteriaceae*.

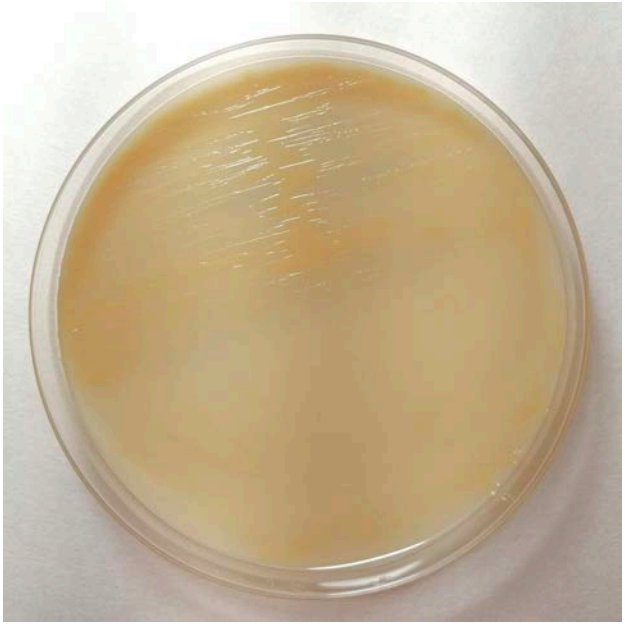

Figura 1 | **Colonies of *M. odoratus* grown in CPSO® chromogenic agar (Biomerieux®).**

The susceptibility profile reported was as follows: susceptible to levofloxacin (MIC < 0.5 mg/L), ciprofloxacin (MIC =1 mg/L) and sulfamethoxazole/trimethoprim (MIC ≤2/76 mg/L), and resistant to piperacillin/tazobactam (MIC>128 mg/L), ceftazidime (MIC ≥64 mg/L), aztreonam (MIC ≥64 mg/L), imipenem (MIC ≥16 mg/L), meropenem (MIC ≥16 mg/L), gentamicin (MIC ≥16 mg/L), tobramycin (MIC ≥16 mg/L), amikacin (MIC ≥64 mg/L), and fosfomycin (MIC ≥32 mg/L). The patient was treated with sulfamethoxazole 800 mg/trimethoprim 600 mg/12 h for 7 days. He responded favorably to treatment and had no further symptoms. No new post-treatment control sample was sent.

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M. odoratus is an emerging uropathogen mainly in immunosuppressed and bladder catheters patients. Accurate identification methods and antibiotic susceptibility are essential for the correct management of these patients.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Carta el Editor

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Peritonitis por *Clostridium baratii* en paciente cirrótico

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Estimado Editor: Los microorganismos del género *Clostridium* son bacterias grampositivas anaerobias estrictas que forman parte de la microbiota del tubo digestivo. *Clostridium baratii* forma parte de este género y puede producir una toxina botulínica por lo que es una causa rara de botulismo infantil [1,2].

Describimos el caso de un paciente varón de 65 años que acude a urgencias por deterioro progresivo del nivel de conciencia de 48 horas de evolución en el contexto de un cuadro recurrente de encefalopatía hepática. Entre sus antecedentes destaca cirrosis hepática de origen mixto (consumo crónico de alcohol e infección por hepatitis B), hipertensión portal, encefalopatía hepática recidivante y ascitis intratable con diuréticos por desarrollo de insuficiencia renal. Está incluido en un programa de paracentesis evacuadoras periódicas como terapia previa a la colocación de una derivación percutánea portosistémica (DPPI).

En el examen físico destacaba un cuadro de desorientación temporo-espacial con bradipsiquia y bradilalia, sin repercusión respiratoria ni hemodinámica y sin clara focalidad infecciosa.

A nivel analítico mostraba una proteína C reactiva de 1,49 mg/dL sin leucocitosis y con niveles de procalcitonina en rango de normalidad.

Se realizó radiografía de tórax que no mostró alteraciones y paracentesis diagnóstica con extracción de líquido ascítico de aspecto turbio y fétido, no fue posible realizar un análisis de la celularidad ni de la bioquímica de este líquido, pero si se remitió al laboratorio de microbiología para cultivo, iniciando en ese momento tratamiento antibiótico de forma empírica con ceftriaxona por alta sospecha de infección abdominal.

La muestra de líquido ascítico se envió en un tubo estéril y en dos frascos de hemocultivos (BacT/ Alert, bioMérieux®). Las muestras se procesaron siguiendo el procedimiento habitual. La botella anaerobia del frasco de hemocultivo fue positiva tras 17 horas de incubación. En la tinción de gram se observaron bacilos grampositivos largos. El cultivo de la botella aerobia y de la muestra del líquido ascítico fueron negativos.

A las 48 horas de incubación se observó el crecimiento de unas colonias grises en agar Brucella con hemina y vitamina K1 (Becton Dickinson®) incubado en atmósfera de anaerobiosis confirmando la sospecha de peritonitis bacteriana. Mediante espectrometría de masas MALDI-TOF (Bruker®), el microorganismo se identificó como *Clostridium baratii* que posteriormente se envió al Centro Nacional de Microbiología (ISCIII, Majadahonda, Madrid) para su confirmación y para la detección de toxina botulínica, que fue negativa. El estudio de sensibilidad antibiótica se realizó mediante Etest (bioMérieux®) en placas de agar Brucella con hemina y vitamina K1, incubadas en atmósfera anaerobia que se leyeron a las 24 y 48 horas, siendo sensible a penicilina (CMI 0,125 mg/L), amoxicilina - ácido clavulánico (CMI 0,06 mg/L), piperacilina- tazobactam (CMI 0,75 mg/L), meropenem (CMI 0,04 mg/L), vancomicina (CMI 0,125 mg/L), metronidazol (CMI 0,125 mg/L) y resistente a clindamicina (CMI 3 mg/L).

