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Sumario

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Cartas al Director

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Review

Clinical practice update of antifungal prophylaxis in immunocompromised children

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ABSTRACT

Due to the rise in the number and types of immunosuppressed patients, invasive fungal infections (IFI) are an increasing and major cause of morbidity and mortality in immunocompromised adults and children. There is a broad group of pediatric patients at risk for IFI in whom primary and/or secondary antifungal prophylaxis (AFP) should be considered despite scant evidence. Pediatric groups at risk for IFI includes extremely premature infants in some settings, while in high-risk children with cancer receiving chemotherapy or undergoing haematopoietic stem cell transplantation (HCT), AFP against yeast and moulds is usually recommended. For solid organ transplanted, children, prophylaxis depends on the type of transplant and associated risk factors. In children with primary or acquired immunodeficiency such as HIV or long-term immunosuppressive treatment, AFP depends on the type of immunodeficiency and the degree of immunosuppression. Chronic granulomatous disease is associated with a particular high-risk of IFI and anti-mould prophylaxis is always indicated. In contrast, AFP is not generally recommended in children with long stay in intensive care units. The choice of AFP is limited by the approval of antifungal agents in different age groups and by their pharmacokinetics characteristics. This document aims to review current available information on AFP in children and to provide a comprehensive proposal for each type of patient.

Key-words: antifungal prophylaxis, children, pediatric patients, HIV, primary immunodeficiency, Solid organ transplantation, haematopoietic stem cell transplantation.

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Revisión de estrategias de profilaxis antifúngica en niños inmunodeprimidos

RESUMEN

Las infecciones fúngicas invasoras (IFI) constituyen un problema creciente en adultos y niños inmunodeprimidos, acompañándose de una elevada morbimortalidad. El número de niños inmunodeprimidos va en aumento. Los grupos de riesgo de IFI en pediatría incluyen a los grandes prematuros, que se benefician de profilaxis con fluconazol, pacientes hemato-oncológicos sometidos a quimioterapia o trasplante de precursores hematopoyéticos con neutropenias prolongadas, en quienes la profilaxis frente a hongos filamentosos suele recomendarse en situaciones de alto riesgo. En niños sometidos a trasplante de órgano sólido, la profilaxis depende del tipo de trasplante y factores de riesgo asociados. En pacientes con inmunodeficiencias primarias o adquiridas como la infección VIH o tratamiento inmunosupresor prolongado, la profilaxis antifúngica dependerá del tipo de inmunodeficiencia primaria y del grado de inmunosupresión. La enfermedad granulomatosa crónica tiene riesgo particularmente elevado de IFI y requiere siempre profilaxis frente a hongos filamentosos. En cambio, en niños con ingresos prolongados en cuidados intensivos la profilaxis frente a IFI habitualmente no está indicada. El tipo de profilaxis está limitado por la diferente aprobación de antifún-

gicos a distintas edades. Este documento pretende revisar la información actual disponible respecto a profilaxis antifúngica en niños, con propuesta para la estrategia más apropiada en cada tipo de paciente.

Palabras clave: profilaxis antifúngica, niños, pacientes pediátrico, VIH, inmunodeficiencia primaria, trasplante de órgano sólido, trasplante de precursores hematopoyéticos

Invasive fungal infection (IFI) is considered an opportunistic infection that occurs almost exclusively in immunocompromised and critically ill children. The impact of an IFI can be devastating and is associated with a high rate of morbimortality despite the availability of new antifungal drugs in recent years.

The number of paediatric patients at risk for IFI is increasing and include: extremely premature infants (especially those with weight less than 1,500 g), children with cancer, undergoing haematopoietic stem cell transplantation (HSCT) or solid organ transplant, neutropenic children, those with long stay in intensive care units (ICU) and children with primary or acquired immunodeficiency such as HIV or long-term immunosuppressive treatment [1-4]. Timely diagnosis and initiation of appropriate antifungal therapy is a key point to improve outcomes. Thus, it is mandatory to consider antifungal prophylaxis (AFP) in most of these situations. Herein, a comprehensive proposals for each patient´s group based on an updated review of AFP strategies in children is provided.

The strength of recommendations and the quality of evidence are graded according to the scoring system proposed by the Infectious Diseases Society of North America (IDSA) [1]. In the process of providing recommendations, we have taken into account the paediatric development regulations and guidelines from the European Medicines Agency (EMA). The EMA accepts the requirement for extrapolation of evidence for efficacy from studies in adults to paediatric patients or from older to younger paediatric patients when the following criteria are met: (a) underlying condition and cause of targeted disease and expected response to therapy are similar; (b) data from clinical studies on pharmacokinetics, safety and tolerance are available for paediatric patients; and (c) supportive paediatric efficacy data exists [2]. In this scoring system, the strength of the recommendation is rated as follows: A: strongly recommended; B: moderately recommended; C: weak author support; D: not recommended by the authors. The quality of evidence is evaluated on a 3-level scale as follows: I: data from at least 1 well-designed and conducted randomized controlled trial; II: data from at least 1 well-designed and conducted clinical trial, without randomization, cohorts or case-control analyses (preferably multicenter), multiple retrospective series, or major findings of noncontrolled studies; III: expert opinions based on clinical experience, descriptive case series, or expert committee reports [1].

1.- ANTIFUNGAL PROPHYLAXIS IN NEWBORNS

The prevalence of invasive candidiasis (IC) is highly variable in neonatal intensive care units (NICU) ranging from 3% to 23% in extreme premature infants, depending on the complexity of the NICU and whether surgery is involved or not. This patient population has a high risk of dissemination to the central nervous system (up to 15-20% in extreme premature babies), even before presenting overt clinical signs of infection, and high mortality rates. Despite high variations among NICU, *Candida albicans* is the most frequent isolated species, followed by *C. parapsilosis* [3-6].

Risk factors for IC in preterm infants include immaturity of the immune system (especially low levels of maternal IgG transmission and impaired functions of opsonization and complement) and epithelial barriers, frequent rupture of these barriers by invasive procedures, such as catheters, intubation or surgery, and the increase in the density of colonization by *Candida* spp. promoted mostly by the frequent use of broad-spectrum antibiotics. In addition, H2-receptor blockers and steroids may facilitate intestinal translocation leading to *Candida* spp. invasion and secondary systemic infection. Transmission through peripartum colonization and horizontal transmission through health-care professionals colonized by *Candida* spp. may also occur [3-6]. Due to its bad prognosis, prophylactic strategies to prevent IF are warranted [7].

General strategies include hand hygiene; individual room for families and newborns; reduction of risk factors for colonization and infection for IC, such as limitation of H2-receptor blockers, steroids and broad-spectrum antibiotics, mainly carbapenems and third-generation cephalosporins; minimizing the use of invasive devices including minimal manipulation of central venous catheters, as well as early introduction of mother´s milk. Although some experts advocate for using lactoferrin alone or combined with probiotics in order to reduce intestinal *Candida* spp. colonization and late onset sepsis in neonates weighing < 1,500 g, evidence is still scarce [7] (CII).

The indication of AFP, although controversial, is generally recommended in preterm babies <1,000 g., in NICU with a prevalence of *Candida* spp. infection above 5-10%. Oral nystatin suspension (1ml: 100.000 U/ml) has been considered as an option, when fluconazole is not available or azole resistance is suspected, but evidence is low [7] (CII). When indicated fluconazole is the recommended option, starting either in the first 24 or 72 hours of life at a dose 3-6 mg/kg i.v. (until catheter withdrawal) or orally twice weekly for 6 weeks. Multiple clinical trials have been performed with several regimens of fluconazole showing the impact of fluconazole in high-risk preterm babies in the incidence of IC, morbidity and mortality [8-12]. Twice weekly regimens do not seem to be inferior to the same daily dose [10]. According to the IDSA guidelines prophylaxis with fluconazole is indicated in admitted neonates with birth weight < 1000 gr when the prevalence of IC is higher than 10% [1]. Similarly, above this threshold in Spain, different Spanish scientific societies (Spanish Society of Infectious Diseases and Clinical Microbiology -SEIMC- and Spanish Society of Paediatric Infectious Diseases-SEIP) advocate fluconazole prophylaxis at 3 mg/kg/day in newborns with birth weight < 1,500 g, continuing it for all the period at risk [4,13]. The ESC-

MID strongly recommends it in NICU with a prevalence higher than 5% of IC in babies <1,000 g at birth at a dose of 3-6 mg/kg twice weekly i.v. or orally [14]. Also, the Latino American working group of invasive fungal infections recommends fluconazole prophylaxis 3 mg/kg twice a week, in newborn weighing < 1,000 g for 6 weeks in NICU with prevalence of IC ≥ 5% [15] (AI). In NICU with a prevalence < 5% fluconazole prophylaxis should be individualized and considered only in preterm neonates with multiple risk factors for IC (<1,000 g, need for a prolonged central vascular central and broad-spectrum antibiotics (CII) [14].

Fluconazole prophylaxis is a safe strategy, and although isolates of *Candida* spp. with reduced susceptibility to azoles have been described, there is no evidence of development of clinical emergence of resistance to azoles in newborns after prolonged exposure. Although there are few studies with long-term follow-up, there is no evidence for any relationship with neurocognitive impairment, blindness, deafness or cerebral palsy at 24 months of life, nor impact on growth. Even though fluconazole may lead to increased liver enzymes, significant liver toxicity is uncommon with prophylaxis dosages [12].

Filamentous fungi are infrequent in the neonatal period, and have only rarely been reported in preterm babies, including *Aspergillus* spp. and *Rhizopus* spp., albeit associated with a very high mortality [16]. Micafungin is approved in newborns including preterm but its activity against moulds is limited. Voriconazole use has been anecdotical, although it is not approved below 2 years of age [16, 17]. Due to its potential retinal toxicity in particular in the immature preterm retina, no clinical trials are planned in this age group [18]. Thus, specific AFP against moulds in this patient group is not recommended [2].

2.- ANTIFUNGAL PROPHYLAXIS IN THE PAEDIATRIC INTENSIVE CARE SETTING

Indications for primary prophylaxis in non-immunocompromised critically ill children are not clear. Despite a relatively high incidence of IFI in patients admitted at the Pediatric Intensive Care Unit (PICU), evidence for primary and/or secondary AFP in non-immunocompromised critically ill children is lacking.

In the intensive care setting, by far the predominant IFI are due to *Candida* spp., being filamentous fungi anecdotical, and therefore, AFP if considered, should be targeted only against yeasts. Some authors, based on different prediction scores, recommend AFP in adults for invasive candidiasis, with fluconazole (CII), or as an alternative an echinocandin like caspofungin in non-immunocompromised high-risk patients, although the grade of recommendation is low (CIII) [19, 20]. Risk factors for invasive candidiasis identified in this population include: abdominal surgery with recurrent perforations, intubated patients for more than 48 h and expected to be ventilated for another 72 hours, multiple *Candida* spp. colonization, systemic antibiotics, central venous catheters, transfusion and *Candida* spp. positive urine cultures [20, 21]. However, these criteria might not be fully applicable to children.

Several studies have tried to identify risk factors for invasive candidemia in non-immunocompromised critically ill children. In one population-based, case-control study in a large tertiary care paediatric center, the following risk factors for *Candida* spp. bloodstream infections were identified: presence of central venous catheter, underlying malignant conditions, and having received vancomycin or an anti-anaerobic antibiotic for more than 3 days. The predicted risk for candidemia for patients with some of these three risk factors in different combinations ranged between 10% and 46%. In this study, the authors concluded that these patients could be candidates for antifungal prophylaxis [4].

Another observational study in 24 Spanish PICUs recorded 125 invasive *Candida* spp. infections and determined that previous bacterial infection, chronic metabolic disease, digestive surgery, pre-PICU stay longer than 15 days, previous colonization, parenteral nutrition and invasive devices were risk factors [22]. Central venous catheters, immunosuppression (including long-term steroids), damage of gastrointestinal tract, broad-spectrum antibiotics, parenteral nutrition, renal failure requiring hemodialysis, mechanical ventilation and genetic susceptibility have been described in other studies as important risk factors [23-25]. Children younger than one year of age have a higher incidence of candidemia [26].

In addition to risk factors, the local incidence of invasive *Candida* spp. infections should be considered to decide whether or not to start AFP. An incidence of 10% is considered the threshold to use prophylaxis with an acceptable risk-benefit analysis. Some authors suggest that in PICUs with rates of invasive candidiasis higher than 5%, AFP may be considered in selected patients with several risk factors (CII).

There are few studies about the benefits of AFP in PICU. Reduction on invasive candidemia incidence with fluconazole prophylaxis has been studied in adult intensive care patients in four meta-analyses, though the incidence of both candidemia and mortality decreased only in two of these studies. Even though evidence is scarce especially in children, prophylaxis may be an option in selected critically ill children, other than immunocompromised and oncological patients, considering the high mortality of these infections. A personalized assessment may be warranted for individual PICU patients based on the presence of specific individual risk factors and local epidemiology

The difficulty to identify risk factors for invasive *Candida* spp. infections and the lack of evidence about the benefits of AFP, make early empirical treatment a preferred strategy for children with a high likelihood of fungal infection in order to decrease the high mortality rates [14] and minimizing the selection of antifungal resistances. Empiric therapy for non-neutropenic patients with risk factors for fungal infections without documented invasive candidiasis is still controversial [27]. Decision should be based on colonization data, presence of risk factors, surrogate markers of fungal infection and ongo-

ing fever despite proper antibiotic treatment. In the absence of microbiological confirmation nor clinical response, therapy should be maintained no more than 4 or 5 days [1].

In adults, daily bathing of the patients admitted in the intensive care units in chlorhexidine has been studied in one trial for its role as a protective factor for *Candida* spp. bloodstream infections [28]. Even though significant impact on *Candida* spp. infection is not proven, the measure is easy, inexpensive and may be beneficial. The impact in paediatric intensive care units is still to be determined.

In conclusion, current recommendations about prophylaxis should be individualized considering the PICU epidemiology, as well as the individual predisposition and colonization (CII).

The balance between overuse of antifungal agents with emerging resistance and efficacy is yet to be determined and better evidence in the paediatric intensive care setting has to be collected.

3.- CANCER PATIENTS AND STEM CELL TRANSPLANT RECIPIENTS

3.1 Risk factors for invasive fungal disease

Children receiving treatment for cancer or undergoing haematopoietic stem cell transplant (HSCT) have a significant risk of developing IFI, with high morbidity and mortality**.** Risk factors for IFI in these patients are conditioned by the breakdown in natural barriers, defects in cell-mediated immunity and mainly deficient the presence of profound and persistent neutropenia (table 1) [29, 30].

Primary AFP is generally recommended for those children whose risk is greater than 10% (table 2) [29, 31, 32]. However, in the choice of an appropriate AFP strategy it is important to consider some modifiers like the local epidemiology, comorbidities or specific treatment modalities. New therapies such as tyrosine kinase inhibitors and other immunomodulatory therapies (i.e. CAR T-cell therapy [33] broad the spectrum of patients at risk for IFI [34], so the assessment of risk should be individualized.

3.2 Primary antifungal prophylaxis

Whereas pharmacokinetic/pharmacodynamic (PK/PD) and safety of the different antifungal agents are targeted in paediatric studies; the evidence for efficacy may need to be extrapolated from studies in adult population. Although there are only few antifungal agents currently approved for AFP in children, an increasing number of reports describe safety and suggest efficacy of agents given to prevent IFI in the pediatric population. In addition, most studies do not address the optimal dosage of an antifungal agent to prevent IFI. The final choice of an antifungal drug for prophylaxis should be individualized based on the patient risk, the agent activity, the toxicity profile, and the PK/PD data [29, 32].

The specific recommendations for AFP are summarized in table 3 based on the different risk groups. There are not specific prophylaxis recommendations for the new drug classes for haematological and oncologic conditions (i.e. tyrosine kinase inhibitors], and it remains unclear if AFP is indicated in these cases [34]. Table 4 presents the different antifungal agents used for AFP. Azoles are the preferred drugs for prevention of IFI, considering anti-mould active agents in high-risk patients. Caution is advised for concomitant use of triazoles with chemotherapy metabolized by cytochrome P450 isoenzymes. Options include itraconazole (AII); posaconazole for patients ≥13 years of age (AII) and voriconazole for patients >2 years of age (AII). Echinocandins and liposomal amphotericin B represent alternatives when azole-

Table 1 Risk factors for IFI

Clinical factors	Severe and persistent neutropenia[a]
	Lymphopenia
	Mucosal damage
	Central venous catheters
	Previous fungal colonization
	Graft versus host disease (GVHD) in HSCT
	CMV infection in HSCT
Pharmacological factors	Steroids in high-doses[b]
	Anti-tumour necrosis factors agents
	Alemtuzumab
	Nucleoside analogues
	CAR T-cell therapy

IFI: invasive fungal infection, HSCT: haematopoietic stem cell transplant, CMV: cytomegalovirus, CAR: chimeric antigen receptor
[a]Absolute neutrophil count of ≤500 cells/µL for >7-10 days
[b]Steroids in pharmacological doses (≥0.3 mg/kg per day prednisone or equivalent)

Table 2 Stratification of risk for IFI

High-risk (≥ 10%)	Acute myeloid leukemia
	Recurrent or high-risk acute lymphoblastic leukemia
	Allogeneic HSCT[a]
	Severe aplastic anemia
Low-risk (≤ 5%)	Standard-risk acute lymphoblastic leukemia
	Autologous HSCT[b]
	Non-Hodgkin's lymphoma
Sporadic	Pediatric solid tumors
	Brain tumors
	Hodgkin's lymphoma

HSCT: Haematopoietic Stem Cell Transplant. GVHD: Graft versus Host Disease. IFI: invasive fungal infection.
[a]Pre-engraftment phase or with associated GVHD.
[b]In the neutropenic phase it could be considered intermediate-risk.

Table 3 **Antifungal primary prophylaxis in children with cancer: recommendations based on risk groups [31, 32, 34-39]**

Underlying condition	Cancer	Comments
Children undergoing allogeneic HSCT with no GVHD		
AFP is recommended during the neutropenic phase until engraftment (BII)	Fluconazole (AI)	Only active against yeasts
AFP is recommended after engraftment until discontinuation of immune suppression and immune recovery (no grading)	Itraconazole (BI)	TDM recommended
	Voriconazole (BI)	TDM recommended
	Micafungin (CI)	
	Liposomal amphotericin B (CIII)	
	Posaconazole (no grading)	For children ≥13 years
		TDM recommended
Children undergoing allogeneic HSCT in the presence of GVHD (acute grade II–IV or chronic extensive) treated with augmented immunosuppression		
AFP against mould and yeast infections is recommended while the immunosuppression is maintained (AII)	Posaconazole (BI)	For children ≥13 years
		TDM recommended
	Voriconazole (BI)	TDM recommended
	Itraconazole (CIII)	TDM recommended
	Liposomal amphotericin B (no grading)	
	Micafungin (no grading)	
Autologous HSCT with anticipated neutropenia >7 days		
AFP should be considered (BI) until immune recovery	Fluconazole (AI)	
	Micafungin (AII)	
	Any mould active agent (DIII)	
Paediatric de novo or recurrent leukemia patients		
AFP should be considered in high risk patients (BII).	Itraconazole (BI)	TDM recommended
No evidence-based recommendations can be made on the	Posaconazole (BI)	For children ≥13 years
duration in patients with persisting neutropenia in this group		TDM recommended
	Liposomal amphotericin B (BII)	
	Fluconazole (CI)	Active only against yeast
	Other options include: Voriconazole (no grading)	
	Micafungin (no grading)	

AFP: Antifungal prophylaxis. TDM: Therapeutic Drug Monitoring. HSCT: Haematopoietic Stem Cell Transplant. GVHD: Graft versus Host Disease

based regimes are contraindicated or not tolerated. Options include liposomal amphotericin B (BII); micafungin (BII); and, with less strength of evidence, aerosolized liposomal amphotericin B (CII) and caspofungin (CII) [31, 34, 35]. In the absence of GvHD, AFP may be continued after engraftment until discontinuation of immunosuppression and signs of immune recovery. In the presence of GvHD requiring augmented immunosuppression (including steroids in therapeutic dosages or anti-inflammatory antibodies), prophylaxis against IA and other relevant IFI is recommended (AII) [2]. Newer agents, such as isavuconazole, are under study in children, and have poor evidence to be recommended for AFP.