El tratamiento se efectuó con metronidazol vía oral 500 mg cada 8 horas durante 5 días y la evolución fue favorable. La peritonitis bacteriana espontánea (PBE) es una infección bacteriana del líquido ascítico sin ninguna fuente de infección intra-abdominal tratable quirúrgicamente [3]. Es muy frecuente en pacientes con cirrosis y ascitis. Su mortalidad llega a superar el 90%, pero su diagnóstico y tratamiento precoz ha logrado reducir esta cifra al 20% [4]. Los pacientes con cirrosis hepática y ascitis tienen riesgo de desarrollar una PBE cuya prevalencia oscila entre 1.5-3.5% en pacientes ambulatorios y hasta un 10% en los pacientes hospitalizados. Se calcula que hasta la mitad de los episodios de PBE están presentes en el momento del ingreso y que el resto de los casos se desarrollan durante la hospitalización. Los pacientes con PBE pueden manifestar datos de infección

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sistémica como: taquicardia, fiebre, hipotensión arterial, encefalopatía hepática, leucocitosis o elevación de reactantes de fase aguda. Sin embargo, es importante destacar que en una gran parte de los pacientes la infección puede ser paucisintomática. El diagnóstico de PBE se basa en el recuento de neutrófilos en líquido ascítico mayor de 250/mm³. Aunque la positividad de los cultivos del líquido ascítico no es un requisito para el diagnóstico de PBE, el diagnóstico microbiológico resulta de gran ayuda para determinar la etiología y la susceptibilidad a los antimicrobiano. Asimismo, los cultivos del líquido ascítico pueden resultar negativos hasta en un 60% de los pacientes con clínica y recuento de neutrófilos sugestivos de PBE, probablemente debido al tratamiento antibiótico empírico y/o profilaxis antibiótica [5].

Debido a la mayor cantidad de líquido ascítico inoculado en frascos de hemocultivos (10 ml) es aconsejable su envío en dichos frascos y en un tubo estéril sin ningún medio de transporte o enriquecimiento, ya que se consigue una mayor rentabilidad de los mismos.

Hemos realizado una búsqueda en PubMed con los siguientes términos «*Clostridium baratii*» y publicados hasta el 20 de agosto de 2022 y no encontramos ningún caso de PBE por *C. baratii* aunque sí hay algunos casos descritos en neumonía [6], absceso pulmonar [7], bacteriemia [8], colecistitis enfisematosa con absceso hepático [9] y sobre todo botulismo [10,11,12].

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CONFLICTO DE INTERESES

Los autores señalan no tener ningún conflicto de interés.

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Carta el Editor

Revista Española de Quimioterapia
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Micosis pulmonar crónica por *Paracoccidioides brasiliensis*

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Estimado Editor: *Paracoccidioides brasiliensis* es un hongo dimórfico endémico de Centro y Sudamérica, responsable de una enfermedad multiorgánica con predominio pulmonar, que es la causa más importante de morbimortalidad [1]. En áreas no endémicas el tratamiento específico se ve demorado por la dificultad del diagnóstico debido a la baja sospecha de los casos (importados en su totalidad), la similitud con otras enfermedades infecciosas y no infecciosas, y el amplio margen de latencia entre la exposición y las manifestaciones clínicas [2,3].

Varón de 61 años trabajador de la construcción, natural de Argentina (viajó a Argentina en 2012 y a Brasil en 2013), que vive desde hace 30 años en España. Como antecedentes: exfumador con diabetes mellitus tipo 2, EPOC de alto riesgo (fenotipo mixto y bronquiectasias bilaterales) y sarcoma epitelioide estadio IV tratado en 2013 con quimioterapia, en remisión desde 2014. Presenta lesiones pulmonares consideradas como metástasis del sarcoma, con afectación en vidrio deslustrado bilateral y varios nódulos pulmonares sin cambios tras años de seguimiento (Figura 1A). Dado que esta circunstancia no se corresponde con un proceso tumoral, en junio de 2020 es diagnosticado de neumonía organizada criptogenética por signos radiológicos, tratada con corticoide sistémico, presentando franca mejoría inicial y posterior progresión de la enfermedad al reducir la dosis de corticoide. A partir de entonces presenta múltiples agudizaciones, aislándose *Klebsiella pneumoniae* en varios cultivos de esputo. En la analítica destaca una ligera anemia y leucocitosis, con incremento de los reactantes de fase aguda. Se realiza punción-aspiración de ganglio linfático, negativa para células malignas. En el PET-TAC se observan adenopatías hipercaptantes y cavitación de las lesiones (Figura 1B), incompatible con neumonía organizada, por lo que se decide biopsiar, enviando muestras a anatomía patológica y microbiología.