3.3 Secondary antifungal prophylaxis

Despite the scant data in children, secondary AFP or continued antifungal treatment after an episode of invasive mould infection is recommended based on the high rate of relapse (30–50%) [31]. The drug of choice should be active against the previous fungal pathogen. Secondary AFP should continue for as long as the patient is neutropenic or immunosuppressed (AII), e.g. allogeneic HSCT (early phase), chemotherapy resulting in severe neutropenia (i.e. <500/mL and at least for 7 days), acute GVHD > stage II, extensive chronic GVHD, or T-cell suppressing therapy, including steroids. Currently evidence is

Table 4 **Agents and antifungal dosing recommended in haemato-oncological or HSCT paediatric patients [31, 32, 37, 40].**

Antifungal agent and dosing	Dosing	Spectrum	Comments
Fluconazole	6–12 mg/kg/day QD IV/PO (maximum 400mg/day)	Only against yeast	
Itraconazole	5 mg/kg/day BD PO (>2 years of age)	Both yeasts and moulds	Not approved in patients <18 years. TDM required
Voriconazole	2 to <12 years or 12–14 years and <50 kg: 16 mg/kg/day BD IV/PO (first day: 18 mg/kg/day IV/PO BD) >15 years or 12–14 years and >50 kg: 8 mg/kg/day BD IV/PO (first day: 12 mg/kg/day BD IV; 400 mg/day BD PO)	Both yeasts and moulds (no against *Zygomycetes*)	Not approved in patients <2 years. TDM required. Increased risk of phototoxicity.
Posaconazole	600 mg/day TDS PO (suspension) in patients >13 years [41] 300 mg/day QD PO (3 x 100 mg delayed-release tablets). First day: 600 mg/day BD. 300 mg/day QD IV (first day: 300 mg/day BD)	Both yeasts and moulds	Limited PK data in patients <13 years. Not approved in the European Union in patients <18 years. TDM required. Coverage for most fungi, including *Zygomycetes*. Delayed released tablet formulation presents better PD data. Taken with food.
Liposomal amphotericin B	1 mg/kg IV every other day or 2.5 mg/kg IV twice weekly	Both yeasts and moulds	Still not approved for prophylaxis in children [32, 42]. Optimal dose of alternate administration is still unknown. Alternative when azole based regime is contraindicated or not tolerated.
Caspofungin	50 mg/m^2/day QD IV (first day: 70 mg/m^2/day QD IV) (maximum 70 mg/day)	Both yeasts and moulds	Caspofungin does not have a label for the prophylactic Indication [43].
Micafungin	1 mg/kg/day (if >50kg : 50mg) QD IV	Both yeasts and moulds	Approved for AFP of *Candida* spp. infections in granulocytopenic children. Less interactions than azoles with other drugs. Considerably higher drug clearance in children 4 months to 5 years compared to older children.

QD: once daily. BD: twice a day. TDS = three times a day. AFP: antifungal prophylaxis. For interactions, see table 6.

lacking regarding the minimal duration of the therapy before the continuation of anticancer treatment of the conditioning regime for allogeneic HSCT [31, 40].

3.4 Practical aspects

3.4.1 Therapeutic drug monitoring (TDM)

The objective of monitoring the plasma levels of antifungals is to optimize their dose, in order to improve efficacy and minimize toxicity. This is important in children, because of their pharmacokinetic variability, and especially in hematology-oncology patients who have multiple conditions (associated with their underlying disease and its treatment) that affects the absorption, distribution, metabolism and clearance of antifungal medications [44]. TDM is generally recommended during prophylaxis with itraconazole, voriconazole and posaconazole (AII). Recommended plasma target ranges are summarized in table 5 [40, 45]. Usually, the first sample should be acquired

Table 5 Recommended plasma target ranges for antifungal drugs

Antifungal	Prophylaxis plasma range	Treatment plasma range	Quality of evidence
Itraconazole	0.5-4 mg/L	1-4 mg/L	AII efficacy
			BII toxicity
Voriconazole	1-6 mg/L (optimal 2-5 mg/L)		AII efficacy
			AII toxicity
Posaconazole	>0.7 mg/L	>1 mg/L	BII efficacy (prophylaxis)
			AII efficacy (treatment)

within 5-7 days of starting therapy (2-5 days for voriconazole) and repeated until steady-state level in the therapeutic range is confirmed, if there are changes in the patient's clinical condition, concomitant medications, or suspected toxicity [40].

3.4.2 Side effects and drug-drug interactions

The main side effects of the antifungals used in prophylaxis and relevant interactions for hematology-oncology patients are summarized in table 6.

3.4.3 Monitoring of fungal biomarkers

Serum galactomannan (GM) screening should not be performed in neonates and children at low risk for IA (DIII). Serum GM should not be used as a screening test in asymptomatic patients undergoing AFP; several studies have shown that it has a low positive predictive value in these cases (BII) [46-48]. Therefore, given the low pre-test risk of IA in the context of effective anti-mould prophylaxis, the result of the test would be either negative or false positive in asymptomatic patients, leading to unnecessary diagnostic tests and treatments. The test remains useful to assist the diagnosis of patients with a clinical suspicion of IFI during prophylaxis [40, 46, 47, 49, 50].

4.- SOLID ORGAN TRANSPLANTATION (SOT)

Patients who received a SOT have a higher risk of IFI, being an important cause of morbidity and mortality. *Candida* spp. are the most frequent IFI, followed by filamentous fungi, especially *Aspergillus* spp. [51]. Not all recipients are at the same risk of IFI. The multicenter epidemiological study TRANSNET, conducted in adult population, shows the highest incidence of IFI in small bowel transplant recipients (11.6%), followed by lung (8.6%), liver (4.7%), heart (4%) and pancreas (3.4%) [51]. Data in children are still scarce, but a recent study, conducted in pediatric population revealed an IFI global incidence of 2%, cardiopulmonary and lung transplant showing the highest incidence (12.5% and 11.4% respectively) [52].

AFP in SOT pediatric recipients can decrease colonization, and therefore, the subsequent development of IFI. Nevertheless, universal AFP is not recommended, and its use will depend on the type of transplanted organ and the receptor risk factors to develop an IFI. There are no recommendations in the pediatric field due to the limited published information, so that most of them are adapted from those published in the adult population. In general, patients at high risk (expected incidence higher than 10%) should receive prophylaxis against filamentous fungi (AII). The drug of choice will depend on the type of transplant organ and the population studied [53].

4.1. Prophylaxis against yeasts

Liver, pancreas and bowel recipients have the highest risk to develop an invasive candidiasis, thereby potentially benefiting from AFP. Liver transplant recipients need to meet at least two risk factors [54, 55] (table 7). Fluconazole or echinocandins are the most recommended drugs [56], considering amphotericin B when patients have risk factors for filamentous fungi (AIII). The most recommended duration is 4 weeks in the liver transplant and at least 4 weeks in the pancreas and bowel transplant. In kidney recipients, invasive candidiasis is the most frequent IFI, although its incidence is low, so prophylaxis is not recommended (DIII) [54].

4.2 Prophylaxis against *Pneumocystis jirovecii* (PJ)

The incidence of PJ pneumonia in a study made in adult population in United Kingdom was 5.8% in lung or cardiopulmonary transplants, 5.5% in heart, 1.2% in liver and 0.3% in the kidney recipients [57]. Prophylaxis against PJ during the first months after the transplantation is recommended in several guidelines for adults and children [58]. Trimethoprim sulfamethoxazole (TMP-SMX) is the drug of choice [57, 59]. In case of intolerance to cotrimoxazole, dapsone is the second line drug more used for prophylaxis, although less effectivity has been observed in some pediatric studies [60]. There is little experience with atovaquone and pentamidine (inhaled or intravenous).

The duration of PJ prophylaxis is not established, ranging between 3 and 12 months after the transplantation according to different scientific societies [59, 61]. Its indication should be prolonged after graft rejection or higher steroid needs (over 20 mg of prednisolone or ≥0.3 mg/kg or equivalent for more than 4 weeks). It has also been proposed to keep it indefinitely in lung or bowel transplant, in patients with chronic CMV infections and in those with a history of a previous infection by PJ (BII) [57].

4.3 Prophylaxis against filamentous fungi

Invasive aspergillosis (IA) is one of the most relevant fungal infections in SOT recipients, with an overall incidence reported of 1-15%, being higher in lung transplant in surveillance studies (44%) [51], and reported mortality rates of ap-

Table 6 Side effects and drug interactions of antifungals used in prophylaxis

Antifungal	Adverse effects	Interactions
Fluconazole	Gastrointestinal disorders Elevation of transaminase levels	Cyclosporine, ifosfamida, irinotecan, vincristine, fentanyl, omeprazole, ondansetron, cotrimoxazole, prednisone, dexamethasone
Itraconazole	Gastrointestinal disorders Elevation of transaminase levels Periferal neuropathy Negative inotropic effect (less frequent)	Cyclosporine, ifosfamida, irinotecan, methotrexate, etoposide, vincristine, fentanyl, deferasirox, omeprazole, ondansetron, ranitidine, dexamethasone, prednisone
Voriconazole	Gastrointestinal disorders Elevation of transaminase levels Visual disturbances Hallucinations Headache Rash Long QT-syndrome	Ciclosporin, etoposide, ifosfamida, irinotecan, vincristine, fentanyl, cotrimoxazole, ibuprofen, omeprazole, ondansetron, dexamethasone, prednisone
Posaconazol	Gastrointestinal disorders Elevation of transaminase levels Headache Dizziness Periferal neuropathy Electrolyte alterations Long QT syndrome (less frequent)	Cyclosporine, etoposide, ifosfamida, irinotecan, vincristine, fentanyl, omeprazole, ranitidine, dexamethasone, prednisone
Micafungin	Gastrointestinal disorders Headache Phlebitis Elevation of transaminase levels Electrolyte alterations	Sirolimus, nifedipine, itraconazole
Amphotericin B	Hypokalemia Nephrotoxocity Headache Elevation of transaminase levels Infusion reactions	Cyclophosphamide, cisplatin, cytarabine, etoposide, hydroxyurea, ifosfamide, irinotecan, mercaptopurine, methotrexate, temozolamide, vincristine, vinorelbine, dexamethasone, prednisone cyclosporine, aminoglycosides, pentamidine

proximately 22% despite novel treatment modalities. In lung transplant recipients, invasive pulmonary disease has an even higher mortality rate (67–82%) [59]. AFP against *Aspergillus* spp. is recommended in lung transplant recipients (AIII) and in those children exhibiting a high-risk profile (e.g. Model for End Stage Liver Disease score >30, liver failure, renal failure, reintervention) (BIII) [2]. Data of IA in heart recipients are scarce. Reduction of IFI has been observed in those patients with prophylaxis, but no consensus exists. Some authors recommend AFP only in patients with risk factors [2, 54]. Inhaled lipid formulations of amphotericin B are the most studied option, although its optimal dose, formulation and duration has not been defined in adult population. Systemic azoles with anti-mould activity may also be used for IA prevention. The effectiveness and safety of voriconazole prophylaxis has been studied in lung transplant recipients [2]. The IDSA guidelines recommends itraconazole or voriconazole in patients colonized by *Aspergillus* spp., in those with a proven fungal infection in the removed organ, sinusal aspergillosis or in those who have received a unipulmonary transplant [35].

Duration of prophylaxis is unclear, but at least 3- to 4-week treatment or until resolution of risk factors seems appropriate [2]. In lung and high-risk heart transplanted children a more prolonged prophylaxis (3-12 months) is warranted. The drug of choice remains controversial. Lipid amphotericin B has shown

Table 7 Risk factors to IFI in children with SOT

Fungal infection	Solid organ transplant	Risk factors
Candida species	Liver	Retrasplant
		Post-transplant renal failure
		More than one episode of acute rejection during the first month, requiring the use of steroids or monoclonal antibodies
		Colonization by *Candida* spp.
Aspergillus species	Liver	Retrasplant
		Post-transplant renal failure requiring dyalisis
		Pretransplant fulminant liver failure
		Surgical re-intervention
	Intestine-pancreas	Immunosupression
		Acute graft rejection
		Hemodyalisis
		Initial graft rejection
		Anastomosis related issues
		Post-transplant need of laparotomy
		Infection by cytomegalovirus
	Heart	Post-transplant hemodyalisis
		Surgical reintervention
		Colonization or previous infection by *Aspergillus* spp. before or after transplantation
		Infection by cytomegalovirus
		Acute graft rejection

SOT: Solid Organ Transplantation

a significant reduction of invasive fungal infections without a mortality reduction but is limited by its potential for nephrotoxicity. Echinocandins are not nephrotoxic, and promising results have been published in preventive studies focusing on high-risk liver transplant recipients [2].

In paediatric kidney transplant recipients AFP to prevent filamentous fungi is not recommended (DIII) [54]. In small bowel and pancreas recipients transplant, only patients are risk are candidates for prophylaxis against moulds.

Table 8 summarized the indications about AFP in pediatric population after SOT [32].

5.- PRIMARY IMMUNODEFICIENCIES

Patients with primary immunodeficiencies (PID) are often prone to develop recurrent and/or severe infections, autoimmune disorders and malignancies. Infection site, causative pathogens, clinical course and outcome depend on a number of factors such as the underlying gene defect, patients' age, existence of comorbidities and also environmental factors potentially related to pathogen exposure [62]. IFI are a hallmark of underlying immune disorders and PID must always be considered in those patients. There are several PID that may present with both invasive and mucocutaneous fungal infections, caused by moulds and/or yeasts [63, 64].

Neutrophil defects, (severe) combined immunodeficiencies and diseases caused by mutations altering relevant cytokine pathways are among the list of PID that may present with severe fungal infections. Thus, primary or secondary AFP is recommended for most of the diseases below listed (table 9). However, evidence regarding the most appropriate medication, duration, dosing schedule, drug monitoring and dose adjustment is scarce and often extrapolated from adults and/or the onco-haematologic setting (table 4). Chronic granulomatous disease (CGD) is the PID with the highest risk for IFI, particularly IA with incidences ranging from 26% to 45%. Additionally, IA is the most common infectious cause of death. Prevention of IA plays a central role in the clinical management of children with CGD and consists of reducing environmental exposure to moulds and the prophylactic use of antifungals. Itraconazole prophylaxis has shown to significantly reduce IFD in CGD patients and is recommended as prophylaxis (AII). Posaconazole is a

Table 8 **Recommendations about antifungal prophylaxis in SOT**

Solid organ transplant	Predominant IFI	Antifungal prophylaxis	Doses	Duration
Liver	*Candida* spp.[a]	Fluconazole oral/ iv	6-8 mg/kg/day	4 weeks
		Caspofungin iv	50 mg/m^2/day	
	Aspergillus spp.[a]	In patients with risk factors for *Aspergillus*: Liposomal amphotericin B iv	1 mg/kg/day	
		Caspofungin iv	50 mg/m^2/day	
Lung	*Aspergillus* spp.	Liposomal amphotericin B (until extubation)	1 mg/kg/day	6-12 months
		Inhaled amphotericin B (in extubated patients)	24 mg: - 1st month 3 times / week – later 1 per week	
		Voriconazole oral/iv[b]	Oral <50Kg: 18mg/kg/day divided in two doses >50 Kg:400 mg/day divided in two doses IV <50Kg: 16 mg/kg/day divided in two doses >50 Kg: 8 mg/day divided in two doses	
		Itraconazole oral/iv[b]	5 mg/kg/day divided in two doses	
Heart	*Aspergillus* spp.	Itraconazole oral/ iv	5 mg/kg/día divided in two doses	3-6 months
		Voriconazole oral/iv	See the previous part	
		Caspofungin iv	50 mg/m^2/day	
		Micafungin iv	1 mg/kg/day	
Pancreas	*Candida* spp.	Fluconazole oral/iv	6-8 mg/kg/day	4 weeks
Intestine	*Candida* spp.	Fluconazole oral/iv	6-8 mg/kg/day	4 weeks
		Liposomal amphotericin B	1 mg/kg/day	
		Caspofungin iv	50 mg/m^2/day	
		Micafungin iv	1 mg/kg/day	
Pneumocystis jirovecii Indicated in all types of SOT		TMP-SMX oral/iv	150 mg/m^2/day divided in two doses 3 consecutive days/ week	3-12 weeks
		Dapsone iv	2 mg/kg/day	
		Pentamidine iv	4 mg/kg/ month	
		Inhaled pentamidine	300 mg/ month	
		Atovaquone iv	30 mg/kg/day	

SOT: Solid Organ Transplantation, IFI: invasive fungal infection. iv=intravenous

The proposed doses have been set following prophylaxis in others indications and after a consensus between the authors.

[a]Recommended in patients with risk factors defined in table 1.

[b]Therapeutic drug monitoring is recommended. Targeted prophylaxis plasma level. Voriconazole: ≥1 mg/l, itraconazole: ≥0,7 mg/l.

favourable alternative (CIII). The use of prophylactic recombinant human interferon-γ has shown to decrease the risk of severe infections (including fungal infections) in CGD by 70%, but controversy remains about its use in routine prophylaxis [2, 65-68].

6.- ANTIFUNGAL PROPHYLAXIS IN CHILDREN WITH HIV-INFECTION

6.1 *Pneumocystis jirovecii* (PJ)

Currently, since the advent of potent combined antiretro-

Table 9 **Indication for primary and/or secondary antifungal prophylaxis in primary immunodeficiencies (adapted from Aguilar C et al.) [63]**

Immunodeficiency	Fungi		Antifungal Prophylaxis
	Invasive /systemic	Mucocutaneous	
Chronic granulomatous disease	Frequent (>30%) *Aspergillus* spp. (pulmonary, bone lesions) and other moulds. Yeasts (rare)	CMC (rare)	Primary prophylaxis Itraconazole (AII)[a] Posaconazole (CIII)[b] Voriconazole not recommended (DIII)
Congenital neutropenia	Rare < 10% *Aspergillus* spp. (pulmonary infections) *Candida* spp. (disseminated infections)		The systematic prescription of antifungal prophylaxis is not justified (DIII). For persistent profound neutropenia despite G-CSF, itraconazole prophylaxis can be considered (BIII).
Hyper-IgM syndrome with cellular defect	*Pneumocystis jirovecii* (pulmonary infections)		TMP-SMX
SCID/CID	*P. jirovecii* (pulmonary infections) *Aspergillus* spp. (pulmonary infections) *Candida* spp.	CMC	TMP-SMX (AII) < 1 month of age consider fluconazole > 1 month consider itraconazole
STAT3 deficiency	*Aspergillus* spp.	CMC	If CMC, consider fluconazole If lung damage consider itraconazole (AIII)
CARD9 deficiency [64] (Only fungi from the phylum *Ascomycota*)	Very common (90%) Mostly *Candida* spp. (CNS infection 30%) Also deep dermatophytosis	Rare (10%) CMC or superficial dermatophytosis	Primary prophylaxis: Fluconazole (AIII) Secondary prophylaxis: according to isolated fungus /infections site
STAT1 gain of function	Rare: mostly *Candida* spp.	Very common Mostly CMC	If recurrent and/or severe CMC: Fluconazole (AIII)
APS-1 (APECED)		Restricted to non-invasive candida infections (CMC)	If recurrent and/or severe CMC: Fluconazole (AIII)
IL-12/IFN-gamma axis defect	Rare: *Candida* spp.	CMC	If recurrent and/or severe CMC: Fluconazole (AIII)
IL-17R deficiencies, ACT1 deficiency	*Candida* spp.	CMC	If recurrent and¨/or severe CMC: Fluconazole (AIII)

ACT1: adaptor for IL-17 receptors; APS1(APECED): autoimmune polyendocrinopathy type1; CARD9: caspase recruitment domain-containing protein 9; CID: combined immunodeficiency; CMC: chronic mucocutaneous candidiasis; G-CSF: granulocyte colony stimulating factor; IFN-gamma: interferon gamma; IL17-R: interleukin-17 receptor; SCID: severe combined immunodeficiency; STAT1: signal transducer and activator of transcription 1; STAT3: signal transducer and activator of transcription 3; TMP-SMX: Trimethoprim-sulfamethoxazole

[a]Itraconazole: broadest experience, dosing regimens are different in Europe and the US.

[b]Posaconazole with promising but only short term results.

viral therapy, PJ is most commonly diagnosed in non-HIV infected children [69]. PJ infection occurs in the general population during the first months of life. More than 80% of children aged 2 to 4 years have antibodies against PJ. Approximately a third of infected immunocompetent children will be asymptomatic or have mild respiratory symptoms. PJ pneumonia (PJP) occurs almost exclusively in the immunocompromised child and is an AIDS-defining illness. PJ infection incidence is highest in the first year of life, in particular between 3 to 6 months. The mode of PJ transmission remains to be established, airborne human to human transmission being the likely cause [70].

Chemoprophylaxis is highly effective in preventing PJP and is recommended in all children older than 6 years with CD4 counts < 200 cells/mm^3 or CD4 percentage <15%; in children 1 to 6 years old with CD4 counts < 500 cells/mm^3 or CD4 percentage <15%; and in infants younger than 12 months regardless of CD4 counts or CD4 percentage (AII) [70].

Infants with indeterminate HIV infection status should receive prophylaxis until HIV-infection has been excluded (AIII). PJP chemoprophylaxis is not recommended in infants found to be definitely or presumed HIV-uninfected. The child should not have other laboratory (e.g., no positive virologic test results) or clinical conditions (e.g., no AIDS-defining conditions that can-

Table 10 Recommended drugs for PJP prophylaxis

Antifungal	Doses and route	Frequency	Evidence
TMP-SMX	150 mg TMP /m²/daily vo	12-24h daily or	AI
1st choice		3 consecutive days or	
	Max dose: 320 mg TMP/daily	3 alternating days	
Atovaquone	Age 1-3 and > 24 months: 30 mg/kg/day/vo	Once daily	AI
2nd choice	4–24 months: 45 mg/kg/day/vo		
	≥13 years: 1,500mg/24h		
	Max dose 1,500 mg/daily		
Dapsone	Age >1 month		BI
3rd choice	2 mg/kg/day	Once daily	
	4 mg/kg/week	Once weekly	
	Max dose 100 mg/daily		
	Max dose 200mg/week		
Pentamidine	4 mg/kg/dose/iv	2-4 weeks	BII
	Age > 5 years:		
	300 mg/dosis/nebulized	Once monthly	BI
	Max dose 300 mg iv		

PJP: *Pneumocystis jirovicii* pneumonia, Max: maximum. iv: intravenous
In case of TMP-SMX contraindication (allergy, intolerance, interactions) 2nd choice prophylaxis includes atovaquone (AI) o dapsone (BI). Aerosolized pentamidine is recommended for children who cannot take TMP-SMX, atovaquone, or dapsone (BI). Intravenous pentamidine can be used in children older than age 2 years when other options are unavailable (BII).

not be explained on the basis of other causes of immunosuppression) or evidence of HIV infection. Presumptive exclusion of HIV infection in non-breastfeeding infants, can be based on two negative virologic test results, one obtained at ≥2 weeks and one obtained at ≥4 weeks of age; a negative test at ≥8 weeks of age or a negative antibody test at ≥6 months of age.

TMP–SMX is the drug of choice for prophylaxis due to its high efficacy, relative safety, low cost, and broad antimicrobial spectrum. It should be administrated during three consecutive or alternating days/week or on a daily base (AI). In case of TMP-SMX contraindication (allergy, intolerance, interactions), second choice prophylaxis includes atovaquone (AI) o dapsone (BI). Aerosolized pentamidine is recommended for children who cannot take TMP-SMX, atovaquone, or dapsone (BI). Intravenous pentamidine can be used in children older than age 2 years when other options are unavailable (BII).

Discontinuation of PJP chemoprophylaxis should be considered for HIV-infected children after having received cART for ≥6 months and have demonstrated for >3 consecutive months a CD4 percentage ≥15% or CD4 count ≥200 cells/mm^3 for patients aged ≥6 years (BII), or CD4 percentage ≥15% or CD4 count ≥500 cells/mm^3 for patients aged 1 to <6 years (BII) [70].

CD4 percentage and CD4 count should be re-evaluated at least every 3 months and prophylaxis reinstituted if the original criteria for prophylaxis are reached (BIII). PJP prophylaxis should not be discontinued in HIV-infected infants aged <1 year.

As PJ transmission occurs easily, isolation should be strongly considered and sharing a room with another patient with an undiagnosed respiratory illness that could be PJP should be avoided, especially during the first 2 years of life (AIII).

As none of the drugs used to treat and prevent PJP completely eliminates PJ, and prophylaxis is only effective while the selected drug is administered, patients who have experienced an episode of PJP should remain on a prophylactic regimen after treatment until they meet criteria for discontinuing prophylaxis (AIII).

Secondary prophylaxis should be discontinued applying the same criteria as for discontinuing primary prophylaxis. PJP prophylaxis must not to be discontinued in HIV-infected infants aged <1 year. Once PJP prophylaxis has been discontinued, children should be evaluated and followed-up despite normal or high CD4 counts or percentages (AIII). Lifelong prophylaxis should be administered if PJ infection reoccurs in a patient with a CD4 count ≥ 200 cells/mm (CIII). Table 10 summarizes the drugs used for PJP prophylaxis [70].

6.2 Cryptococcosis

As the incidence of cryptococcosis is low in HIV infected children, neither routine testing of asymptomatic children for serum cryptococcal antigen (CIII), nor primary prophylaxis is recommended (BIII). Secondary prophylaxis for a duration of at least 12 months is indicated using fluconazole (AI) or itraconazole (BI) [70].

6.3 Hystoplasmosis

Routine primary prophylaxis for histoplasmosis in children is not recommended (BIII). Prevention of exposure is attempted by avoiding risk factors predisposing to infection such as exposure to contaminated areas, which can result in the inhalation of histoplasma spores.

Prevention of recurrence is attempted using induction therapy (amphotericin B), followed by a consolidation therapy (itraconazole) for a total of at least 12 months. In case of

sustained immunosuppression (CD4 percentage <15% at any age or <150 cells/mm^3 in children aged ≥6 years) as well as in patients suffering from relapse despite appropriate therapy, treatment may be prolonged (AII). Whilst experience with voriconazole is limited in children, fluconazole has been shown to be less effective than itraconazole (CII).

Recommendations regarding discontinuation of secondary prophylaxis are based on data from clinical trials in adults. Once immune reconstitution (CD4 counts >150 cells/mm^3 in children aged>6 years or >15% at any age) is achieved, histoplasma serum antigen is <2 ng/mL (when available) and itraconazole has been given for ≥1 year; treatment may be stopped (CIII). Histoplasma antigen is not available in most Spanish centers. Therapy is to be continued in case of relapse occurring despite appropriate treatment (BIII) [70].

6.4 *Candidiasis*

Candidiasis due to *Candida* spp. is the most frequent fungal infection in HIV infected patients, being mainly localized and limited to the mucosa and the skin (oropharyngeal and oesophageal candidiasis, vulvovaginitis and dermatitis). Invasive candidiasis is less frequent.

Exposure to *Candida* spp. cannot be prevented as they are commensals of the mucosa and the skin. However, the limitation and rational use of antibiotics is fundamental in order to avoid overgrowth of *Candida* spp. Primary and secondary prevention are not indicated [70].

REFERENCES

1. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner et al. Clinical practice guideline for the mana,gement of Candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis.2016;62: e1–50.19. DOI: 10.1093/cid/civ1194
2. Warris A, Lehrnbecher T, Roilides E, Castagnola E, Brüggemann RJM, Groll AH. ESCMID-ECMM guideline: diagnosis and management of invasive aspergillosis in neonates and children. Clin Microbiol Infect. 2019: S1198-743X(19)30282-4. DOI: 10.1016/j.cmi.2019.05.019.
3. Benjamin DK Jr, Poole C, Steinbach WJ, Rowen JL, Walsh TJ. Neonatal candidemia and end-organ damage: a critical appraisal of the literature using meta-analytic techniques. Pediatrics 2003; 112:634-640. DOI. 10.1542/peds.112.3.634
4. Zaoutis TE, Prasad PA, Localio AR, Coffin SE, Bell LM, Walsh TJ, Gross R. Risk factors and predictors for candidemia in pediatric intensive care unit patients: implications for prevention. Clin Infect Dis 2010; 51:e38-e45. DOI: 10.1086/655698
5. Aguado JM, Ruiz I, Muñoz P, Mensa J, Almirante B, Vázquez L, Rovira M, et al por el Grupo de Estudio de Micología Médica de la SEIMC (GEMICOMED). Recomendaciones sobre el Tratamiento de la Candidiasis Invasiva y Otras Infecciones por Levaduras de la Sociedad Española de Microbiología Clínica y Enfermedades Infecciosas (SEIMC). Actualización 2010. Enferm Infecc Microbiol Clin 2011; 29:345-61. DOI: 10.1016/j.eimc.2011.01.008
6. Puig-Asensio M, Padilla B, Garnacho-Montero J, Zaragoza O, Aguado JM, Zaragoza R, et al. Epidemiology and predictive factors for early and late mortality in Candida bloodstream infections: a population-based surveillance in Spain. CANDIPOP Project. Clin Microbiol Infect. 2014;20:O245-54. DOI: 10.1111/1469-0691.12380
7. Vaseleiou E, Apsemidou A, Vyzantiadis TA, Tragiannidis A. Invasive candidiasis and candidemia in pediatric and neonatal patients: A review of current guidelines. Curr Med Mycol. 2018; 3:28-33. DOI 10.18502/cmm.4.3.173
8. Healy CM, Campbell JR, Zaccaria E, Baker CJ. Fluconazole prophylaxis in extremely low birthweight neonates reduces invasive candidiasis mortality rates without emergence of fluconazole resistant Candida. Pediatrics 2008; 1 21:703-10. DOI: 10.1542/peds.2007-1130
9. Kaufman DA. Challenging issues in neonatal candidiasis. Current Medical Research and Opinion, 2010. 26:1769-1778. DOI: 10.1185/03007995.2010.487799
10. Cleminson J, Austin N, McGuire W. Prophylactic systemic antifungal agents to prevent mortality and morbidity in very low birth weight infants.Cochrane Database of Systematic Reviews 2015, Issue 10. Art. No.: CD003850. DOI: 10.1002/14651858.CD003850.pub5
11. Ferreira da Silva J, Moreira Camargos PA, Corrêa LP, de Castro Romanelli RP. Fluconazole prophylaxis in preterm infants: a systematic review. Braz J Infect Dis 2017; 21:333–338. DOI: 10.1016/j.bjid.2017.01.008
12. Greenberg RG, Benjamin DK Jr. Neonatal candidiasis: diagnosis, prevention, and treatment. J Infect. 2014;69 Suppl.1:S19–22. DOI: 10.1016/j.jinf.2014.07.012
13. Figueras C, Diaz de Heredia C, Garcia JJ, Navarro ML, Ruiz Contreras J, Rossich R, et al for The Spanish Society of Paediatric Infectious Diseases (SEIP) recommendations on the diagnosis and management of invasive candidiasis. An Pediatr (Barc) 2011;74, 337.e1–e17. DOI: 10.1016/j.anpedi.2010.12.012
14. Hope WW, Castagnola E, Groll AH, Roilides E, Akova M, Arendrup MCet al. ESCMID guideline for the diagnosis and management of Candida diseases 2012: prevention and management of invasive infections in neonates and children caused by *Candida* spp. Clin Microbiol Infect 2012;18 Suppl. 7:38–52. DOI: 10.1111/1469-0691.12040
15. Santolaya ME, Alvarado Matute T, de Queiroz Telles F, Colombo AL, Zurita J, Tiraboschi IN, et al. Recommendations for the management of candidemia in neonates in Latin America. Grupo Proyecto Epico. Rev Iberoam Micol. 2013;30 Suppl. 1:158–70. DOI: 10.1016/j.riam.2013.05.011
16. Frick MA, Boix H, Camba Longueira F, Martin-Gomez MT, Rodrigo-Pendás JÁ, Soler-Palacin P. Primary Cutaneous Aspergillosis in a Preterm Infant. Pediatr Infect Dis J 2016;35:704-6. DOI:10.1097/INF.0000000000001136
17. Fortún J, Carratalá J, Gavaldá J, Lizasoaín M, Salavert M, de la Cámara R, et al por el Grupo de Estudio de Micología Médica de la SEIMC (GEMICOMED). Recomendaciones sobre el Tratamiento de la Enfermedad Fúngica Invasiva por *Aspergillus* spp y otros hongos filamentosos de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC). Actualización 2010. Enfermedades Infecciosas y Microbiología Clínica 2011 Enferm Infecc Microbiol

Clin 2011; 29:435-54. DOI: 10.1016/j.eimc.2011.01.010

18. Steinbach WJ. Antifungal agents in children. Pediatr Clin North Am 2005;52:895-915. DOI: 10.1016/j.pcl.2005.02.009
19. Science M, Robinson PD, MacDonal T, Rasseckh SR, Dupuis LL, Sung L, Guideline for primary antifungal prophylaxis for pediatric patients with cancer or hematopoietic stem cell transplant recipients. Pediatr Blood Can 2014; 612: 393-400. DOI:10.1002/pbc.24847
20. Corneley OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortholary O, Meesserman W, et al. ESCMID guideline for the diagnosis and management diseases 2012: non-neutropenic adult patients. Clin Microbiol Infect 2012. 18 (suppl. 7): 19-37. DOI:10.1111/1469-0691.12039
21. Colombo AL, Almeida Junior JN, Slavin MA, Chen SCA, Sorrell TC. Candida and invasive mould diseases in non-neutropenic critically ill patients and patients with haematological cancer. Lancet Infect Dis 2017; e344-e356. DOI:10.1016/S1473-3099(17)30304-3
22. Jordan I, Balaguer M, Lopez-Castilla JD, Belda S, Shuffelman C, García Teresa MA, et al. on behalf of the ERICAP Study Group. Per-Species risk factors and predictors of invasive Candida infections in patients admitted to Pediatric Intensive Care Units: development of ERICAP Scoring Systems. Pediatr Infect Dis J 2014; 33: e187-193. DOI:10.1097/INF.0000000000000274
23. Blyth CC, Chen SC, Slavin MA, Nguyen Q, Marriott D, Ellis D, et al. Australian Candidemia Study. Not just Little adults: Candidemia epidemiology, molecular characterization and antifungal susceptibility in neonatal and pediatric patients, Pediatrics 2009;123:1360. DOI: 10.1542/peds.2008-2055.
24. Benedict K, Roy M, Kabbani S, Anderson F, Farley MM, Harb B, et al. Neonatal and Pediatric candidemia: Results from Population-Based Active Laboratory Surveillance in Four US Location 2009-2015. J Pediatric Infect Dis Soc 2018;7:e78. DOI:10.1093/jpids/piy009
25. Kumar V, Cheng SC, Johnson MD, meekens SP, Wojtowicz A, Giamarellos-Bourboulis E , et al. Immunochip SNP array identifies novel genetic variants conferring susceptibility to candidemia. J Infect Dis 2012;205:934. DOI: 10.1038/ncomms5675.
26. Pana ZD, Kotzadamis D, Roilides E. Invasive Candidiasis in Pediatric Intensive Care Unit: More Challenges. Pediatr Infect Dis J 2018;37:1309. DOI:10.1097/INF.0000000000002186
27. Martin-Loeches I, Antonelli M, Cuenca-Estrella M, Dimopoulos G, Einav S, De Waele JJ, et al. ESICM/ESCMID task force on practical management of invasive candidiasis in critically ill patients. Intensive Care Med 2019;45;789-805. DOI.org/10.1007/s00134-019-05599-w.
28. Climo MW, Yokoe DS, Warren DK, Perl TM, Bolon M, Herwaldt LA, Weinstein RAet al. Effect of daily chlorhexidine bathing on hospital-acquired infection. N Engl J Med 2013; 368:533–42. DOI:10.1056/NEJMoa1113849
29. Dvorak CC, Fisher BT, Sung L, Steinbach WJ, Nieder M, Alexander S, Zaoutis TE. Antifungal Prophylaxis in Pediatric Hematology/Oncology: New Choices & New Data. Pediatr Blood Cancer. 2012;59:21–6. DOI:10.1002/pbc.23415
30. Papachristou S, Iosifidis E, Roilides E. Invasive Aspergillosis in Pediatric Leukemia Patients: Prevention and Treatment. J Fungi. 2019;5:14. DOI:10.3390/jof5010014
31. Groll AH, Castagnola E, Cesaro S, Dalle JH, Engelhard D, Hope W, et al. Fourth European Conference on Infections in Leukaemia (ECIL-4): Guidelines for diagnosis, prevention, and treatment of invasive fungal diseases in paediatric patients with cancer or allogeneic haemopoietic stem-cell transplantation. Lancet Oncol. 2014;15:e327–40. DOI:10.1016/S1470-2045(14)70017-8
32. Lehrnbecher T. Antifungal prophylaxis in pediatric patients undergoing therapy for cancer: Drugs and dosing. Curr Opin Infect Dis. 2015;28:523–31. DOI:10.1097/QCO.0000000000000210
33. Hill JA, Li D, Hay KA, Green ML, Cherian S, Chen X, et al. Infectious complications of CD19-targeted chimeric antigen receptor-modified T-cell immunotherapy. Blood. 2018;131:121–30. DOI:10.1182/blood-2017-07-793760
34. Mellinghoff SC, Panse J, Alakel N, Behre G, Buchheidt D, Christopeit M, et al. Primary prophylaxis of invasive fungal infections in patients with haematological malignancies: 2017 update of the recommendations of the Infectious Diseases Working Party (AGIHO) of the German Society for Haematology and Medical Oncology (DGHO). Ann Hematol. 2018;97:197–207. DOI:10.1007/s00277-017-3196-2
35. Patterson TF, Thompson GR, Denning DW, Fishman JA, Hadley S, Herbrecht R, et al. Practice guidelines for the diagnosis and management of aspergillosis: 2016 update by the infectious diseases society of America. Clin Infect Dis. 2016;63:e1–60. DOI:10.1093/cid/ciw326
36. Tissot F, Agrawal S, Pagano L, Petrikkos G, Groll AH, Skiada A, et al. ECIL-6 guidelines for the treatment of invasive transplant patients : Haematologica. 2017;102:433–44. DOI:10.3324/haematol.2016.152900
37. Science M, Robinson P, MacDonald T, Rassekh SR, Dupuis LL SL, Stem. Guideline for primary antifungal prophylaxis for pediatric patients with cancer or hematopoietic cell transplant recipients. Pediatr Blood Cancer. 2014;61:393–400. DOI:10.1002/pbc.24847
38. Maertens JA, Girmenia C, Brüggemann RJ, Duarte RF, Kibbler CC, Ljungman P, et al. European guidelines for primary antifungal prophylaxis in adult haematology patients: summary of the updated recommendations from the European Conference on Infections in Leukaemia. J Antimicrob Chemother. 2018:1–10. DOI:10.1093/jac/dky286
39. Taplitz RA, Kennedy EB, Bow EJ, Crews J, Gleason C, Langston AA, et al. Antimicrobial prophylaxis for adult patients with cancer-related immunosuppression: ASCO and IDSA clinical practice guideline update. J Clin Oncol. 2018;36:3043–54. DOI:10.1200/JCO.18.00374
40. Ullmann AJ, Aguado JM, Arikan-Akdagli S, Denning DW, Groll AH, Lagrou K, et al. Diagnosis and management of Aspergillus diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline. Clin Microbiol Infect. 2018;24:e1–38. DOI:10.1016/j.cmi.2018.01.002
41. Döring M, Cabanillas Stanchi KM, Queudeville M, Feucht J, Blaeschke F, Schlegel P, et al. Efficacy, safety and feasibility of antifungal prophylaxis with posaconazole tablet in paediatric patients after haematopoietic stem cell transplantation. J Cancer Res Clin Oncol. 2017;143:1281–92. DOI:10.1007/s00432-017-2369-7
42. Bochennek K, Tramsen L, Schedler N, Becker M, Klingebiel T, Groll AH, et al. Liposomal amphotericin B twice weekly as antifungal prophylaxis in paediatric haematological malignancy patients. Clin Microbiol Infect. 2011;17:1868–74. DOI:10.1111/j.1469-0691.2011.03483.x