El estudio histológico muestra necrosis y presencia de granulomas con células gigantes multinucleadas. Con la tinción de plata metenamina se observan formas redondeadas de diferente tamaño sugestivas de infección fúngica, mientras que con la tinción de Ziehl-Neelsen no se identifican bacilos ácido-alcohol resistentes. Dada la procedencia del paciente, se solicita serología para hongos endémicos (inmunodifusión de Ouchterlony) y PCR multiplex de neumonía fúngica (*Pneumocystis jiroveci, Cryptococcus neoformans e Histoplasma capsulatum)*. Se detectaron anticuerpos frente a *P. brasilensis*, por lo que se solicitó una PCR, con resultado positivo. El cultivo de hongos en agar Sabouraud fue negativo a los 30 días.

Hasta la obtención de los resultados el paciente fue tratado con fluconazol 21 días, con mejoría clínica, iniciándose posteriormente itraconazol 200 mg oral 6 meses y corticoides. El paciente respondió bien al tratamiento, con disminución del componente de partes blandas dentro de las lesiones cavitadas y negativización de la PCR, por lo que se suspende el itraconazol. Al año, el paciente sigue con mejoría clínica, sin disnea de esfuerzo y tos mínima con escasa expectoración.

Una revisión reciente muestra que de los 83 casos publicados en Europa en los últimos 40 años, 35 son españoles [3]. La infección crónica es más frecuente en adultos entre 30-60 años, con mayor prevalencia en hombres, probablemente porque los estrógenos inhiben el paso a la forma infectiva del hongo [4]. Puede considerarse una enfermedad ocupacional por afectar mayoritariamente a trabajadores del campo o de la construcción, que se infectan al inhalar conidias del suelo [5,6], con el antecedente de viaje a zona endémica, principalmente a Brasil, Colombia y Venezuela [2,3]. Como factores predisponentes de la infección se encuentran el tabaco, el alcohol y la malnutrición [7]. La quimioterapia recibida por el paciente pudo ser el desencadenante de la infección, confinada exclusivamente

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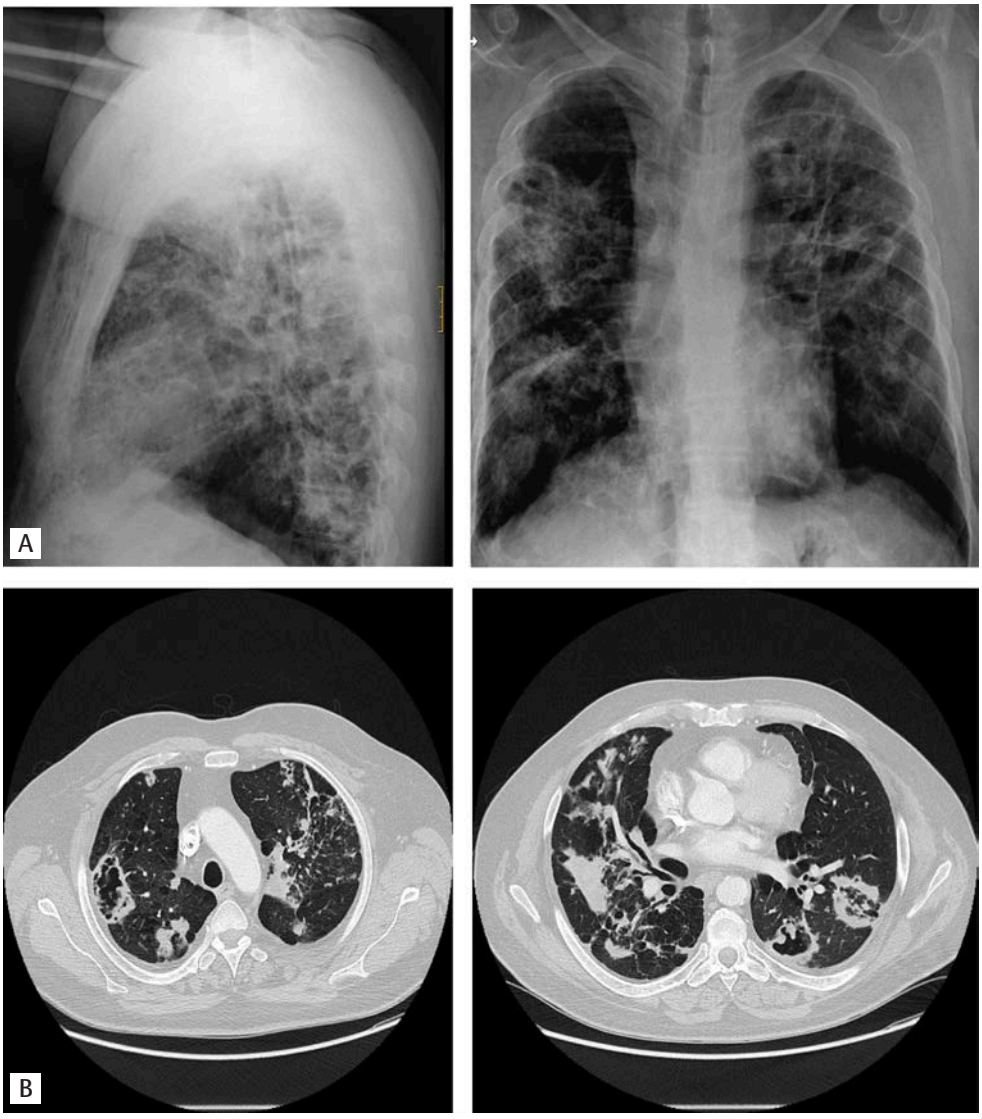

Figura 1 | **(A) Radiografía pulmonar anteroposterior y lateral y (B) TAC en paciente con paracoccidiomicosis pulmonar. Muestran extensas consolidaciones coalescentes bilaterales, muchas de ellas cavitadas, y múltiples nódulos redondeados dispersos por ambos hemitórax**

en el pulmón. El tratamiento de elección en los casos leves-moderados es itraconazol al permitir la administración oral en ciclos cortos (3-6 meses), con pocos efectos adversos y menor tasa de recaídas que otros antifúngicos [8]. Anfotericina B, asociada o no con sulfonamidas, se administra como alternativa, siendo de elección en los casos graves [8].