43. Döring M, Hartmann U, Erbacher A, Lang P, Handgretinger R, Müller I. Caspofungin as antifungal prophylaxis in pediatric patients undergoing allogeneic hematopoietic stem cell transplantation: A retrospective analysis. BMC Infect Dis. 2012;12. DOI:10.1186/1471-2334-12-151
44. Soler-Palacín P, Frick MA, Martín-Nalda A, Lanaspa M, Pou L, Roselló E, de Heredia CD, Figueras C. Voriconazole drug monitoring in the management of invasive fungal infection in immunocompromised children: a prospective study. J Antimicrob Chemother 2012;67:700-6. DOI: 10.1093/jac/dkr517
45. Lewis R, Brüggemann R, PaDOIn C, Maertens J, Marchetti O, Groll A, et al. Triazole Antifungal Therapeutic Drug Monitoring Final slide set posted on the ECIL website on ECIL 6 meeting. 2015; Available from: https://www.ebmt.org/Contents/Resources/Library/ECIL/Documents/2015 ECIL6/ECIL6-Triazole-TDM-07-12-2015-Lewis-R-et-al.pdf
46. Duarte RF, Sánchez-Ortega I, Cuesta I, Arnan M, Patiño B, Fernández de Sevilla A, et al. Serum galactomannan-based early detection of invasive aspergillosis in hematology patients receiving effective antimoulds prophylaxis. Clin Infect Dis 2014;59:1696–702. DOI:10.1093/cid/ciu673
47. Vena A, Bouza E, Álvarez-Uría A, Gayoso J, Martín-Rabadán P, Cajuste F, et al. The misleading effect of serum galactomannan testing in high-risk haematology patients receiving prophylaxis with micafungin. Clin Microbiol Infect 2017;23:1000.e1-1000.e4.DOI.org/10.1016/j.cmi.2017.05.006
48. Lehrnbecher T, Robinson PD, Fisher BT, Castagnola E, Groll AH, Steinbach WJ, et al. Galactomannan, β-D-Glucan, and Polymerase Chain Reaction-Based Assays for the Diagnosis of Invasive Fungal Disease in Pediatric Cancer and Hematopoietic Stem Cell Transplantation: A Systematic Review and Meta-Analysis. Clin Infect Dis. 2016;63:1340–8. DOI:10.1093/cid/ciw592
49. Lehrnbecher T, Hassler A, Groll AH, Bochennek K. Diagnostic approaches for invasive aspergillosis-Specific considerations in the pediatric population. Front Microbiol. 2018;9:1–8. DOI:10.3389/fmicb.2018.00518
50. Tong T, shen J, Xu Y. Serum galactomannan for diagnosing invasive aspergillosis in pediatric patients: A meta-analysis. Microb Pathog [Internet]. 2018;118:347–56. DOI.org/10.1016/j.micpath.2018.03.059
51. Pappas PG, Alexander BD, Andes DR, Hadley S, Kauffman CA et al. Invasive fungal infections among organ transplant recipients: results of the Trasplant- Associated Infection Surveillance Network (TRANSNET). Clin Infect Dis. 2010; 50: 1101-1111. DOI:10.1086/651262
52. Saxena SG, Gee J, Klieger S, Kajon A, Petersen H, Zaoutis T, Fisher B. Invasive Fungal Disease in Pediatric Solid Organ Transplant Recipients. J Pediatr Infectious Diseases Society 201; 7, 219-225. DOI:10.1093/jpids/pix041
53. Garcia-Vidal C, Alastruey-Izquierdo A, Aguilar-Guisado M, Carratalà J, Castro C, Fernández-Ruiz M, et al. Executive summary of clinical practice guideline for the management of invasive diseases caused by Aspergillus: 2018 Update by the GEMICOMED-SEIMC/REIP. Enferm Infecc Microbiol Clin. 2018 Jun 27. pii: S0213-005X(18)30200-3. DOI:10.1016/j.eimc.2018.03.018
54. Brizendine KD, Vishin S, Baddley JW. Antifungal prophylaxis in solid organ transplant recipients. Expert Rev. Anti Infect Ther 2011; 9 (5): 571- 581. DOI:10.1586/eri.11.29
55. Zaragoza R, Aguado JM, Ferrer R, Rodríguez AH, Maseda E, Llinares P Antifungal prophylaxis in solid organ transplant recipients. Rev Iberoam Micol 2016; 33: 187-195. DOI:10.1016/j.riam.2016.02.001
56. Fortún J, Muriel A, Martín-Dávila P, Montejo M, Len O, Torre-Cisneros J, Carratalá J, et al. Caspofungin versus fluconazole as prophylaxis of invasive fungal infection in high-risk liver transplantation recipients: A propensity score analysis. Liver Transpl. 2016;22:427-35. DOI: 10.1002/lt.24391.
57. Brakemeier S, Pfau A, Zukunft B, Budde K, Nickel P. Prophylaxis and treatment of Pneumocystis Jirovecci after solid organ transplantation. Pharmacol Res.2018; 134: 61-67. DOI:10.1016/j.phrs.2018.06.010
58. Patterson TF, Thompson GR, Denning DW, Fishman JA, HAdley S et al. Practice guideline for the diagnosis and management of Aspergillosis: 2016 updateby the Infectious Diseases Society of America. Clin Infect Dis. 2016;63:e1-e60. DOI:10.1093/cid/ciw326
59. Kabir V, Maertens J, Kuypers D. Fungal infections in solid organ transplantation: an update on diagnosis and treatment. Transplat RV (Orlando). 2019; 33: 77-86. DOI:10.1016/j.trre.2018.12.001
60. Prasad P, Nania JJ, Shankar SM. Pneumocystis pneumonia in children receiving chemotherapy. Pediatr Blood Cancer. 2008; 50: 896-8. DOI:10.1002/pbc.21202
61. Fishman JA, Gans H; AST Infectious Diseases Community of Practice. Pneumocystis jiroveci in Solid Organ Transplantation - Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019:e13587. DOI: 10.1111/ctr.13587
62. Picard C, Gaspar H, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, et al. International Union of Immunological Societies: 2017 Primary Immunodeficiency Diseases Committee Report on Inborn Errors of Immunity.J Clin Immunol. 2018;38:96-128. DOI:10.1007/s10875-015-0201-1
63. Aguilar C, Malphettes M, Donadieu J, Chandesris O, Coignard-Biehler H, Catherinot E, et al. Prevention of infections during primary immunodeficiency. Clin Infect Dis. 2014;59:1462-70. DOI:10.1093/cid/ciu646
64. Corvilain E, Casanova JL, Puel A. Inherited CARD9 Deficiency: Invasive Disease Caused by Ascomycete Fungi in Previously Healthy Children and Adults. J Clin Immunol. 2018;38:656-693. DOI:10.1007/s10875-018-0539-2
65. Gallin JI, Alling DW, Malech HL, Wesley R, Koziol D, Marciano B, et al. Itraconazole to prevent fungal infections in chronic granulomatous disease. N Engl J Med. 2003;348:2416-22. DOI:10.1056/NEJMoa021931
66. Slack Ma, Thomsen IP. Prevention of Infectious Complications in Patients with Chronic Granulomatous Disease. J Pediatric Infect Dis Soc. 2018;7 (suppl_1): S25-S30. DOI:10.1093/jpids/piy016
67. Mouy R, Veber F, Blanche S, Donadieu J, Brauner R, Levron JC, et al. Long-term itraconazole prophylaxis against Aspergillus infections in thirty-two patients with chronic granulomatous disease. J Pediatr 1994; 125:998–1003. DOI:10.1016/s0022-3476(05)82023-2
68. Welzen ME, Brüggemann RJ, Van Den Berg JM, Gilissen JH, Pajkrt D, Klein N, et al. A twice daily posaconazole dosing algorithm for

children with chronic granulomatous disease. Pediatr Infect Dis J. 2011;30:794-7. DOI:10.1097/INF.0b013e3182195808

69. García-Moreno J, Melendo-Pérez S, Martín-Gómez MT, Frick MA, Balcells-Ramírez J, Pujol-Jover M. Pneumocystis jirovecii pneumonia in children. A retrospective study in a single center over three decades. Enferm Infecc Microbiol Clin. 2019 (in press) DOI:10.1016/j.eimc.2019.05.005
70. Siberry GK, Abzug MJ, Nachman S; Panel on Guidelines for Prevention and Treatment of Opportunistic Infections in HIV-Exposed and HIV-Infected Children—a working group of the Office of AIDS Research (OAR) Advisory Council. J Pediatric Infect Dis Soc 2013; 2:293-308. DOI:10.1093/jpids/pit074

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Microbiota biliar en pacientes colecistectomizados: Revisión de la antibioterapia empírica

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RESUMEN

Introducción. La colecistitis constituye una importante causa de ingreso hospitalario. En colecistitis moderada o severa, el retraso en el tratamiento puede acarrear complicaciones graves. Nuestro objetivo es analizar los microorganismos aislados en bilis de pacientes colecistectomizados y su patrón de sensibilidad para evaluar el tratamiento empírico en aquellos casos en que la extirpación quirúrgica de la vesícula deba demorarse.

Pacientes y métodos. Estudio descriptivo prospectivo de los cultivos biliares de pacientes sometidos a colecistectomía desde mayo de 2013 hasta febrero de 2015, en el Servicio de Cirugía del Hospital General Universitario de Castellón.

Resultados. Se estudiaron 196 pacientes, 83 mujeres (42,3%) y 113 hombres (57,7%), con una media de edad de 61,5 años. Los antibióticos más utilizados como tratamiento empírico fueron piperacilina/tazobactam (77,8%) y amoxicilina/clavulánico (14,8%). En el 46,4% de los pacientes (91/196) los cultivos de bilis fueron positivos. Se aislaron un total de 165 microorganismos. La mayoría eran bacilos gramnegativos (60,5%), principalmente *Enterobacterales* (91/54,5%), siendo *Escherichia coli* el microorganismo más frecuente (24%) seguido de *Klebsiella* spp. (12,5%). Se aislaron 3 *E. coli* productoras de betalactamasa de espectro extendido (BLEE) y 1 *Klebsiella pneumoniae* BLEE. No se aislaron microorganismos productores de carbapenemasa ni *Staphylococcus aureus* resistente a meticilina.

Conclusión. La microbiota biliar, con predominio de *Enterobacterales*, es similar a la encontrada en estudios europeos.

Palabras clave: Colecistitis, microbiota biliar, antibioterapia

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Biliary microbiote in cholecystectomized patients: Review of empirical antibiotherapy

ABSTRACT

Introduction. Cholecystitis is an important cause of hospital admission. In moderate or severe cholecystitis, the delay in treatment can lead to serious complications. Our objective is to analyze the microorganisms isolated in bile from cholecystectomized patients and their sensitivity pattern, to evaluate the empirical treatment in those cases in which the surgical removal of the gallbladder should be delayed.

Patients and methods. Prospective descriptive study of biliary cultures of patients undergoing cholecystectomy from May 2013 to February 2015, in the Surgery Department of the Hospital General Universitari de Castelló.

Results. We studied 196 patients, 83 women (42.3%) and 113 men (57.7%), with an average age of 61.5 years. The most used antibiotics as empiric treatment were piperacillin/tazobactam (77.8%) and amoxicillin/clavulanic (14.8%). In 46.4% of patients (91/196) bile cultures were positive. 165 microorganisms were isolated. The majority were Gram-negative bacilli (60.5%), mainly of the *Enterobacterales* order (91/54.5%), with *Escherichia coli* being the most frequent microorganism (24%) followed by *Klebsiella* spp. (12.5%). 3 *E. coli* with extended-spectrum beta-lactamase (ESBL) and 1 *K. pneumoniae* with ESBL were isolated. Microorganisms producing carbapenemase and methicillin-resistant *Staphylococcus aureus* were not isolated.

Conclusion. The bile microbiota, with a predominance of *Enterobacterales* is similar to that found in european studies.

Key-words: Cholecystitis, biliary microbiota, antibiotic therapy

INTRODUCCIÓN

La inflamación de las vías biliares supone una de las principales causas de ingreso hospitalario. Se diferencian dos for-

mas clínicas: la colecistitis, relacionada con la inflamación de la vesícula biliar, y la colangitis, que afecta a los conductos biliares.

La colecistitis aguda aparece, en el 90-95% de los casos, tras la obstrucción completa y prolongada del cuello de la vesícula o del conducto cístico por cálculos (*colelitiasis*), pero también tras isquemia, trastornos de la motilidad, infecciones o colagenopatías [1]. Si no se instaura tratamiento precoz puede aparecer infección secundaria a la colestasis y complicaciones como empiema vesicular, colecistitis gangrenosa, absceso abdominal o hepático, perforación de la vesícula con peritonitis e incluso sepsis. Según las Tokio Guidelines (TG) [1] hay tres grados en la colecistitis aguda que van desde la inflamación local sin signos generales, Grado I, a la disfunción orgánica severa o Grado III (tabla 1). El tratamiento puede ser desde conservador con antibioterapia (acompañada o no de drenaje biliar por colecistostomía en aquellos pacientes de riesgo) hasta quirúrgico realizando colecistectomía. El propósito de la antibioterapia es limitar la respuesta inflamatoria local y sistémica, y prevenir tanto la infección postquirúrgica como la formación de abscesos intrahepáticos. En la colecistitis leve su papel es fundamentalmente profiláctico, mientras que en la moderada o severa es terapéutico mientras no pueda extirparse la vesícula. Los antibióticos deberían administrarse en las primeras 6 horas tras la sospecha de infección biliar excepto en pacientes sépticos, en los que su administración no debería demorarse más de 1 hora. Para instaurar el tratamiento empírico deben considerarse, entre otros factores, los microorganismos probables, la sensibilidad local, los antecedentes de hospitalización o la toma de antibióticos en los 6 meses previos, por aumento en el riesgo de aparición de resistencias.

El porcentaje de positividad de los cultivos en colecistitis aguda ronda el 29-54% [2]. Los microorganismos habitualmente aislados pertenecen al orden *Enterobacterales* (con predominio de *Escherichia coli* y *Klebsiella pneumoniae*). Aproximadamente el 5% de las infecciones comunitarias y el 10% de las nosocomiales lo son por *E. coli* o *K. pneumoniae* productoras de betalactamasa de espectro extendido (BLEE) [3]. La mayoría de las guías recomiendan emplear antibióticos de amplio espectro en pacientes con infección grave, que hayan recibido antibióticos previamente o con comorbilidad importante [4].

El objetivo de nuestro estudio es analizar los microorganismos aislados en la bilis de pacientes colecistectomizados así como su patrón de sensibilidad, con el propósito de evaluar el tratamiento empírico para aquellos casos en que no sea posible (o deba demorarse) la extirpación quirúrgica de la vesícula biliar.

Tabla 1 **Criterios de severidad para la infección de origen biliar**

-Grado I o leve: Inflamación local sin signos generales

-Grado II o moderada: Fiebre>38, leucocitosis>18000/mL, masa dolorosa palpable, evolución>72h, signos inflamatorios (coleperitoneo, absceso perivesicular o hepático, colecistitis gangrenosa o enfisematosa).

-Grado III o grave: Hipotensión que requiere fármacos, alteración de consciencia, disfunción respiratoria, renal (Creatinina >2 mg/dl), hepática (INR >1,5) o hematológica (plaquetas <100.000/mL)

PACIENTES Y MÉTODOS

Estudio descriptivo prospectivo de los cultivos biliares de pacientes sometidos a colecistectomía desde mayo de 2013 hasta febrero de 2015, en el Servicio de Cirugía del Hospital General Universitario de Castellón. Los criterios de inclusión fueron: pacientes diagnosticados de colelitiasis sintomática (desde cólico biliar simple hasta colecistitis aguda), con indicación quirúrgica para colecistectomía tanto de forma programada como urgente, incluyendo la vía laparoscópica y la abierta.

Se excluyeron los pacientes diagnosticados de colangitis, las colecistectomías realizadas por neoplasias pancreáticas o tumores de la vía biliar, las colecistectomías por pólipos vesiculares y las colecistostomías percutáneas.

Las muestras se tomaron intraoperatoriamente y se remitieron, en las 2 horas siguientes, al Servicio de Microbiología para cultivo bacteriológico. Se procesaron según los protocolos estandarizados del laboratorio, realizándose identificación bacteriana y estudio de sensibilidad (CMI) mediante los sistemas automatizados Vitek 2 (bioMérieux®) y/o Phoenix (Becton Dickinson®).

Se analizaron variables *epidemiológicas*: sexo, edad, hospitalización previa, comorbilidad, toma de antibióticos los 6 meses previos, antibióticos al ingreso, antibiótico profiláctico; *clínicas*: diagnóstico, manipulación previa de las vías biliares, estancia postoperatoria o exitus; y *microbiológicas*: cultivo de bilis, hemocultivos, microorganismos aislados y porcentaje de microorganismos multirresistentes.

Los datos se incluyeron para su análisis estadístico en el paquete GNU GPL (General Public License) PSPP, versión 1.2.0. Las variables cuantitativas se expresaron como media, desviación estándar (DE) y/o rango, o bien como valor absoluto y porcentaje. Para la comparación de variables cualitativas o categóricas se empleó el test de chi-cuadrado (χ^2). Las diferencias se consideraron estadísticamente significativas cuando $p < 0,05$.

RESULTADOS

Se estudiaron 196 pacientes, 83 mujeres (42,3%) y 113 hombres (57,7%), con una media de edad de 58,68 años (rango:16-91). 109 (55,6%) habían estado hospitalizados los 12 meses previos y 112 (57,1%) presentaban alguna comorbilidad (hipertensión arterial, diabetes mellitus, dislipemia, cardiopatía, enfermedad pulmonar obstructiva crónica o neoplasia).

Respecto a la antibioterapia, 92 pacientes (47%) habían tomado algún antibiótico en los 6 meses previos a la cirugía. Los 54 pacientes (27,6%) operados de urgencia por colecistitis aguda llevaron antibióticos como parte del tratamiento duran-

Tabla 2 **Microorganismos aislados en bilis (n=165)**

GRAMNEGATIVOS	100	% (60,6)	GRAMPOSITIVOS	44	% (26,6)
Enterobacterales	91	54,5	*Enterococcus* spp.	19	11,5
E. coli	40	24	*E. faecalis*	9	5,4
Klebsiella spp.	21	12,5	*E. faecium*	3	1,8
Enterobacter spp.	13	7,8	*E. casseliflavus/gallinarum*	3	1,8
Citrobacter spp.	7	4,2	Otras especies	4	2,4
Proteus spp.	4	2,4			
Providencia rettgeri	2	1,2			
Pantoea agglomerans	2	1,2			
Serratia spp.	2	1,2			
Otros BGNs	9	5,4	*Streptococcus* spp.	19	11,1
Pseudomonas aeruginosa	3	1,8			
Aeromonas spp.	3	1,8			
Haemophilus influenzae	1	0,6			
Campylobacter jejuni	1	0,6			
Shewanella putrefaciens	1	0,6			
			Otros grampositivos	6	3,6
ANAEROBIOS	**17**	**% (10,2)**	**LEVADURAS**	**4**	**% (2,4)**
Clostridium spp.	8	4,8	*C. albicans*	2	1,2
BGN anaerobio	5	3	*C. glabrata*	1	0,6
Bacteroides spp.	2	1,2	*C. tropicalis*	1	0,6
Prevotella spp.	2	1,2			

BGN: bacilos gramnegativos

te el ingreso y los 142 (72,4%) pacientes intervenidos de forma programada, profilaxis con 2 g de cefazolina 30 minutos antes de la colecistectomía. Para el tratamiento de la colecistitis aguda los antibióticos más utilizados fueron: piperacilina/tazobactam (77,8%), amoxicilina/clavulánico (14,8%), ertapenem (3,7%) y ciprofloxacino más metronidazol (3,7%) en alérgicos a la penicilina. La duración media del tratamiento antibiótico en la colecistitis fue de 5 días (DE: 2,40). En 25 casos (12,8%) se realizó colangiopancreatografía retrógrada endoscópica (CPRE) por coledocolitiasis. La estancia media postoperatoria fue de 4,2 días (DE: 5,09; rango: 1-44). 4 pacientes (2%) fallecieron, 2 de ellos por shock séptico.

En el 46,4% de los pacientes (91/196) los cultivos de bilis fueron positivos: 52 (57,1%) con un microorganismo, 22 (24,2%) con dos y 17 (18,7%) con tres o más. Se encontró bactibilia en el 88% de los pacientes con CPRE previa (25) frente al 40,9% de los casos sin CPRE (171), p<0,001. En aquellos pacientes con ingresos previos la presencia de bactibilia fue del 57,8% (63/109) frente al 33,3% en los que no estuvieron ingresados (29/87), p=0,001. En cuanto a la influencia del tratamiento antibiótico, la bactibilia fue del 61,5% en los pacientes con antibioterapia en los 6 meses previos (56/91) frente al 34% en los que no existía este antecedente (36/105), p<0,001. Aquellos pacientes a los que se les administró antibioterapia al ingreso presentaron bactibilia en un 54% (32/59) versus el 44% (60/137) en los no tratados, no encontrándose diferencias estadísticamente significativas entre antibioterapia al ingreso y bactibilia, p=0,18.

Se aislaron un total de 165 microorganismos. La mayoría eran bacilos gramnegativos (60,5%), en su mayor parte del orden *Enterobacterales* (91/54,5%), siendo *E. coli* el microorganismo más frecuente (24%) seguido de *Klebsiella* spp. (12,5%). Otros bacilos gramnegativos fueron *Pseudomonas aeruginosa* en 3 muestras, *Aeromonas* spp. (3) o *Campylobacter jejuni* (1). En cuanto a los grampositivos se aisló *Enterococcus* spp. en 19 muestras (11,5%), destacando 3 *E. faecium* y 3 *E.casseliflavus/gallinarum*, *Streptococcus* spp. en 19 (11,4%) y *Staphylococcus* spp. en un sólo paciente. El 10,2% de los microorganismos eran anaerobios y el 2,4% levaduras (tabla 2).

Se aislaron 4 microorganismos multirresistentes (2,4%) (3 *E. coli* BLEE y 1 *K. pneumoniae* BLEE), 3 en pacientes con hospitalización y antibioterapia previas (75%). No se aislaron microorganismos productores de carbapenemasa ni *S. aureus* resistente a meticilina.

Se solicitaron hemocultivos en 18 ocasiones (19,5%). En 6 se detectó crecimiento pero sólo dos de ellos se consideraron significativos (2,1%), con aislamiento del mismo germen que en bilis.

DISCUSIÓN

Las posibles causas de bactibilia podrían estar relacionadas con la presencia o no de colecistitis aguda y la gravedad de la misma, el uso de antibióticos, la manipulación previa de la vía biliar o la comorbilidad asociada.

El porcentaje de bactibilia en pacientes con manipulación previa de la vía biliar es dos veces mayor que cuando no existe este antecedente (88% vs 40,3%). Rupp et al. [5], encuentran un 71,6% de bactibilia tras CPRE (con predominio de grampositivos, a diferencia de nuestra serie). Aún así, en las TG18 no se recomienda profilaxis antibiótica ante este procedimiento [6].

El cultivo de bilis debería solicitarse en los grados II y III puesto que en pacientes sin clínica de infección existe controversia sobre la necesidad de instaurar tratamiento antibiótico ante el hallazgo de bactibilia. No obstante, trabajos como el de Troyano-Escribano et al. [7], sobre 368 pacientes sometidos a colecistectomía electiva (excluyendo a aquellos con signos de infección biliar aguda) mostraron bactibilia en el 28,7%, (sobre todo tras cirugía abierta) aunque este hallazgo no se

Tabla 3 **Antibióticos empleados en infecciones del tracto biliar y su capacidad de penetración en bilis. (**Tomada de Ansaloni et al. [2])

Elevada penetración (ABSCR ≥ 1)	Baja penetración (ABSCR <1)
Piperacilina/tazobactam (4,8)	Ceftriaxona (0,75)
Tigeciclina (> 10)	Cefotaxima (0,23)
Amoxicilina/clavulánico (1,1)	Meropenem (0,38)
Ciprofloxacino (> 5)	Ceftazidima (0,18)
Ampicillina/Sulbactam (2,4)	Vancomicina (0,41)
Cefepima (2,04)	Amikacina (0,54)
Levofloxacino (1,6)	Gentamicina (0,30)
Penicilina G(>5)	
Imipenem (1,01)	

(ABSCR Antibiotics Bile/Serum Concentration Ratio).

relacionó con la aparición de complicaciones postquirúrgicas. La positividad de los cultivos de bilis en la colecistitis aguda puede alcanzar el 54%, aunque es menor que en la colangitis (hasta el 93%) [8]. Una vez aislados los microorganismos causales, debería modificarse el antibiótico o desescalarse según antibiograma [6]. En nuestro caso, en un 46,9% de las muestras se encontró bactibilia. En ninguna ocasión se modificó el antibiótico por el resultado del cultivo ya que muchas veces los pacientes ya habían sido dados de alta con un tratamiento antibiótico secuencial.

Las TG18 [6] revisan la duración del tratamiento antibiótico en la colecistitis aguda: entre 24 h tras la colecistectomía en los Grados I y II sin complicaciones, y de 4 a 7 días en el Grado III una vez controlado el foco mediante colecistectomía. En nuestra serie todas las colecistitis fueron tratadas una media de 6 días independientemente del grado, conducta que debería modificarse ya que, tras el control del foco, la prolongación del tratamiento antibiótico no aporta beneficios adicionales, sino que favorece la selección de bacterias resistentes.

Se define el tratamiento empírico como aquel administrado hasta disponer del resultado del cultivo [6] y las recomendaciones en las guías clínicas varían en función de la gravedad de la infección. No obstante cada vez existen más microorganismos multirresistentes en la comunidad (como las enterobacterias portadoras de BLEE, generalmente resistentes a betalactámicos, cefalosporinas y fluoroquinolonas) por lo que deben considerarse los factores de riesgo (como el tratamiento antibiótico previo o la estancia hospitalaria mayor de 15 días) [4] y la flora local, que puede variar enormemente. Por ejemplo, para Kwon et al. [9], en Seúl, la especie más frecuentemente aislada fue *Enterococcus* spp. (a expensas de *E. faecium,* habitual en receptores de transplante hepático) lo que les llevó a replantearse el tratamiento empírico.

La Guía de la Infectious Diseases Society of America (IDSA) recomienda el tratamiento con carbapenémicos, piperacilina/tazobactam o, si la prevalencia de microorganismos multirresistentes no supera el 10-20%, ceftazidima o cefepima con metronidazol [10]. Al analizar nuestros datos encontramos un 4,8 % de microorganismos multirresistentes. Además, ante la presencia de fístula o anastomosis bilio-entérica se recomienda cubrir anaerobios con metronidazol si se están empleando cefalosporinas o aztreonam. Las fluoroquinolonas, dado el aumento de resistencias, no se recomiendan como tratamiento empírico; sólo estaría indicado su empleo en alérgicos a betalactámicos para continuar el tratamiento vía oral (si se demuestra sensibilidad en el antibiograma).

A parte del espectro debería tenerse en cuenta la penetración del antibiótico en bilis, sobre todo ante obstrucciones. En la tabla 3 se citan los antibióticos más adecuados [2].

El antibiótico más utilizado en nuestra serie fue piperacilina/tazobactam, un betalactámico con buena penetración biliar y efecto anaerobicida, activo en medio ácido (en presencia de abscesos) y que no requiere ajuste de dosis ante insuficiencia hepática. Además no induce la producción de betalactamasas [3]. Encontramos un porcentaje de resistencia frente a piperacilina/tazobactam del 4,8 % (5 bacilos gramnegativos y 3 *E. faecium*). En segundo lugar se empleó amoxicilina/clavulánico, con una resistencia elevada (del 24,2 %), ya que cada vez son más frecuentes las enterobacterias productoras de BLEE, AmpC plasmídicas o betalactamasas resistentes al inhibidor (IRT). En cuanto a los carbapenémicos, frente a ertapenem (el tercer antibiótico más empleado) la resistencia fue del 15%, si tenemos en cuenta la resistencia intrínseca tanto en *Pseudomonas* como en *Enterococcus*, frente a meropenem del 11,5 % (a expensas de *Enterococcus*) y frente a imipenem del 1,2% (dos *E. faecium*).

Podríamos sustituir ertapenem por imipenem, con mayor porcentaje de sensibilidad y mejor penetración biliar. La resistencia a cefepima (antibiótico con buena penetración en bilis) fue del 12,1 %.

La resistencia a ciprofloxacino fue del 9,1 % y a metronidazol del 1,2%. En cambio a amikacina fue del 1,2%, pero por su escasa penetración biliar, no se indicaría como tratamiento empírico.

En la colecistitis aguda Grado III se recomienda emplear antibióticos activos frente a *Pseudomonas aeruginosa*, ya que clásicamente se ha encontrado en un 20% de los cultivos. En la TG18 [6], se constata una disminución hasta el 3,6% y para autores como Armiñanzas et al. [11] menos de un 1%. Nosotros hemos encontrado un 1,8%. No obstante, la recomendación de cubrirla se mantiene dado el exceso de mortalidad que supondría el no hacerlo. Si la resistencia a ceftazidima supera el 20% (en nuestra serie no encontramos resistencias), se recomienda el empleo de carbapenémicos (excepto ertapenem), piperacilina/tazobactam y aminoglucósidos [6].

Cefazolina se empleó como profilaxis en cirugía programada, por ser el antibiótico de elección, eficaz y razonablemente seguro, con una pauta corta de administración que minimiza tanto las reacciones adversas como el desarrollo de resistencias o la infección por *Clostridioides difficile* [12]. Presenta actividad frente a microoorganismos que frecuentemente colonizan el

lecho quirúrgico. En cirugía limpia-contaminada como la abdominal es activo frente a microorganismos de la flora cutánea (*Staphylococcus* coagulasa-negativo o *S. aureus* sensible a meticilina), *Streptococcus spp* y ciertas enterobacterias. En nuestra serie sólo hubo dos infecciones de la herida lo que sugiere que este antibiótico es efectivo para evitar la infeccción secundaria al acto quirúrgico.

El hallazgo de *Aeromonas caviae* en bilis es excepcional. Se adquiere en climas templados, tras contacto con agua dulce o salobre, mordedura por animales acuáticos o consumo de alimentos contaminados. Puede presentarse como colangitis aguda en pacientes con obstrucción previa de la vía biliar (por litiasis o estenosis) [13]. En nuestra serie se aisló en 3 muestras (3,2%), siempre en cultivo monomicrobiano. En la historia clínica no constaban factores de riesgo destacables.

Campylobacter spp. se aisla con muy baja frecuencia en cuadros de colecistitis. Necesita condiciones especiales para su crecimiento, y no se recomienda su cultivo a menos que en la tinción de Gram se visualicen bacilos gramnegativos curvos [14]. En nuestro caso se aisló *C. jejuni* resistente a ciprofloxacino en una mujer de 64 años con colecistitis aguda litiásica y antecedentes de pancreatitis aguda, sin constancia de diarrea previa. La evolución fue favorable con amoxicilina/clavulánico.

Enterococcus spp. muestra algunas dificultades en el tratamiento por presentar resistencia intrínseca a antibióticos muy empleados como cefalosporinas y baja sensibilidad a quinolonas o cotrimoxazol.

Su patogenicidad en la infección biliar es incierta [10] aunque se recomienda su tratamiento en pacientes inmunodeprimidos [2]. *E. faecalis* suele ser sensible a ampicilina, mientras que en *E. faecium* (un 1,8% en nuestra serie) es frecuente la resistencia a betalactámicos por alteraciones en la PBP5 (incluyendo piperacilina/tazobactam, ampliamente utilizado en infecciones intraabdominales), por lo que debería emplearse vancomicina, linezolid o daptomicina. El uso de carbapenémicos debería limitarse a imipenem para *E. faecalis*, puesto que *E. faecium* suele ser resistente. La aparición de cepas con resistencia adquirida a glucopéptidos (VanA/B) todavía es infrecuente en nuestro país [15]. En nuestra serie encontramos un 1,8% de especies intrínsecamente resistentes a vancomicina como *E. casseliflavus, E. gallinarum o E. flavescens,* todos sensibles a ampicilina.

Respecto a los anaerobios no se recomienda clindamicina como tratamiento empírico por la resistencia que presenta *Bacteroides* spp. Cueto et al [16], cuyo trabajo estudia la flora biliar en colecistitis aguda, no constatan ningún anaerobio, aunque sí recomiendan su cobertura antibiótica. En nuestro caso se aislaron anaerobios en el 16,6% de los pacientes (en cultivo puro sólo en uno). No encontramos relación con fístulas bilio-entéricas pero destacaba el antecedente de CPRE previa en el 21,4% de los casos por lo que deberían cubrirse, especialmente, en estos pacientes.

Se aisló *Candida* spp. en 4 muestras (2,4%), porcentaje similar a otros trabajos: Cueto et al. [16] encuentran un 3,4%, mientras que Kwon [9] un 2,6%. En ningún caso se aislaron levaduras en otras localizaciones. Se relacionan con antibioterapia previa, inmunodepresión y manipulación de las vías biliares. En nuestro caso todos los pacientes presentaron hospitalización y antibioterapia previa y se les realizó CPRE. La guía IDSA [10] recomienda el tratamiento antifúngico sólo ante cuadros graves, con fluconazol si se aisla *C. albicans* o con una equinocandina si se trata de especies resistentes a azoles, como *C. krusei* o *C. glabrata.* En nuestra serie no fue necesario añadir tratamiento antifúngico.

En la TG18 [6] se recomienda la toma de hemocultivos en colecistitis de Grado II y III (y no en la de Grado I de adquisición comunitaria). La guía IDSA tampoco la recomienda en la comunitaria ya que no varía el manejo terapéutico (de 1.062 hemocultivos, sólo un 1,6% generaron un cambio) [10]. Únicamente se extrajeron hemocultivos en caso de fiebre durante el postoperatorio, independientemente del grado de colecistitis, ya que habitualmente son negativos en las colecistitis no complicadas [8]. En nuestro caso sólo el 2,1% de los hemocultivos se consideraron significativos.

A nuestro estudio cabe añadir algunas limitaciones. La primera es que se incluye el análisis microbiológico de vesículas extirpadas por diversas patologías. Sería interesante (con un mayor número de pacientes) poder estratificar por etiología y comparar la posible variación en la microbiota encontrada. Otra limitación es que en cuadros como las colecistitis, que combinan el tratamiento médico con el quirúrgico, es difícil saber hasta qué punto el papel de la antibioterapia es determinante en la curación. Como conclusiones, la microbiota biliar de nuestro estudio no difiere de otros trabajos a nivel europeo, aunque sí de aquellos realizados en países asiáticos. El porcentaje de microorganismos multirresistentes es bajo respecto a otras series. Piperacilina/tazobactam es una buena elección en nuestro medio para el tratamiento de pacientes con colecistitis leve y moderada, puesto que su elevada penetración biliar, el efecto anaerobicida y, sobre todo, la baja capacidad para inducir la producción de betalactamasas lo convierten en un antibiótico óptimo para estos cuadros. Habría que reducir, no obstante, el tiempo de tratamiento en colecistitis no graves. La resistencia observada ante amoxicilina/clavulánico debería hacernos replantear su uso como tratamiento empírico.

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BIBLIOGRAFÍA

1. Kimura Y, Takada T, Strasberg SM, Pitt HA, Gouma DJ, Garden OJ et al. TG13 current terminology, etiology and epidemiology of acute colangitis and cholecystitis. J Hepatobiliary Pancreat Sci. 2013; 20:8-23. doi: 10.1007/s00534-012-0564-0
2. Ansaloni L, Pisano M, Coccolini F, Peitzmann AB, Fingerhut A, Catena F et al. 2016 WSES guidelines on acute calculous cholecystitis. World J Emerg Surg. 2016; 11:25. doi: 10.1186/s13017-016-0082-5
3. Mensa J, Gatell JM, García-Sánchez JE, Letang E, López-Suñé E, Marco F. Guía de Terapéutica Antimicrobiana. 2018. Ed.Antares.
4. Guirao X, Arias J, Badía JM, García-Rodríguez JA, Mensa J, Álvarez-Lerma F et al. Recomendaciones en el tratamiento antibiótico empírico de la infección intraabdominal. Cir Esp. 2010; 87(2):63-81. doi:10.1016/j.ciresp.2009.09.014
5. Rupp C, Bode K, Weiss KH, Rudolph G, Bergemann J, Kloeters-Plachky P et al. Microbiological assesment of bile and corresponding antibiotic treatment: a Strobe-Compliant Observational Study of 1401 Endoscopic Retrograde Cholangiographies. Medicine (Baltimore). 2016; 95 (10): e2390 doi: 10.1097/MD.0000000000002390.
6. Gomi H, Solomkin J, Schlossberg D, Okamoto K, Takada T, Strasberg M et al. Tokyo Guidelines 2018: antimicrobial theraphy for acute colangitis and cholecystitis. J Hepatobiliary Pancreat Sci. 2018; 25:3-16. doi: 10.1002/jhbp.518.
7. Troyano Escribano D, Balibrea J, Molinos S, Ausina V, Fernández Llamazares J, Oller B. Bactibilia and antibiotic resistance in elective cholecystectomy: an updated ecologic survey. Surg Infect. 2015; Jun;16(3):287-92. doi: 10.1089/sur.2014.023
8. García-Sánchez JE, García-García MI, García-Garrote F, Sánchez-Romero I. Diagnóstico microbiológico de las infecciones intraabdominales. Enferm Infecc Microbiol Clin.2013; 31(4):230-239. doi: 10.1016/j.eimc.2012.01.023
9. Kwon W, Jang JY, Kim EC, Park JW, Han IW, Kang MJ et al. Changing trend in bile microbiology and antibiotic susceptibilities: over 12 years of experience. Infection. 2013; 41:93-102. doi: 10.1007/s15010-012-0358-y
10. Solomkin JS, Mazuski JE, Bradley JS, Rodvold KA, Goldstein EJC, Baron EJ et al. Diagnosis and Management of Complicated Intra-abdominal Infection in Adults and Children: Guidelines by the Surgical Infection Society and the Infectious Diseases Society of America. Clin Infect Dis 2010;15(2):133-64. doi: 10.1086/649554.
11. Armiñanzas C, Tigera T, Ferrer D, Calvo J, Herrera LA, Pajarón M et al. Papel de la bacteriobilia en las complicaciones postoperatorias. Rev Esp Quimioter. 2016; 29(3): 123-129.
12. Asensio A. Infección de la localización quirúrgica. Profilaxis antimicrobiana en cirugía.Enferm Infecc Microbiol Clin. 2014;32(1):48–53. doi: 10.1016/j.eimc.2013.11.003
13. Vargas JC, Fernández M, Muñoz I, Sanza A. Pileflebitis por *Aeromonas caviae* secundaria a colecistitis aguda. Enferm Infecc Microbiol Clin. 2016; 34(1):69-77.
14. Udayakumar D, Sanaullah M. Campylobacter cholecystitis. Int J Med Sci. 2009 ;6(6):374-5. PMID: 19960123.
15. Cercenado E. *Enterococcus*: resistencias fenotípicas y genotípicas y epidemiología en España. Enferm Infecc Microbiol Clin. 2011; 29(Supl 5):59-65. doi:10.1016/S0213-005X(11)70045-3
16. Cueto-Ramos R, Hernández-Guedea M, Pérez-Rodríguez E, Reyna-Sepúlveda F, Muñoz-Maldonado G. Identificación de flora bacteriana en cultivos de bilis y pared de vesícula biliar de pacientes sometidos a colecistectomía laparoscópica en el Hospital Universitario «Dr. José Eleuterio González». Cir Cir. 2017; 85(6):515-521. doi: 10.1016/j.circir.2016.10.030

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Reacciones adversas asociadas a la vacunación en pacientes inmunodeprimidos y en situaciones especiales de una Unidad de Vacunas hospitalaria

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RESUMEN

Objetivos. Describir el tipo de vacunas administradas en la Unidad de Vacunas de un hospital de referencia y calcular la tasa de notificación global y específica de las reacciones adversas asociadas.

Métodos. Estudio observacional retrospectivo, realizado en el periodo entre noviembre de 2014 y noviembre de 2017, de los pacientes que desarrollaron una reacción adversa a medicamento (RAM) tras la administración de una vacuna y que fue notificada al Sistema Español de Farmacovigilancia. Las variables analizadas fueron edad, sexo, grupo de riesgo, tipo de vacuna, coadministración y tipo de RAM. Se llevó a cabo un análisis univariante y bivariante. Se calculó la tasa de notificación de RAM global y específica para cada vacuna.