Nuestro paciente fue erróneamente diagnosticado durante años debido a la baja sospecha clínica, siendo la combinación del examen histológico y la serología fundamentales para el diagnóstico. No obstante, el gold estándar sigue siendo el aislamiento del hongo en cultivo y la visualización directa de la levadura multigemante, con su aspecto característico en rueda de timón, hallazgo considerado patognomónico de la infección [9]. Las técnicas de biología molecular presentan la ventaja de poder utilizarse en muestras clínicas tanto como método de diagnóstico precoz como de seguimiento de la respuesta al tratamiento, a ser posible a partir de muestras de calidad, de especial relevancia cuando el agente etiológico, como las levaduras, puede colonizar ciertas mucosas sin causar infección [10].

En conclusión, ponemos de manifiesto una infección crónica por *P. brasiliensis* en paciente argentino que recibió quimioterapia el mismo año que viajó a Brasil. La presencia de nó-

dulos pulmonares en pacientes procedentes de Latinoamérica requiere el diagnóstico diferencial con otros hongos endémicos, tuberculosis, sarcoidosis y carcinoma de células escamosas [2,10]. El estudio histológico y/o microbiológico de una muestra de calidad como la biopsia suele ser clave para el correcto diagnóstico y tratamiento de la infección.

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CONFLICTO DE INTERESES

Los autores declaran no tener conflicto de intereses.

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Letter to the Editor

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Cellulitis due to *Pasteurella stomatis* and *Actinomyces canis* following dog bite

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Sir,

The *Pasteurella* genus consists of Gram-negative coccobacilli that form part of the commensal flora of dogs and cats, mainly. It is the cause of infection in many animal species, although it can also produce infections in humans, principally infections of the skin and soft tissues after bites, scratches or contact with animal saliva. *Actinomyces canis* is a previously isolated Gram-positive facultative anaerobe bacillus of dogs, but it had not been reported as a cause of infections in humans until now [1].

Patient of 12 years of age who suffered a dog bite in the left lower limb, being tended in his outpatient clinic by disinfection of the wound and oral antibiotic treatment with trimethoprim/ sulfamethoxazole and clindamycin (160 mg/800 mg/12 h y 600 mg/8 h, respectively). He did not have a medical history of interest except for suspicion of allergy to amoxicillin after a pruriginous skin reaction several years before. He stopped taking clindamycin due to oral intolerance, being examined by his doctor three days afterwards with evident phlogogenous signs consisting of the left lower limb being slightly oedematous, increase in temperature and perilesional erythema, as well as purulent secretion.

At this time he was sent to the emergency room, where the physicians observed four symmetrical wounds of around 1.5 cm with torn skin, regular edges, and bloody and purulent secretion. In addition, the perilesional area evidenced redness, heat and swelling, which encompassed up to 7x5 cm (figure 1A). The patient did not have fever or functional impairment. An ultrasound was performed which showed mild subcutaneous oedema with absence of collections or of involvement of deep muscle levels (figure 1B). Two samples were obtained from the wounds, being inoculated in chocolate and TSA with 5% of sheep blood agars in aerobiosis with 5% of CO_2, Brucella-blood agar in anaerobiosis, as well as CNA and McConkey agar in aerobiosis. A blood culture sample was also obtained that was left incubating during seven days. After conducting the provocation test with cephalosporins with negative result, he was admitted with intravenous treatment of cefotaxime and clindamycin (1.5 g/8 h and 600 mg/8 h, respectively). In the Gram tincture, polymicrobial flora was observed consisting of Gram-negative and Gram-positive bacilli. At 24h there was growth in chocolate agar and TSA with 5% of sheep blood (Figure 2), being identified through MALDI-TOF mass spectrometry (Bruker, Massachusetts, USA) as *Pasteurella stomatis* with a value of 2,24. In the following 24 hours, growth was observed in Brucella-blood agar (BD®) being again identified through MALDI-TOF MS as *A. canis* with a value of 2.23. The antibiotic susceptibility was performed through gradient strips or E-test® in Mueller-Hinton fastidious agar (BD®) for *P. stomatis* and E-test® in Brucella-blood (BD®) for *A. canis*. *P. stomatis* was susceptible to penicillin (MIC = 0.38 mg/L), amoxicillin/clavulanic acid (MIC = 0.38 mg/L), cefotaxime (MIC= 0.02 mg/L), doxycycline (MIC = 0.5 mg/L), ciprofloxacin (MIC = 0.06 mg/L) and trimethoprim/sulfamethoxazole (MIC = 0.125 mg/L). In turn, *A. canis* was susceptible to penicillin (MIC < 0.016 mg/L), amoxicillin/clavulanic (MIC < 0.016 mg/L) and clindamycin (MIC = 0.25 mg/L), and resistant to metronidazole (MIC > 32 mg/L).

It was later confirmed that the patient was not allergic to amoxicillin, receiving four more days of IV treatment with amoxicillin/clavulanic acid (1.2 g/8 h), being released with oral suspension of amoxicillin/clavulanic acid (9ml/8 h at 100 mg /12.5 mg/ml) with good evolution after follow-up by his physician.

Infections from animal bites in humans are usually polymicrobial, with a mixture of aerobic and anaerobic microorganisms that reflect the oral flora of the animal that produces the lesion [2,3]. Besides bites and scratches, contact with colonised or infected animal saliva is also a possible transmission

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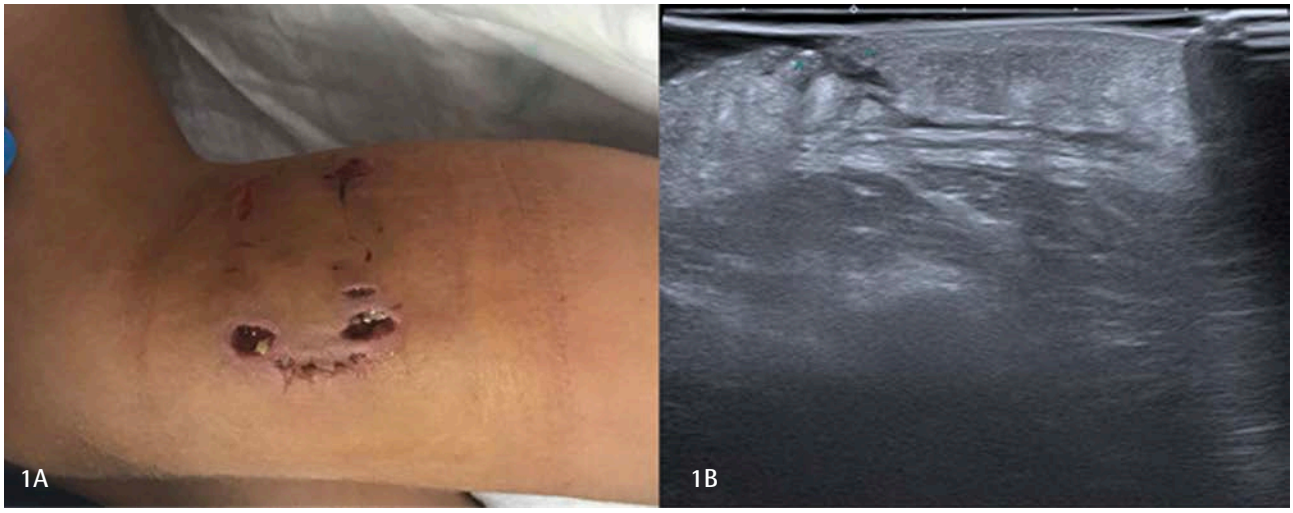

Figure 1 **1A: four symmetrical wounds of around 1.5 cm with torn skin, regular borders, bloody and purulent secretion. In addition, the perilesional area presented redness, heat and swelling, which encompassed up to 7x5 cm.**
1B: the thickening of the subcutaneous region associated with four skin wounds with subcutaneous trajectory in relation to the entrances of the bite. Mild laminar oedema in the deep subcutaneous region. There is no involvement of the muscle plane and no collections are observed.

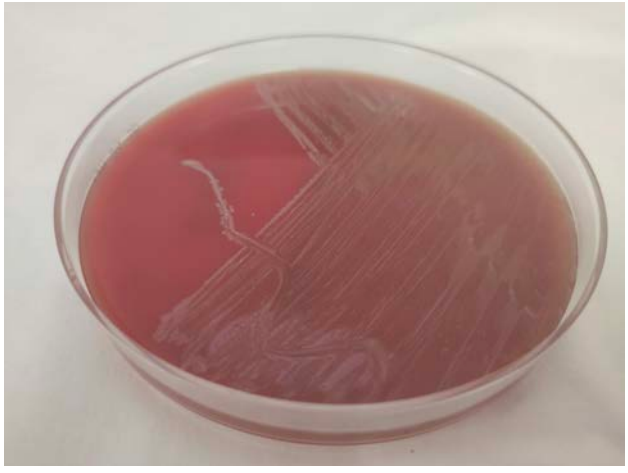

Figure 2 **Growth of white colonies on TSA with 5% of sheep blood agar after 24 h of incubation under aerobic conditions with 7.5% of CO_2. The growth of *A. canis* on culture plates could not be shown since the image was not obtained during the episode and after trying to recover it from the bacterial library it was no longer viable for culture.**

route, finding, for example, that multiple infections by *Pasteurella* spp. were not preceded by bites or scratches [4,5].

The most frequent infections produced by *Pasteurella* spp. are those of the skin and soft tissues, with the respiratory and invasive infections being rare, and with *Pasteurella multocida* being the most frequently isolated species [4-6]. The invasive infections such as sepsis, meningitis or peritonitis are usually produced more frequently in immunocompromised patients, with comorbidities or in patients in extreme age groups [3,6,7]. There are few reported cases up to now of human infections due to *P. stomatis,* while *A. canis* only seemed to produce infections in animals [5,8-10].