Resultados. Se administraron un total de 18.123 vacunas de las que el 20,7% correspondían a la vacuna frente al virus de la hepatitis B. Se notificaron 53 sospechas de RAM. En el 64,2% de las ocasiones se había administrado solamente una vacuna. El 88,7% de las notificaciones correspondieron a vacunas inactivadas. La vacuna frente neumococo polisacárida de 23 serotipos fue la que generó el mayor número de notificaciones. La tasa de notificación global de RAM fue de 0,42%. La vacuna hexavalente fue la que registró la tasa de notificación más elevada (2,81%). El 49,1% de las RAM fueron de tipo sistémico.

Conclusiones. La tasa de notificación global fue baja aunque superior a la registrada por otros autores. La correcta notificación de posibles reacciones adversas postvacunales es imprescindible para contribuir a la seguridad vacunal y para aumentar la confianza de la población en las vacunas.

Palabras clave: vacunas, reacción adversa, inmunosupresión, farmacovigilancia.

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Vaccine-related adverse reactions in immunocompromised patients and in special situations of a hospital Vaccine Unit

ABSTRACT

Objectives. The aim of the study was to describe the type of vaccines administered in the Vaccine Unit at a reference hospital. Calculate the overall and specific reporting rate of adverse reactions.

Methods. Retrospective observational study for the period between November 2014 and November 2017, on patients who developed an adverse drug reaction (ADR) after the administration of a vaccine and who were notified to the Spanish Pharmacovigilance System. The variables analyzed were age, sex, risk group, vaccine class, co-administration and type of ADR. A univariate and bivariate analysis was performed. The global and vaccine specific rate of ADR notification was calculated.

Results. A total of 18,123 vaccines were administered, of which 20.7% corresponded to hepatitis B virus vaccine. Fifty-three RAM suspects were reported. In 64.2% of cases only one vaccine was administered. Inactivated vaccines accounted for 88.7% of notifications. The highest number of notifications was generated by the 23 serotypes pneumococcal polysaccharide vaccine. The overall reporting rate was 0.42%. The hexavalent vaccine had the highest reporting rate (2.81%). 49.1% of the ADR were systemic.

Conclusions. The overall reporting rate was low but higher than that of other authors. Proper reporting of possible adverse post-vaccine reactions is essential to contribute to vaccine safety and to increase public confidence in vaccines.

Keywords: vaccines, adverse effects, immunosuppression, pharmacovigilance

INTRODUCCIÓN

Las vacunas son uno de los grandes logros para la salud pública mundial ya que cada año evitan alrededor de 2 a 3

millones de fallecimientos [1]. Durante los dos últimos siglos han permitido erradicar la viruela, reducir las tasas de mortalidad infantil y evitar anomalías congénitas y discapacidades permanentes [1-3].

La seguridad de las vacunas es un hecho contrastado con una sólida base de evidencia científica. Los ensayos clínicos previos a la comercialización, así como los controles de calidad durante la fabricación, hacen que se hayan convertido en fármacos muy seguros [4-6]. Al igual que el resto de medicamentos, las vacunas pueden producir un efecto indeseable no intencionado y perjudicial denominado reacción adversa a medicamento (RAM) [7]. Cuando la RAM no es de tipo local resulta complicado establecer causalidad. Sin embargo, se sospecha que la RAM puede estar relacionada con la vacuna si se cumplen algunas de las siguientes condiciones: secuencia temporal compatible, conocimiento previo, efecto de reexposición, anafilaxia, confirmación por parte de un laboratorio de la relación de las lesiones con la vacuna o la realización de un estudio epidemiológico [4, 8, 9].

Por otro lado, la notificación a los sistemas de farmacovigilancia de cualquier sospecha de RAM es clave [10] ya que permite identificar efectos adversos previamente desconocidos, valorar los riesgos de los medicamentos comercializados, proponer medidas de salud pública e informar a toda la población sobre la seguridad de los medicamentos. En España, el Sistema Español de Farmacovigilancia de Medicamentos de Uso Humano (SEFV-H) es responsable de registrar y gestionar las sospechas de RAM [11]. Tanto profesionales sanitarios como pacientes que sospechen cualquier RAM pueden notificarla a través de la web https://www.notificaram.es [11, 12].

En la sociedad actual, existen grupos poblacionales con dudas hacia la vacunación lo que ha generado una baja tolerancia ante la aparición de cualquier RAM tras la administración de una vacuna [13]. Por ello, trabajar en la seguridad de las vacunas es clave para mejorar la confianza de la población en esta herramienta preventiva y que tanto la población infantil como la adulta, mantenga elevadas coberturas de vacunación [3].

Así pues, los objetivos de la presente investigación son: a) describir la distribución de las vacunas administradas en la Unidad de Vacunas de un hospital autonómico de referencia; b) calcular la tasa de notificación de RAM global y para cada una de las vacunas al SEFV-H y c) describir las características clínicas de los pacientes en los que se identificó una RAM asociada a la vacunación.

MATERIAL Y MÉTODOS

Tipo y período de estudio. Estudio observacional retrospectivo correspondiente al periodo entre el 1 de noviembre de 2014 y el 30 de noviembre de 2017.

Ámbito de estudio. El estudio ha sido realizado en la Unidad de Vacunas de un hospital autonómico de referencia.

Población de estudio. Se han incluido los pacientes adultos y pediátricos en régimen de hospitalización o ambulatorio y en situación de inmunosupresión secundaria a una enfermedad o un tratamiento tales como: infección por el virus de la inmunodeficiencia humana, trasplante de órgano sólido y de progenitores hematopoyéticos, quimioterapia, tratamiento inmunomodulador, asplenia anatómica o funcional e inmunodeficiencia congénita o primaria. Por otro lado, también se han incluido otras situaciones especiales entre las que se encuentran las gestantes hospitalizadas en el tercer trimestre hospitalizadas, los prematuros hospitalizados y los familiares o convivientes de pacientes inmunodeprimidos que acudían como acompañantes y a los que se les indicaba vacunación.

Variables de estudio. Se han tenido en cuenta variables sociodemográficas (edad y sexo), el grupo de riesgo o situación especial del paciente, la clasificación de la vacuna (atenuada/inactivada y tipo de vacuna*) y si se coadministraron **más de una vacuna en el mismo acto (sí/no)**. Las principales variables dependientes fueron la presencia de RAM (sí/no) y el tipo de reacción (local/sistémica).

* Se incluyó el preparado de la prueba del Mantoux (tuberculina) dentro de la variable "tipo de vacuna administrada" a sabiendas de que no es una vacuna ya que al inicio del estudio este producto era gestionado desde la Unidad de Vacunas y se tenía en cuenta tanto en el stock como en la farmacovigilancia.

Fuentes de datos. Para el cálculo de la frecuencia de administración de cada una de las vacunas se consultaron los

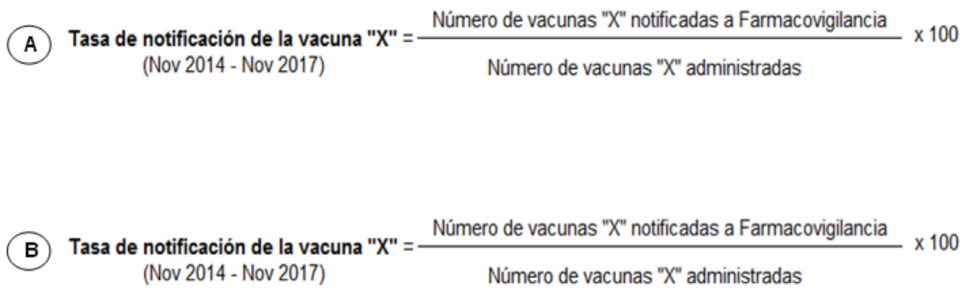

Figura 1 Fórmulas para el cálculo de la tasa de notificación global (A) y tasa de notificación específica (B) para cada una de las vacunas ("X")

Tabla 1 **Vacunas administradas durante el periodo de estudio según el tipo de vacuna y su nombre comercial.**

Tipo de vacuna	Nombre comercial	Nº de vacunas administradas	Porcentaje sobre el total de vacunas administradas[a]
Hepatitis B adulto adyuvada	FENDRIX®	3.768	20,79%
Gripe	CHIROFLU®	2.987	16,48%
Neumococo conjugada de 13 serotipos	PREVENAR13®	2.806	15,48%
Neumococo polisacárida de 23 serotipos	PNEUMOVAX23®	1.693	9,34%
Hepatitis A adulto	VAQTA50®	1.390	7,67%
Haemophilus influenzae tipo b	HIBERIX®	973	5,37%
Meningococo C	NEISVAC-C®	828	4,57%
Tétanos-difteria adulto	DIFTAVAX®	468	2,58%
Hepatitis B adulto	ENGERIX20®	411	2,27%
Tétanos-difteria adulto	DITANRIX®	380	2,10%
Meningococo B	BEXSERO®	337	1,86%
Hexavalente (Tétanos-difteria-tos ferina- *H. influenzae* b-hepatitis B-polio)	INFANRIXHEXA®	314	1,73%
Varicela	VARIVAX®	307	1,69%
Triple vírica (Sarampión-rubeola-parotiditis)	MMRVAXPRO®	231	1,27%
Hepatitis A adulto	HAVRIX1440®	227	1,25%
Hexavalente (Tétanos-difteria-tos ferina- *H. influenzae* b-hepatitis B-polio)	HEXYON®	178	0,98%
Polio	IMOVAX®	174	0,96%
Tétanos-difteria adulto	MASSBIOLOGICS®	137	0,76%
Hepatitis A infantil	HAVRIX720®	104	0,57%
Tétanos-difteria-tos feria de baja carga	BOOSTRIX®	97	0,54%
Virus del papiloma humano bivalente	CERVARIX®	97	0,54%
Hepatitis B infantil	ENGERIX10®	59	0,33%
Tuberculina		57	0,31%
Gripe	CHIROMAS®	35	0,19%
Pentavalente (Tétanos-difteria-tos ferina- *H. influenzae* b-polio)	PENTAVAC®	18	0,10%
Triple vírica (Sarampión-rubeola-parotiditis)	PRIORIX®	16	0,09%
Tetravírica (Sarampión-rubeola-parotiditis-varicela)	PROQUAD®	10	0,06%
Meningocócica ACWY	NIMENRIX®	9	0,05%
Tétanos-difteria-tos feria de alta carga	INFANRIX®	5	0,03%
Virus del papiloma humano tetravalente	GARDASIL®	4	0,02%
Rabia	ANTIRRÁBICA MERIEUX®	3	0,02%
TOTAL		18.123	100%

[a](Nº de vacunas administradas / Total de vacunas administradas) x100

registros de stock vacunales de enfermería. En el caso del cálculo de la tasa de notificación global y específica para cada tipo de vacuna se utilizó el registro informatizado de sospechas de RAM relacionadas con la vacunación iniciado en el año 2014 en la propia Unidad en el que también se incluye información clínica de cada RAM.

Aspectos éticos. La presente investigación fue aprobada por el Comité de Ética de la Investigación de la Comunidad Autónoma (referencia 149/18).

Análisis estadístico. Se realizó la estadística descriptiva de cada variable (análisis univariante), expresando las frecuencias absolutas y relativas de las variables cualitativas investigadas. Se calculó la media como medida de tendencia central, así como la desviación típica y el valor mínimo y máximo para las

Tabla 2 **RAM sospechosas notificadas a farmacovigilancia durante el periodo de estudio según el tipo de vacuna y su nombre comercial.**

Tipo de vacuna	Marca de la vacuna	Frecuencia de notificaciones	Porcentaje de notificaciones[a]	Tasa de notificación específica (figura 1)
Hexavalente (Tétanos-difteria-tos ferina- *H. influenzae* b-hepatitis B-polio)	HEXYON®	5	6,58%	2,81%
Neumococo polisacárida de 23 serotipos	PNEUMOVAX23®	23	30,26%	1,36%
Triple vírica (Sarampión-rubeola-parotiditis)	MMRVAXPRO®	3	3,95%	1,30%
Tétanos-difteria-tos feria de baja carga	BOOSTRIX®	1	1,32%	1,03%
Varicela	VARIVAX®	3	3,95%	0,98%
Hexavalente (Tétanos-difteria-tos ferina- *H. influenzae* b-hepatitis B-polio)	INFANRIXHEXA®	3	3,95%	0,96%
Polio	IMOVAX®	1	1,32%	0,57%
Hepatitis A adulto	HAVRIX1440®	1	1,32%	0,44%
Tétanos-difteria adulto	DIFTAVAX®	2	2,63%	0,43%
Neumococo conjugada de 13 serotipos	PREVENAR13®	10	13,16%	0,36%
Hepatitis B adulto adyuvada	FENDRIX®	13	17,11%	0,35%
Meningococo B	BEXSERO®	1	1,32%	0,30%
Meningococo C	NEISVAC-C®	2	2,63%	0,24%
Hepatitis B adulto	ENGERIX20®	1	1,32%	0,24%
Hepatitis A adulto	VAQTA50®	2	2,63%	0,14%
Gripe	CHIROFLU®	4	5,27%	0,13%
H. influenzae tipo b	HIBERIX®	1	1,32%	0,10%
TOTAL		76	100%	

[a](Frecuencia de notificaciones / Nº total notificaciones) x100

variables cuantitativas. Se llevó a cabo un análisis bivariante para conocer si las variables de estudio seleccionadas tenían o no asociación. Para las variables cualitativas dicotómicas se aplicó el test exacto de Fisher dado que el tamaño muestral era pequeño. Se calculó la tasa de notificación global y la específica para cada una de las vacunas ("X") en el periodo de estudio mediante las fórmulas A y B de la figura 1. Se consideró estadísticamente significativo si $p<0,05$. Se utilizó el programa Predictive Analytics SoftWare versión 18.0.

RESULTADOS

Durante el periodo de estudio se administraron un total de 18.123 vacunas. De ellas, el 20,79% fueron frente a la hepatitis B (con vacuna adyuvada con AS04C), cerca de un 16,48% correspondían a la vacunación antigripal estacional y alrededor del 25% frente a neumococo [15,48% correspondió a la vacuna conjugada de 13 serotipos (VNC13) y casi un 9,34% a la polisacárida de 23 serotipos (VNP23)]. Del total de las vacunas administradas, 17.559 (96,89%) fueron inactivadas y 564 (3,11%) fueron vacunas vivas-atenuadas. La tabla 1 describe las vacunas administradas según la clasificación.

Durante los tres años se realizaron 53 notificaciones de sospechas de RAM asociadas a la vacunación que incluyeron un total de 76 vacunas dado que en algunos casos se había coadministrado dos o más vacunas. De los 53 pacientes afectados, el 35,85% (19) fueron hombres y el 64,15% (34) mujeres. La media de edad (desviación típica) fue de 47,40 (± 19,95) años, con un rango de edad entre 3 y 87 años. De ellos, 21 padecían una enfermedad autoinmune, pero solo 10 tenían un tratamiento inmunosupresor en el momento en el que se les administró la vacuna. De las reacciones adversas notificadas, en el 64,2% de las ocasiones se había administrado solamente una vacuna, mientras que en el 35,9% se administraron 2 o más vacunas en el mismo acto vacunal. Por otro lado, el 88,7% de las notificaciones fue debido a una vacuna inactivada y, en el 11,4% de las notificaciones, se había administrado o coadministrado una vacuna atenuada.

La tasa de notificación global fue del 0,42%. La tabla 2 incluye la descripción de las vacunas que fueron consideradas sospechosas de RAM y que fueron incluidas en las notificaciones a farmacovigilancia, así como el porcentaje y tasa de notificación según el tipo de vacuna. En este sentido, se observa cómo la vacuna que más número de veces generó una notificación fue la VNP23, en 23 ocasiones (30,26%), mientras que la vacuna que presentó mayor tasa de notificación específica fue la hexavalente (2,81%).

En el análisis bivariante no se encontró asociación esta-

Tabla 3 **Análisis bivariante.**

Sexo	Tipo de vacuna					Tipo de reacción adversa				
	Inactivada	Viva	Viva + inactivada	Total	p	Local	Sistémica	Local + sistémica	Total	p
Hombre	18	0	1	19		6	10	3	19	
Mujer	29	3	2	34	0,405	13	16	5	34	0,888
Total	47	3	3	53		19	26	8	53	

dísticamente significativa entre la variable sexo y el tipo de reacción adversa (p=0,888) ni el tipo de vacuna administrada (p=0,405) (tabla 3). Tampoco la hubo entre las variables tipo de vacuna y tipo de reacción (p=0,135). Por último, no se encontró correlación entre el número de vacunas administradas y el tipo de reacción adversa registrada posteriormente (p=0,219).

En relación con la clínica de RAM observadas, 19 fueron de tipo local (35,8%), 26 de tipo sistémico (49,1%) y, en 8 (15,1%), se observaron síntomas locales y sistémicos al mismo tiempo. A pesar de que 15 pacientes precisaron asistencia inmediata o ambulatoria, solo en un caso se precisó hospitalización y el desenlace de todas las reacciones fue favorable, desapareciendo los síntomas a las pocas horas o días y sin dejar secuelas ni discapacidades. La reacción más frecuentemente observada fue inflamación en 16 del total de notificaciones (30,19%), relacionada con otros síntomas como impotencia funcional, prurito, malestar general, dolor o tumefacción, seguida del eritema que apareció en 9 pacientes (16,98%) y la fiebre en 8 (15,09%). Dentro de las reacciones locales se identificaron 3 posibles casos de hipersensibilidad al aluminio contenido en la vacuna como adyuvante. De las reacciones sistémicas, 9 pacientes padecieron *flushing*, dificultad respiratoria y taquicardia. Estas reacciones adversas estaban posiblemente relacionadas con la VNP23. Por otro lado, en 5 casos se observó reactivación de la patología de base (ptosis palpebral en paciente con *miastenia gravis* tras la administración de VNC13, diarrea en paciente con enfermedad de Crohn tras vacuna frente a la VNC13 y parestesia y dificultad para la deambulación en una paciente con esclerosis múltiple tras la administración de vacuna de varicela, etc).

Las figuras 2, 3 y 4 muestran algunas de las reacciones locales que fueron notificadas a farmacovigilancia.

DISCUSIÓN

Con los resultados obtenidos a través de la metodología expuesta se considera que se ha dado cumplida respuesta al objetivo de la investigación. Por un lado, se ha descrito la distribución de las vacunas administradas en una Unidad de Vacunas de un hospital autonómico de referencia en el contexto de la asistencia a pacientes inmunodeprimidos y/o en situaciones especiales. Por otro, se ha calculado la tasa de notificación a farmacovigilancia global y específica para cada una de las vacunas administradas en el período de estudio. Igualmente, se han detallado las características clínicas de las RAM identificadas.

En primer lugar, destacar que la mayoría de las vacunas que fueron administradas en el período de estudio fueron inactivadas. Esto se debe a que los pacientes que acuden a la Unidad de Vacunas están inmunodeprimidos y, por tanto, las vacunas atenuadas están contraindicadas o se administran en situaciones muy concretas [14]. Además, la mayoría de los pacientes eran adultos por lo que muchos de ellos habían sido inmunizados de manera natural o artificial en la infancia [15]. La vacuna más frecuentemente administrada fue la del VHB debido a la necesidad de vacunar con cuatro dosis a la mayoría de los adultos inmunodeprimidos [16, 17] que no fueron inmunizados en la infancia puesto que la introducción de esta vacuna en el calendario vacunal se aprobó en el año 1982 [15]. Al mismo tiempo, la vacuna antigripal se encuentra entre las más administradas ya que la indicación es generalizada para estos pacientes en cada campaña estacional [18]. Por último, la VNC13 fue la tercera por orden de frecuencia; esta es de especial interés por un mayor riesgo de neumonía neumocócica y enfermedad neumocócica invasora en este grupo [19].