As for the handling of animal bites, the first is to clean the area with soap and water. The post-exposure antibiotic prophylaxis for the majority of the bites continues being controversial. The bites on the hand are the only location that seems to benefit from antibiotic prophylaxis and its use in these patients significantly decreases the infection rates [2,11]. In case of extreme pain, exposure of the muscle or underlying bone or signed of infection, if the state of vaccination against rabies of the dog is unknown or when the patient was vaccinated for the last time against tetanus, it is important to go to the healthcare centre or nearest hospital as soon as possible for its evaluation and treatment [12]. Amoxicillin/clavulanic acid and doxycycline or the combination of quinolones with clindamycin could be used as antibiotic prophylaxis. In case of abscess or other signs of complication of the wounded skin and soft issue infections, as well as in the case of invasive infections, cultures would be required to be able to establish an appropriate antibiotic treatment [11].

In conclusion, the cleaning and debridement of the wounds, as well as the identification of the patients who need antibiotic prophylaxis, against tetanus or rabies are keys for handling and to avoid serious complications. In those cases in which there is a suspicion or signs of infectious complication of the wounds, the patient should be examined again, microbiological samples obtained and an empirical antibiotic should be recommended until knowing the results. The virulence of the specie that inhabit the oral flora of the animals must continue to be researched to know which could provoke serious infections in humans.

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CONFLICTS OF INTERESTS

Authors declare no conflicts of interest.

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Carta el Editor

Revista Española de Quimioterapia
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Una vacuna inactivada para la inmunización primaria frente a varicela

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Estimado Editor: Actualmente las vacunas autorizadas en España para la vacunación frente a varicela, Varivax®, Varilrix® y Proquad®, se componen del virus atenuado de la varicela cepa Oka con ≥1.350 unidades formadoras de placa (UFP), $\geq 10^{3,3}$ UFP y ≥3,99 $\log_{10}$ UFP respectivamente. Además, existen dos vacunas para prevenir el herpes zóster (HZ) y la neuralgia postherpética (NPH): una vacuna atenuada (Zostavax®) compuesta por ≥19.400 UFP de cepa Oka, aprobada por la Agencia Europea del Medicamento (EMA) en 2006, que ha estado disponible en España desde entonces, aunque en agosto de 2022 cesó su comercialización en nuestro país; y otra recombinante de subunidades compuesta por la glicoproteína E y el adyuvante $AS01_B$ (Hz/su, Shingrix®), aprobada por la EMA en 2018 y disponible en España recientemente. Tanto Shingrix® como Zostavax® han sido autorizadas para la prevención del HZ y NPH en personas de ≥50 años; y Shingrix®, además, en ≥18 años con factores de riesgo de HZ [1]. Sin embargo, ni Zostavax® ni Shingrix® han sido autorizadas para la vacunación frente a varicela.

Se describe un caso de un paciente candidato a trasplante renal que, tras completar primovacunación frente a varicela con vacuna atenuada, no generó niveles protectores de IgG-anti virus varicela zoster (VVZ). Si bien, posteriormente, tras completar primovacunación con vacuna recombinante frente a HZ alcanzó niveles considerados como protectores.

Se trata de un varón de 61 años, natural de Bulgaria que emigró de su país a los 43 años. Exfumador de 20 cigarrillos/día, sin otros hábitos tóxicos, con hipertensión arterial, diabetes tipo I, enfermedad renal crónica estadio V, retinopatía, polineuropatía y vasculopatía periférica secundarias, amputación infracondílea de miembro inferior izquierdo y a nivel metatarsiano de pie derecho, úlcera gastroduodenal y hepatitis B pasada. El paciente fue derivado para inmunización pre-trasplante renal. Previamente a la vacunación presentaba los siguientes parámetros analíticos: leucocitos ($8,45x10^9$/L), neutrófilos ($4,73x10^9$/L), linfocitos ($2,22x10^9$/L), plaquetas ($256x10^9$/L), creatinina (3,03mg/dL), AST/GOT (9U/L), ALT/GPT (11U/L), GGT (23U/L), HBsAg (-), HBsAc (58mUI/mL), HBcAc (+), VHC-IgG (-), VIH-1-2 (-) e IgG-anti VVZ (-). Además, ante este último hallazgo, se resalta que el paciente negó antecedente de infección pasada de varicela. Junto a otras vacunas, se administró una dosis de Varivax® constatándose la ausencia de IgG-anti VVZ en la serología realizada a los 14 días; y nuevamente, tras administrar una segunda dosis a las 4 semanas, se constató la ausencia de IgG-anti VVZ en la serología realizada a los 30 días, catalogándose como no-respondedor. A los tres meses de la pauta de Varivax®, se administraron dos dosis de Shingrix® separadas por ocho semanas, hallándose un título de IgG-anti VVZ de 3.158 mUI/mL en la serología realizada a los 37 días de completar dicha pauta. El paciente no había recibido tratamiento inmunosupresor ni hemoderivados plasmáticos durante el proceso de vacunación ni en los 6 meses anteriores.