En general, puede considerarse que la tasa de notificación global observada en el presente estudio es baja (0,42%), sobre todo si se compara con las RAM descritas para otros fármacos como, por ejemplo, el acenocumarol (2,25%) [20] o la carbamacepina (2-4%) [21]. No obstante, lo descrito en esta investigación es superior a lo observado por autores como Batalla et al, donde la tasa de notificación fue 0,01% [22] y 7,2 por cada 100.000 vacunas administradas [23]. La elevada tasa de notificación de la vacuna hexavalente se explica por tener mayor carga antigénica frente a difteria y tos ferina (presentación infantil) y estar siendo aplicada en población adulta [24]. En cuanto a la tasa de notificación de la VNP23, está descrito en la literatura científica que se trata de una vacuna con mayor reactogenicidad, sobre todo local [25, 26].

Merece la pena destacar que en 1977, Rawlins y Thompson, establecieron una clasificación para los efectos adversos que engloba varios mecanismos partícipes en su aparición, dividiéndolos en reacciones tipo A y tipo B [27]. Las reacciones tipo A son las más frecuentes, aparecen con dosis normales y son dosis-dependiente, provocadas por un efecto predecible y exagerado, son leves y definidas, frecuentemente locales y desaparecen en un corto espacio de tiempo, se corresponden con el 80-90% de las RAM [3, 27]. Por el contrario, las reacciones tipo B son inusuales e impredecibles, su mecanismo de aparición no guarda relación con la farmacocinética a dosis normales del fármaco y representan el 10-20% de los efectos adversos [27]. Atendiendo

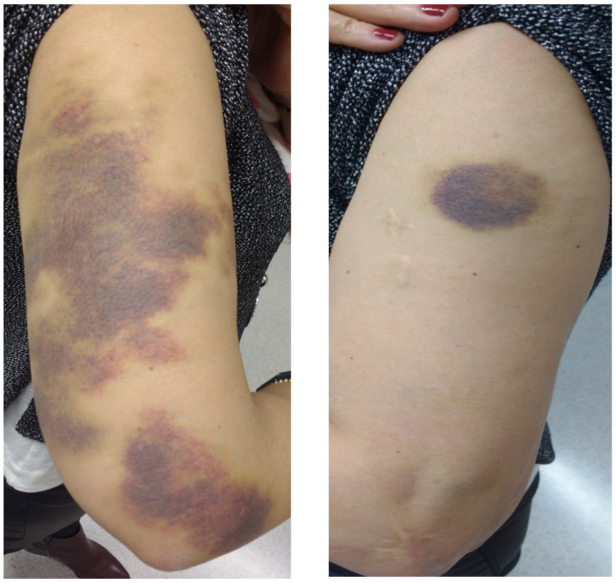

Figura 2 | **Hematomas en ambos miembros superiores relacionados con la administración de las vacunas VHB y VNP23.**

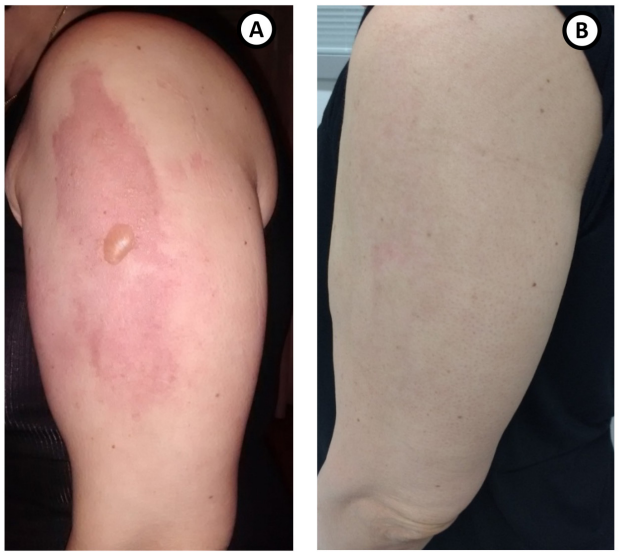

Figura 3 | **Reacción adversa local relacionada con la administración de VNP23 y B) resolución de la reacción adversa a los 30 días**

a esta clasificación las reacciones adversas notificadas en la presente investigación podrían corresponder con las de tipo B dado que estas se consideran aberrantes con respecto a lo esperado y la frecuencia de aparición es inferior al 20%.

La aparición de una reacción adversa postvacunal se cree que es multifactorial y no se conocen con exactitud con mecanismos de respuesta. Por lo que respecta a los resultados relacionados con la variable sexo, existe concordancia con lo descrito en la literatura científica. Esto es, al igual que refieren Harrisa et al [28], en la presente investigación se observa que el sexo femenino registra mayor frecuencia de RAM asociadas a la vacunación, aunque las diferencias no resultan estadísticamente significativas. Quizás factores genéticos, inmunológicos u hormonales puedan explicar estos resultados [28]. Aparte de lo anterior, parece lógico pensar que no exista relación entre el sexo y el tipo de reacción ni el tipo de vacuna.

En lo concerniente a la coadministración de vacunas en un mismo acto, se sabe que esta práctica es segura y efectiva por lo que no está relacionada con la mayor aparición ni intensidad de las RAM, siempre que las vacunas se administren en diferentes zonas anatómicas, no se trate de vacunas conjugadas y polisacáridas frente a la misma enfermedad y se realice con distintas jeringas [29].

A pesar de que las RAM observadas se resolvieron sin secuelas ni discapacidades, desde el punto de vista de la seguridad vacunal resulta de gran interés el control y seguimiento de las mismas con el fin de detectar posibles señales o alertas relacionadas con estos fármacos [3, 30]. Todos los datos referentes a esta investigación han sido contrastados con el Centro Autonómico de Farmacovigilancia.

Por último, el presente trabajo no se encuentra exento de limitaciones. Se trata de un estudio realizado en una única Unidad de Vacunas que, aún siento el punto de vacunación de referencia en la comunidad autónoma, no permite generalizar los resultados. Además, el período de estudio solamente abarca tres años lo cual quiere decir que la baja frecuencia de administración de algunas vacunas como la tetravírica o la vacuna frente al VPH tetravalente, por ejemplo, puede no ser suficiente como para detectar RAM postvacunales diferentes a lo esperado.

Como propuestas de futuro parece interesante destacar que, en base a la importancia y la necesidad tanto ética como legal de la farmacovigilancia, debería reforzarse la formación en la identificación, notificación y manejo de las RAM relacionadas con los medicamentos en general y las vacunas en particular, de todos los profesionales sanitarios (enfermería, medicina, farmacia y odontología, sobre todo). Igualmente, la formación específica y acreditada a lo largo de la carrera profesional contribuiría al incremento del número de notificaciones. Además, el *feedback* periódico por parte de los responsables del Centro de Farmacovigilancia de la Consejería de Sanidad hacia los profesionales sobre los casos notificados a nivel autonómico o las actuaciones llevadas a cabo por su parte podría reforzar dicha conducta.

En conclusión, la vacuna frente al VHB registró la mayor frecuencia absoluta de administración. La vacuna hexavalente y la VNP23 fueron las que obtuvieron una mayor tasa de notificación específica. La correcta notificación de posibles RAM postvacunales es imprescindible para contribuir a la seguridad vacunal y para aumentar la confianza de la población en las vacunas.

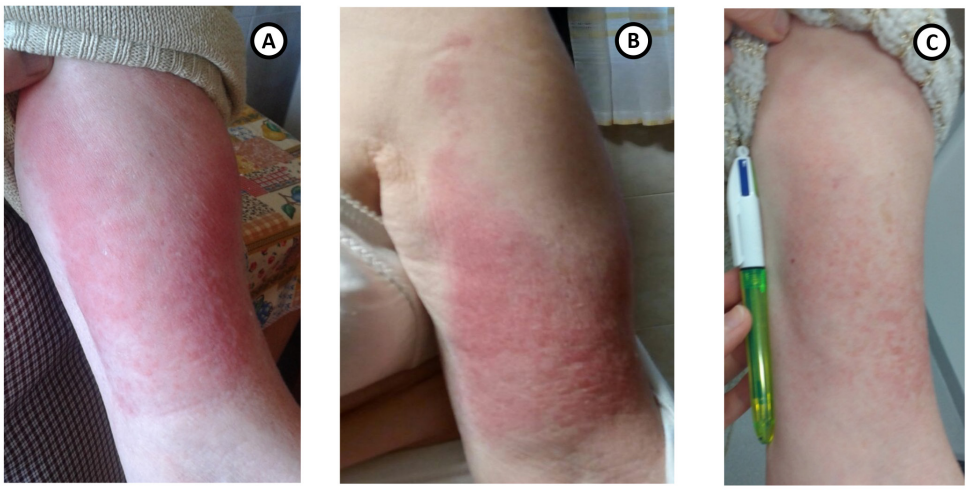

Figura 4 | **Reacción local a las 24 horas de la vacunación con VNC13, B) Evolución de la reacción a las 48 horas y C) Remisión de la lesión a las 72 horas**

FINANCIACIÓN

No existen relaciones financieras, laborales o de otra índole. Es decir, no hemos recibido "beneficios en dinero, bienes, hospitalidad o subsidios" de fuente alguna que tenga un interés particular en los resultados de la investigación.

CONFLICTOS DE INTERESES

Los autores declaran que no tienen Conflictos de intereses

BIBLIOGRAFÍA

1. Centers for Disease Control and Prevention . Atlanta: MMWR. Update: Vaccine side effects, adverse reactions, contraindications and precaution. Recommendations of the Advisory Committee on Immunization Practices (ACIP). [acceso 13 de febrero de 2018]. Disponible en: http://www.cdc.gov/mmwr/preview/mmwrhtml/00046738.htm
1. Galindo Santana BM, Arroyo Rojas L, Concepción Díaz D. Seguridad de las vacunas y su repercusión en la población. Rev Cubana Salud Pública. 2011;37:149-58.
2. Centers for Disease Control and Prevention. Epidemiology and Prevention of Vaccine-Preventable Diseases. Hamborsky J, Kroger A, Wolfe S, eds. 13th ed., Supplement. Washington D.C. Public Health Foundation, 2017.
3. Centers for Disease Control and Prevention. Atlanta. Capítulo 4: Vaccination safety. Epidemiology and Prevention of Vaccine-Preventable Diseases. The Pink Book: Course Textbook. 2015. [Acceso 11 de marzo de 2019]. Disponible en: https://www.cdc.gov/vaccines/pubs/pinkbook/safety.html
4. Cunningham AL, Garçon N, Leo O, Friedland LR, Strugnell R, Laupèze B, et al. Vaccine development: from concept to early testing. Vaccine2016; 34(52):6655-64. doi: 10.1016/j.vaccine.2016.10.016.
5. Plotkin S, Robinson JM, Cunningham G, Igbal R, Larsen S. The complexity and cost of vaccine manufacturing – an overview. Vaccine 2017; 35(33):4064-71. doi: 10.1016/j.vaccine.2017.06.003
6. Comité Asesor de Vacunas (CAV-AEP) [Internet]. Madrid: Manuel Merino Moína; [acceso 23 de febrero de 2018]. Manual de Vacunas en línea de la AEP; Capítulo 3. Seguridad de las vacunas. Contraindicaciones y precauciones. Disponible en: http://vacunasaep.org/documentos/manual/cap-3
7. Escudero C, García-Fernández C, Ibáñez MD. Reacciones alérgicas a las vacunas. Vacunas. 2008; 9:156-60.
8. Eseverri JL, Ranea S, Marin, A. Reacciones adversas a vacunas. Allergol Immunopathol. 2003; 31:125-38. PMID: 12783762
9. Virga C, Aguzzi A. FARMACOVIGILANCIA: estudio de las reacciones adversas a medicamentos (RAM). Archivos venezolanos de farmacología y terapéutica. 2011;30:61-3.
10. Agencia Española de Medicamentos y Productos Sanitarios. Madrid: Ministerio de Sanidad Servicio Sociales e Igualdad; [acceso el 03 de octubre de 2018]. Notificación de sospechas de reacciones adversas a medicamentos. Disponible en: www.notificaram.es
11. Agencia Española de Medicamentos y Productos Sanitarios [Internet]. Madrid: Ministerio de Sanidad Servicio Sociales e Igualdad; [acceso el 26 de febrero de 2018]. Información para las notificaciones de sospechas de reacciones adversas a medicamentos por parte de profesionales sanitarios. Disponible en: https://www.aemps.gob.es/vigilancia/medicamentosUsoHumano/SEFV-H/NRA-SEFV-H/notificaSospechas-RAM-profSanitarios.htm
12. Martínez-Diz S, Martínez Romero M, Fernández-Prada M, Cruz Piqueras M, Molina Ruano R, Fernández Sierra MA. Demandas y expectativas de padres y madres que rechazan la vacunación y perspectiva de los profesionales sanitarios sobre la negativa a vacunar. An Pediatr (Barc). 2014;80:370-8. doi: 10.1016/j.anpedi.2013.08.009

13. Comité Asesor de Vacunas (CAV-AEP). Madrid: Manuel Merino Moína; [acceso 2 de mayo de 2018]. Manual de Vacunas en línea de la AEP; Capítulo 14. Vacunación en niños inmunodeprimidos o con tratamiento inmunosupresor. Disponible en: https://vacunasaep.org/documentos/manual/cap-14
14. Comité Asesor de Vacunas (CAV-AEP). Madrid: Manuel Merino Moína; [acceso 10 de febrero de 2018]. Manual de Vacunas en línea de la AEP; Capítulo 7. Calendarios de vacunación en España. Disponible en: https://vacunasaep.org/documentos/manual/cap-7
15. Shouval D. Prevention of hepatitis B reactivation in immunosuppressed patients with cancer. Medwave. 2007;7:e3326.
16. Idrovo V, Yanette C, Suárez Q. Epidemiología e historia natural de la hepatitis B. Rev Col Gastroenterol. 2009;24:4-11.
17. Ministerio de Sanidad Servicios Sociales e Igualdad. Recomendaciones de vacunación frente a la gripe. Temporada 2017-18. Madrid: MSSSI;2017 [acceso 18 de mayo de 2018]. Disponible en: https://www.mscbs.gob.es/profesionales/saludPublica/prevPromocion/vacunaciones/docs/Recomendaciones_vacunacion_gripe.pdf
18. Picazo J, González-Romo F, García Rojas A. Consenso sobre la vacunación anti-neumocócica en el adulto con patología de base. Rev Esp Quimioter. 2013;26:232-52. PMID: 24080892
19. Castro C, Bustos L, Ocampo R, Molina E, Cabrero P, Vergara R, et al. Effectiveness and safety of oral anticoagulation treatment with acenocoumarol in non-valvular atrial fibrillation. Rev Med Chil. 2017;145:1243-51. doi: 10.4067/S0034-98872017001001243.
20. Morales Acedo MJ, Mora García E, Martínez Blanco J. Reacciones adversas de la carbamacepina. A propósito de un caso. Semergen. 2000; 26:325-7.
21. Batalla J, Parrón I, Boldú M. Programa de sospecha de reacciones adversas a vacunas de Cataluña. Resultados del primer año. Vacunas. 2000; 1:119-25.
22. Batalla J, Urbiztondo L, Martínez M. Incidencia de los efectos adversos asociados con la vacunación sistemática y los programas de vacunación en Cataluña. Vacunas. 2004; 4:127-31.
23. Huerta I. Calendario de vacunaciones del adulto y vacunación en situaciones especiales. Oviedo: Servicio de Vigilancia Epidemiológica. Principado de Asturias. Dirección General de Salud Pública;2014 [acceso 18 de mayo de 2018] Disponible en: https://www.asturias.es/Astursalud/Ficheros/AS_Salud%20Publica/AS_Prevencion/Vacunas%202014/Guia_indicaciones_Calendario_Adultos_2014.pdf
24. WHO. Twenty-three-valent pneumococcal polysaccharide vaccine. WHO position paper. WklyEpidemiol Rec. 2008; 83(42):373-84. PMID: 18927997
25. Li G, Liang Q, Shi J. Safety and immunogenicity of 23-valent pneumococcal polysaccharide vaccine in 2 to 70 year old healthy people in China: A phase III double blind, randomized clinical trial. Hum Vaccin Immunother. 2015; 11:699-703. doi: 10.1080/21645515.2015.1011015
26. Rawlins MD, Thompson JW. Mechanism of adverse drug reactions. En: Davies DM, ed. Textbook of adverse drug reactions, 4 ed. Oxford: Oxford University Press, 1991:18-45.
27. Harrisa T, Nair J, Fediureka J. Assessment of sex-specific differences in adverse events following immunization reporting in Ontario, 2012–15. Vaccine. 2017; 35(19):2600-4. doi: 10.1016/j.vaccine.2017.03.035
28. Comité Asesor de Vacunas (CAV-AEP) [Internet]. Madrid: Manuel Merino Moína; [acceso 17 de mayo de 2018]. Manual de Vacunas en línea de la AEP; Capítulo 2. Coadministración de las vacunas entre sí y con otros productos biológicos. Disponible en: https://vacunasaep.org/documentos/manual/cap-2
29. Plotkin S, Robinson JM, Cunningham G, Iqbal R, Larsen S. The complexity and cost of vaccine manufacturing – An overview. Vaccine. 2017; 35(33):4064-71. doi: 10.1016/j.vaccine.2017.06.003

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Hepatitis C: nuevos diagnósticos y seroconversiones en una clínica de infecciones de transmisión sexual en Madrid

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RESUMEN

Objetivo. El objetivo es evaluar las nuevas infecciones por el virus de la hepatitis C (VHC) en función de su orientación sexual, situación serológica respecto al virus de la inmunodeficiencia humana (VIH), región geográfica de origen y la coinfección con otras infecciones de transmisión sexual (ITS).

Material y métodos. Estudio realizado en el Centro Sanitario Sandoval, clínica de referencia de ITS en Madrid. Se incluyeron todas las personas seronegativas al VHC que fueron reanalizadas para este virus, entre enero de 2010 y diciembre de 2016.

Resultados. Se diagnosticaron 59 nuevos diagnósticos de infección por el VHC. La proporción de hombres que tienen sexo con hombres (HSH) dentro de los nuevos diagnósticos fue del 37% en 2010 y del 75% en 2016 y, fue aún mayor en el grupo de coinfectados por el VIH/VHC (94%). Se detectaron 67 seroconvertores al VHC (1,2%). El 100% eran HSH. El 89% de los seroconvertores al VHC eran seropositivos para el VIH.

Conclusiones. La infección por el VHC sigue siendo un problema de salud vigente, especialmente en colectivos de riesgo, como los HSH seropositivos para el VIH.

Palabras clave: VHC; nuevos diagnósticos; seroconvertores; VIH; HSH

Hepatitis C: New diagnosis and seroconversions in a Madrid sexually transmitted diseases clinic

ABSTRACT

Indroduction. The aim of this study was to evaluate the incidence of new hepatitis C virus (HCV) infections, based on their sexual orientation, human immunodeficiency virus (HIV) status, geographical regions and coinfection with other sexually transmitted diseases (STDs).

Material and methods. This study was carried out at the Sandoval Health Center, reference clinic of Sexually Transmitted Diseases (STDs) in Madrid. All HCV seronegative individuals who were reanalyzed for this virus were included, between January 2010 and December 2016.

Results. A total of 59 new diagnoses of HCV were diagnosed. The proportion of men who have sex with men (MSM) diagnosed with HCV was 37% in 2010 and 75% in 2016 and was even higher in the group of coinfected with HIV/HCV (94%). A total of 67 seroconverters for HCV were detected (1.2%) of which 100% were MSM. The proportion of HCV seroconverters with HIV was 89%.

Conclusions. HCV infection continues to be a current health problem, especially in HIV-positive MSM.

Key-words: HCV; new diagnostics; seroconverters; HIV; MSM

INTRODUCCIÓN

La infección por el virus de la hepatitis C (VHC) constituye un problema de salud que afecta aproximadamente a 185 millones de personas en todo el mundo, de las que cada año fallecen 350.000 [1]. Hasta un 75-80% de las personas que entran en contacto con el VHC desarrollarán una infección crónica, con un espectro de afectación hepática que varía desde mínimos cambios histológicos, hasta extensa fibrosis con cirro-

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sis (entre un 5-25% evolucionarán a cirrosis en décadas) con o sin desarrollo de carcinoma hepatocelular (CHC). Además, la infección por VHC es una de las principales indicaciones de trasplante hepático (TH) en nuestro medio.

Pese a la alta prevalencia de la infección, la mayoría de los individuos afectados no conocen que están infectados [2].