Varios estudios han comparado la inmunogenicidad de Zostavax® y Shingrix® observándose una mayor y más consistente inmunogenicidad humoral y celular (hasta 3,5 y 10 veces respectivamente) de la vacuna recombinante frente a la atenuada [2-4]. Por otro lado, en un meta-análisis se observó una mayor eficacia en la prevención del HZ y NPH en todos los grupos de edad de la vacuna recombinante frente a la atenuada [5]. Estos hallazgos junto a la mayor cantidad de virus, de hasta 14 veces más, en el preparado de Zostavax® en comparación con las vacunas frente a varicela (Varivax® o Varilrix®), nos plantean la necesidad de evaluar como indicación el uso de Shingrix® como inmunización primaria frente a varicela en personas seronegativas con contraindicación o no respondedoras a las vacunas atenuadas. De hecho, en esta línea un único ensayo abierto con 31 pacientes *naive* receptores de trasplante de órgano sólido ha evaluado Shingrix® para inmunización primaria frente a varicela, observándose una respues-

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ta humoral en el 55% de los participantes y un incremento de más de 7 veces en la respuesta celular [6].

Asimismo, es reseñable que la nueva vacuna recombinante para prevención de HZ y NPH (Shingrix®), ha mostrado un buen perfil de seguridad e inmunogenicidad en términos de intensidad y duración, y ofrece una oportunidad no solo para la inmunización primaria en personas seronegativas a VVZ con contraindicación o no respondedoras a las vacunas atenuadas, sino también en población general [7]. En consecuencia, y dado que no se ha evidenciado la capacidad de Shingrix® para generar una robusta y permanente inmunidad celular en ausencia de estímulo previo al VVZ completo -ni en su forma salvaje ni como virus atenuado-, recomendamos fuertemente impulsar líneas de investigación sobre el uso de Shingrix® como indicación de inmunización primaria frente a varicela. En nuestra opinión, esta indicación podría contribuir a superar las limitaciones de la actual estrategia de vacunación, y un avance para alcanzar la eliminación del reservorio humano del VVZ.

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CONFLICTO DE INTERÉS

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Letter to the Editor

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Nirmatrelvir/ritonavir as a potential treatment for prolonged SARS-CoV-2 infection in immunocompromised patients

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Sir,

At the present time, there are no recommendations on the management of SARS-CoV-2 pneumonia in immunocompromised patients or on the usefulness of antiviral treatment beyond the first days of illness in patients without viral clearance. We describe the good response to nirmatrelvir/ritonavir in an oncohematologic patient, who is in his fifth month of infection with SARS-CoV-2 pneumonia and in his fifth admission due to recurrent pneumonia attributed to the infection itself.

68-year-old male diagnosed with diffuse large B-cell lymphoma in 2009 and treated with three cycles of chemotherapy that was terminated due to infectious complications. In 2014 and 2019 he presented relapses of the disease that required new treatment. He has been in complete remission for two years and with bimonthly maintenance rituximab (RTX) (end of treatment 11/8/21). Vaccinated with 3 doses against SARS-CoV-2 (Pfizer-BioNTech®, last dose 11/27/21).

On 1/6/22 he started febrile symptoms and cough, and was diagnosed with COVID-19 by detection in nasopharyngeal exudate sample of SARS-CoV-2 RNA by real-time PCR (Simplexa™-COVID-19 & Flu A/B Direct Kit, Diasorin®) and associated dyspnea on day +13. In analytical tests, C reactive protein (CRP) 37.70 mg/L (1.00-10.00), procalcitonin (PCT) 0.09 ng/mL (0.0-0.1), ferritin 464 ng/mL (20-300), absence of lymphopenia and a faint bilateral infiltrate on chest X-ray (CXR). He was admitted and received 60 mg/24h of methylprednisolone (MTP) for 6 days, with good clinical, analytical and radiological evolution. On admission, a serological study of IgG was performed by chemiluminescent microparticle assay (CMIA) against SARS-CoV 2 protein S (SARSCoV-2 IgG II Quant, Alinity, Abbott®) and IgM against SARS-CoV-2 protein S by chemiluminescence assay (CLIA) (LIAISON® SARS-CoV-2 IgM, Diasorin®), both results being negative. At discharge, prednisone (PDN) was prescribed in a descending regimen.

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After 10 days and being on 20 mg of PDN daily, he consulted again for dyspnea and cough, with the appearance of new infiltrates on CXR. Both SARSCoV-2 antigen and PCR were positive (Ct=17), without detecting IgG antibodies against SARS-CoV-2 protein S. High-resolution computed axial tomography (HRCT) was performed and revealed the presence of ground glass in the periphery of both lower lung lobes. No clinical improvement was observed until the corticosteroid dose was increased to 40 mg of MTP daily, and he was discharged on day 12 with 30 mg of PDN. At discharge, COVID-19 CRP positive (Ct=18) (day +36 since onset of symptoms).

Coinciding with the decrease to 10 mg of PDN, he presented again dyspnea and fever (38ºC), for which he was admitted (day +58 of symptoms), having completed the tapering corticosteroid regimen. Previously, a PET-CT scanner was performed with marked bilateral pulmonary hypermetabolism compatible with an active infectious process. On admission he presented lymphopenia, discrete elevation of CRP and bilateral peripheral interstitial involvement on chest peripheral interstitial involvement on chest X-ray. He still did not develop IgG against S protein and PCR was still positive (Ct=29). Fibrobronchoscopy was performed, in which only the detection by RT-PCR of SARS-CoV-2 (E and Y genes) (Xpert®Xpress SARS-CoV-2/Flu/ RSV, Cepheid®Xpress RSV, Cepheid®) in the bronchioalveolar lavage was outstanding (Ct=17). Corticosteroids were reintroduced and a single dose of sotrovimab 500 mg was administered. With this he showed immediate improvement. However, at discharge the SARS-CoV-2 PCR remained positive (Ct=20) and a further decrease in corticosteroids was scheduled.

With the decrease of PDN, he presented respiratory symptoms requiring admission, similar to the three previous admissions: reappearance of radiological infiltrates with positive PCR of SARS-CoV-2 (Ct=27). After a few days of admission and in the absence of improvement, corticotherapy was increased (40 mg MTP) with resolution of clinical, analytical and radiological alterations. At discharge PDN 20 mg was maintained and the SARS-CoV-2 PCR was positive (Ct=22).

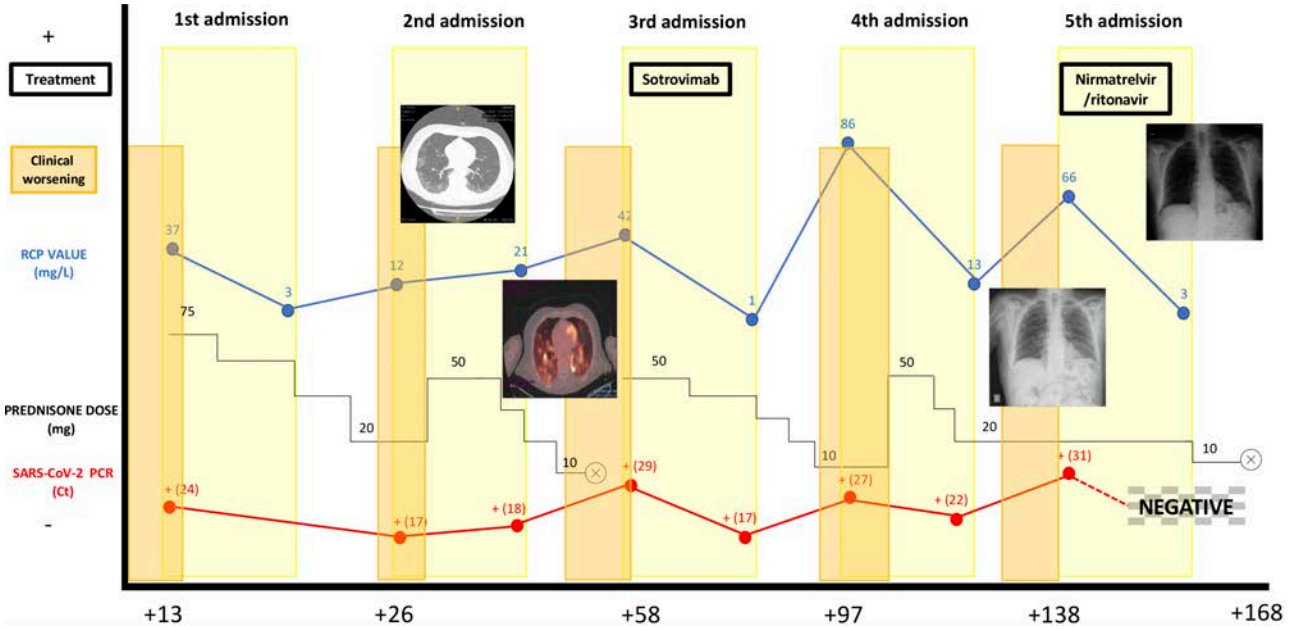

Figure 1 | **Clinical, analytical and radiological evolution of the patient**

After 9 days of PDN 20 mg daily, the previous symptoms reappeared. He was admitted on day +138 of symptoms. At this time, radiographic worsening with positive SARS-CoV-2 antigen and PCR (Ct=31). After verbal consent from the patient, nirmatrelvir/ritonavir was administered off-label for 5 days and the PDN 20 mg dose was maintained. This resulted in immediate clinical improvement as well as analytical and radiological normalization. The first negative PCR result for SARS-CoV-2 since the onset of the disease was obtained.

After discharge from this fifth and last hospitalization, corticosteroids were progressively reduced until they were withdrawn without relapse of the disease. He is currently at day +75 since taking nirmatrelvir/ritonavir and the patient remains asymptomatic. The patient's evolution is summarized in Figure 1.

Corticosteroid therapy could contribute to reduce viral clearance [1] and cases with persistence of viral activity and relapse of pneumonia are beginning to be described in patients with immunosuppressive treatment, especially with RTX (anti-CD20 monoclonal antibody) [2-4]. After a review of the literature, we found the description of 3 cases in which the administration of antiviral treatment beyond the first days of symptoms in patients treated with anti-CD20 drugs and with evidence of prolonged persistence of viral activity [5-7]. In all of them, the antiviral used was remdesivir, and although the symptoms subsided, later relapse of the disease occurred with PCR positivization. In these cases, remdesivir seems to contribute to attenuate the infection but not to eliminate it completely [5-7]. In contrast, we found no similar experience with nirmatrelvir/ritonavir. Therefore, the present work describes the first experience to date of an exceptional clinical response to late treatment with nirmatrelvir/ritonavir in an immunosuppressed patient with anti-CD20 drug and evidence of prolonged SARSCoV-2 infection.

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CONFLICTS OF INTEREST

IPC has participated in several scientific meetings paid for by the Gilead laboratory on the antiviral treatment of COVID-19 during the current year 2022. The rest of the authors declare that they have no conflict of interest directly or indirectly related to the contents of the manuscript.

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