Los regímenes de tratamiento de la infección por el VHC, basados en la combinación de agentes antivirales de acción directa (AAD), consiguen unas tasas de respuesta virológica sostenida por encima del 90%, incluso en pacientes que fracasaron previamente a las terapias basadas en interferón [3].

Sin embargo, a pesar de estos avances en la eficacia del tratamiento, se siguen observando nuevas infecciones, sobre todo en personas seropositivas para el virus de la inmunodeficiencia humana (VIH). En los países con terapia antirretroviral disponible para el VIH, la mortalidad por el VHC supera a la producida por el VIH y por el virus de la hepatitis B (VHB).

El objetivo del presente trabajo es evaluar la incidencia de nuevas infecciones por el VHC en personas inicialmente seronegativas, en función de su orientación sexual, situación serológica respecto al VIH, región geográfica de origen y la coinfección con otras infecciones de transmisión sexual (ITS), especialmente el linfogranuloma venéreo (LGV).

MATERIAL Y MÉTODOS

Estudio retrospectivo realizado en el Centro Sanitario Sandoval (CSS), clínica de referencia de ITS en la Comunidad de Madrid. Se incluyeron todas las personas seronegativas al VHC que fueron nuevamente analizadas para este virus y todas las personas con diagnóstico de VHC sin serología previa conocida (nuevos diagnósticos), entre el 01 de enero de 2010 y el 31 de diciembre de 2016.

A todos los pacientes seronegativos para el VHC se les realizó anualmente una nueva serología y todos ellos respondieron a un cuestionario epidemiológico estructurado, con objeto de conocer variables sociodemográficas y conductuales.

La determinación del anticuerpo para el VHC (Anti-VHC) se llevó a cabo mediante un inmunoensayo de micropartículas por quimioluminiscencia (CMIA), Abbott diagnostics division®. La carga viral se determinó mediante Versant® HCV RNA 1(kPCR), Siemens.

RESULTADOS

Del total de 26.805 consultas atendidas en el año 2016, se diagnosticaron microbiológicamente 3.362 episodios de ITS, de los cuales 287 correspondían a nuevos diagnósticos de infección por el VIH y 59 a nuevos diagnósticos de infección por el VHC (lo que representaba un 2% del total de las ITS diagnosticadas).

Desde el año 2010 y hasta el año 2016 se reanalizaron un total de 4.740 muestras para detectar la presencia de anticuerpos para el VHC, pertenecientes a 4.271 hombres, 414 mujeres y 55 transexuales (TSX). La edad media de los mismos fue de 36 años (desviación estándar: 7,18).

De todas las muestras reanalizadas (4.740) se detectaron 67 seroconvertores para el VHC (1,4%). El 100% fueron hom-

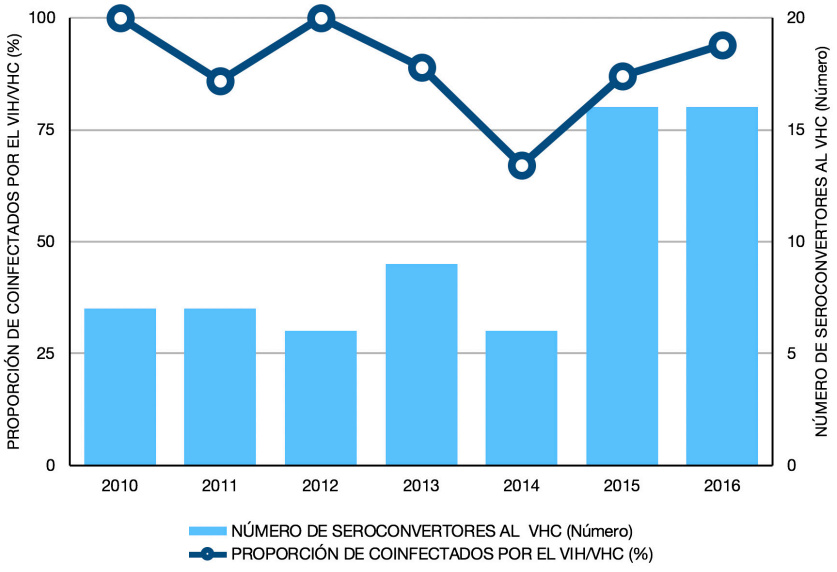

Figura 1 Número de seroconvertores por año y proporción de coinfectados por el VIH, en el Centro Sanitario Sandoval (CSS) (2010-2016)

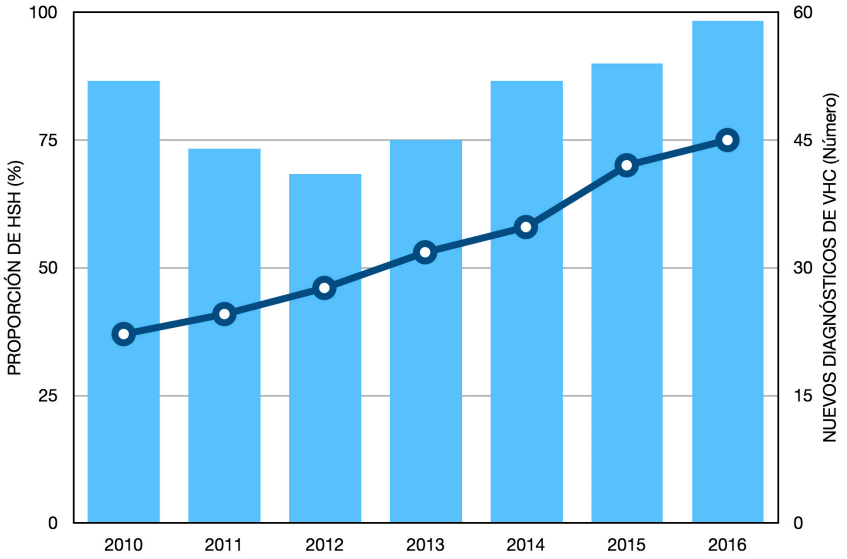

Figura 2 | **Nuevos diagnósticos de infección por el VHC y proporción de HSH, en el Centro Sanitario Sandoval (CSS) (2010-2016).**

bres que tienen sexo con hombres (HSH) frente al 0% de heterosexuales (HTX).

En relación a la región geográfica de procedencia de los seroconvertores, un 68% (46/67) eran españoles y un 34% (23/67) no españoles, de los cuales un 69% (16/23) procedían de Latinoamérica.

En cuanto a la coinfección con otras ITS, se analizó principalmente la relación con el LGV (*Chlamydia trachomatis* serovares L1-L3). Del total de los 67 seroconvertores para el VHC, 13 (19%) estaban coinfectados por LGV.

En el total de los 67 seroconvertores se cuantificó el ARN-VHC. En 21 personas la carga viral fue indetectable y detectable en los 46 restantes (rango: 111 UI - >40.000.000 UI).

Según la situación respecto al VIH, el 89% (60/67) de los seroconvertores al VHC fueron seropositivos para el VIH (figura 1).

Durante el periodo de estudio, se incrementó el diagnóstico de nuevos casos de infección por el VHC en un 13,5%.

Entre los nuevos diagnósticos de infección por el VHC, la proporción de HSH aumentó progresivamente, desde el año 2010 hasta el 2016, siendo más marcada en la segunda mitad del periodo analizado (de 2013 a 2016).

Así, la proporción de HSH dentro de los nuevos diagnósticos de infección por el VHC fue del 37% en el año 2010, del 53% en el año 2013 y ascendió hasta el 75% en el año 2016. Estos datos traducen un incremento, estadísticamente significativo ($p < 0,025$), del 131% (figura 2). La proporción de HSH fue aún mayor cuando se analizó en el grupo de coinfectados por el VIH/VHC (94%).

DISCUSIÓN

La principal vía de transmisión del VHC es la sangre. No obstante, en los últimos años estamos asistiendo a un aumento de la transmisión sexual del VHC [4].

La transmisión del VHC entre parejas heterosexuales (HTX) con relación monógama es muy rara, con una prevalencia que oscila entre el 0 y el 27% [5].

Según algunos estudios [6], cuando se excluye a las parejas con exposición parenteral conocida, la prevalencia es < 5%. Sin embargo, esta prevalencia aumenta en HSH especialmente en aquellos coinfectados por el VIH donde puede estar en torno al 3-39%, frente al 0-19% de los no coinfectados por el VIH [7]. De acuerdo con nuestros resultados, la prevalencia de infección por el VIH en los seroconvertores al VHC fue del 89% y el 100% de los seroconvertores eran HSH.

En esta línea de investigación, se ha planteado que el contacto con parejas infectadas por el VIH podría facilitar la transmisión del VHC, dado que algunos trabajos han demostrado que los pacientes coinfectados tienen cargas virales mayores que los monoinfectados [8].

Se han descrito factores biológicos, sociales y conductuales relacionados con el incremento en la transmisión sexual de la infección por el VHC en HSH, especialmente en coinfectados por el VIH.

En relación a los factores biológicos, no hay evidencias claras de que los pacientes VIH seropositivos sean más susceptibles a la infección por el VHC como consecuencia de la deficiencia del sistema inmune [9]. En algunos estudios se ha postulado que, pacientes HSH-VIH positivos podrían tener mayor carga viral de VHC en semen que aquellos pacientes HSH-VIH negativos, independientemente de la carga viral del VHC en plasma [10]. Otro factor biológico implicado estaría relacionado con la inflamación gastrointestinal crónica asociada a pacientes VIH seropositivos, ya que podría debilitar la mucosa a ese nivel y favorecer la transmisión del VHC [11].

Entre los factores conductuales y de comportamiento, el aumento del consumo de drogas recreativas (intravenosas o no) para mantener relaciones sexuales, término que se define como "CHEMSEX", está contribuyendo al aumento de la transmisión sexual del VHC.

Otro factor conductual favorecedor de la transmisibilidad del VHC serían las prácticas sexuales de riesgo, mucho más traumáticas entre los HSH, así como las relaciones sexuales desprotegidas, con múltiples parejas, el empleo de juguetes sexuales que pueden provocar laceración o lesión de la mucosa rectal y la presencia de otras infecciones de transmisión sexual, especialmente a nivel ano-genital.

En relación con estos factores analizados, y con el objetivo de reducir la tasa de infección por el VIH en aquellos paciente con riesgo "sustancial", el estudio llevado a cabo por Ayerdi-Aguirrebengoa et al. [12], argumenta que los HSH y HTX seronegativos, con pareja positiva para el VIH, ITS bacteriana en los últimos 6 meses, múltiples parejas sexuales, no uso sistemático del preservativo y trabajadores del sexo, serían candidatos a profilaxis preexposición (PrEP) para el VIH, y así disminuir, en la medida de lo posible, la probabilidad de seroconversión al VIH. Esta medida, también estaría indicada en los usuarios de drogas inyectables y en los transexuales con prácticas de riesgo.

La globalización y el uso de aplicaciones móviles para la búsqueda de contactos sexuales, son algunos de los factores sociales más importantes implicados en el incremento de la transmisión sexual del VHC. En nuestro estudio, la prevalencia de seroconvertores al VHC, según su región geográfica de procedencia, fue del 68% en españoles y del 34% en inmigrantes, la mayor parte de los cuales (hasta un 69%) procedían de Latinoamérica.

En relación con lo expuesto previamente, en España, Berenguer et al. [13] han analizado los cambios en la prevalencia y las características epidemiológicas de los pacientes coinfectados en los últimos años. Entre los factores relacionados se incluyen el aumento del uso de drogas intravenosas como mecanismo de transmisión del VIH, la emergencia de nuevos casos de infección por el VHC en HSH con prácticas sexuales de riesgo y la aparición de nuevos tratamientos más efectivos para tratar la infección por el VHC.

Considerando lo expuesto previamente, sería necesario implementar intervenciones específicas dirigidas a prevenir la transmisión del VHC, así como otras ITS, en especial en los HSH-VIH positivos que presenten prácticas de riesgo para la infección por el VHC y por el VIH.

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CONFLICTO DE INTERESES

Los autores declaran que no presentan conflictos de intereses.

REFERENCIAS

1. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global Epidemiology of Hepatitis C Virus Infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology. 2013;57(4):1333-42. DOI: 10.1002/hep.26141
2. Albillos Martínez A, Cañada Merino JL, Molero García JM, Pérez Cachafeiro S, Pérez Escanilla F, Simón Marco MA, Turnes Vázquez J; AEEH, SEMERGEN, semFYC, SEMG. Consenso de recomendaciones para el diagnóstico precoz, la prevención y la atención clínica de la hepatitis C en Atención Primaria. Madrid: Luzán 5; 2017.
3. Gutierrez JA, Lawitz EJ, Poordad F. Interferon-free, direct-acting antiviral therapy for chronic hepatitis C. J Viral Hepat. 2015;22(11):861-70. DOI: 10.1111/jvh.12422
4. Midgard H, Weir A, Palmateer N, Lo Re III V, Pineda JA, Macías J, et al. HCV epidemiology in high-risk groups and the risk of reinfection. J Hepatol.2016;65(1 Suppl):S33-S45. DOI:10.1016/j.jhep.2016.07.012
5. Terrault NA, Dodge JL, Murphy EL, Tavis JE, Kiss A, Levin TR, Gish RG, Busch MP, Reingold AL, Alter MJ. Sexual transmission of hepatitis C virus among monogamous heterosexual couples: the HCV partners study. Hepatology. 2013;57(3):881-9. DOI: 10.1002/hep.26164
6. Chan DP, Sun HY, Wong HT, Lee SS, Hung CC. Sexually acquired hepatitis C virus infection: a review. Int J Infect Dis. 2016;49:47-58. DOI: 10.1016/j.ijid.2016.05.030
7. Chen YC, Wiberg KJ, Hsieh YH, Bansal A, Bolzan P, Guy JA, et al. Favorable socioeconomic status and recreational polydrug use are linked with sexual Hepatitis C Virus transmission among Human Immunodeficiency Virus-infected men who have sex with men. Open Forum Infect Dis. 2016;3(3):ofw137. DOI:10.1093/ofid/ofw137
8. Neukam K, García-Rey S, Cifuentes C, Macías J, Mira JA, Vázquez MJ, et al. HIV-coinfection leads to a modest increase in plasma HCV-RNA load in patients with chronic HCV infection. Antiviral Res. 2012;95(3):212-5. DOI: 10.1016/j.antiviral.2012.06.009
9. European AIDS Treatment Network (NEAT) Acute Hepatitis C Infection Consensus Panel. Acute hepatitis C in HIV-infected individuals: recommendations from the European AIDS Treatment Network (NEAT) Acute Hepatitis C Infection Consensus Conference. AIDS. 2011;25(4):399-409. DOI: 10.1097/QAD.0b013e328343443b

10. Samuel S. Turner, Sara Gianella, Marcus J-S. Yip, Wouter O. van Seggelen, Robert D. Gillies, Andrew L. Foster, Zachary R. Barbati, Davey M. Smith, and Daniel S. Fierer. Shedding of Hepatitis C Virus in Semen of Human Immunodeficiency Virus-Infected Men. Open Forum Infect Dis. 2016;3(2):ofw057. DOI: 10.1093/ofid/ofw057

11. Taylor LE, Swan T, Mayer KH. HIV coinfection with hepatitis C virus: evolving epidemiology and treatment paradigms. Clin Infect Dis. 2012;55 Suppl 1:S33-42. DOI: 10.1093/cid/cis367

12. Oskar Ayerdi-Aguirrebengoa, Mar Vera-García, Teresa Puerta-López, Montserrat Raposo-Utrilla, Camen Rodríguez-Martín y Jorge Del Romero-Guerrero. ¿A quién proponer la profilaxis preexposición al virus de la inmunodeficiencia humana? Enferm Infecc Microbiol Clin. 2017;35(5):299–302. DOI: 10.1016/j.eimc.2016.06.006

13. Berenguer J, Rivero A, Jarrín I, Núñez MJ, Vivancos MJ, Crespo M, et al. Human Immunodeficiency Virus/Hepatitis C Virus coinfection in Spain: prevalence and patient characteristics. Open Forum Infect Dis. 2016;3(2):ofw059. DOI: 10.1093/ofid/ofw059

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Concordancia entre la prueba de la tuberculina y el Interferon Gamma Release Assay-IGRA en pacientes con enfermedades inflamatorias mediadas por la inmunidad

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RESUMEN

Introducción. Las terapias inmunosupresoras en el tratamiento de las enfermedades inflamatorias mediadas por la inmunidad (EIMI) predisponen a la tuberculosis, por lo que el cribado de infección tuberculosa latente (ITL) y su tratamiento reduce la probabilidad de progresión a tuberculosis activa. El objetivo del estudio fue analizar la concordancia entre la prueba de la tuberculina (PT) e "Interferon Gamma Release Assay-IGRA" en relación con el tipo de EIMI y tratamiento inmunosupresor (IS).

Material y métodos. Estudio transversal en pacientes con EIMI candidatos o en tratamiento IS remitidos para cribado de ITL, de Abril del 2017 hasta Mayo del 2018. Variables resultado fueron PT e IGRA. Variables explicativas: EIMI, IS, edad, sexo, vacunación BCG previa y factores de riesgo de tuberculosis.

Resultados. Se estudiaron 146 pacientes (33 [22,6%] vacunados con BCG, 1 [0,7%] con diagnóstico previo de tuberculosis y 22 [15,1%] originarios de país endémico). Índice de Kappa (k) fue de 0,338 entre PT e IGRA para la totalidad de la muestra. Menor concordancia en pacientes con enfermedad de Crohn (k=0,125), en los tratados con corticoides (k=0,222), vacunados con BCG (k=0,122) y en pacientes procedentes de países endémicos de tuberculosis (k=0,128).

Conclusiones. La concordancia entre la PT y el IGRA se ve afectada en pacientes con EIMI y en mayor medida en la enfermedad inflamatoria intestinal, con la corticoterapia, con la vacunación con BCG o en los procedentes de países endémicos.

Palabras clave: tuberculosis, interferon gamma Release Assays, Tuberculin test, Latent Tuberculosis Infection, Autoimmune Diseases, Immunosuppressive Agents.

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Concordance between the test of the tuberculin and Interferon Gamma Release Assay-IGRA in patients with immune-mediated inflammatory diseases

ABSTRACT

Introduction. The immunosuppressive therapies in the treatment of the immune-mediated inflammatory diseases (EIMI) predispose individuals to the tuberculosis, so the screening of latent tuberculosis infection (ITL) and the treatment reduces the likelihood of a progression to an active tuberculosis. The aim of the study was to analyze the concordance between the test of the tuberculin (PT) and "Interferon Gamma Release Assay-IGRA" in relation to the type of EIMI and the immunosuppressive treatment (IS).

Material and methods. Transversal study of patients with EIMI candidates or in treatment IS forwarded to the ITL screening, from April 2017 until May 2018. The outcome variables were PT and IGRA. The explicative variables were: EIMI, IS, age, gender, prior BCG vaccination and tuberculosis risk factors.

Results. A total of 146 patients were analyzed (33[22.6%] vaccinated with BCG, 1 [0.7%] with a pre-diagnosis of tuberculosis, and 22 [15.1%] from an endemic country). Kappa index (k) was 0,338 between PT and IGRA for the whole sample. A lower concordance was found in patients with the Crohn's disease (k=0.125), in the ones treated with corticosteroids (k=0.222), vaccinated with BCG (k=0.122) and in patients from tuberculosis endemic countries (k=0.128).

Conclusion. The concordance between PT and IGRA is affected in patients with EIMI, and to a greater extent to patients with the inflammatory bowel disease, with the corticotherapy, with the BCG vaccination, or in the ones from endemic countries.

Keywords: tuberculosis, interferon gamma Release Assays, Tuberculin test, Latent Tuberculosis Infection, Autoimmune Diseases, Immunosuppressive Agents.

INTRODUCCIÓN

El auge de las terapias biológicas en los últimos años ha supuesto una revolución en el tratamiento de las enfermedades inflamatorias mediadas por la inmunidad (EIMI). La farmacovigilancia ha evidenciado un aumento de los casos de tuberculosis (TB) asociada en este grupo de pacientes, especialmente aquellos que se encuentran en tratamiento con fármacos anti-factor de necrosis tumoral (TNF) [1, 2]. Por este motivo resulta de vital importancia el diagnóstico precoz de infección tuberculosa latente (ITL) y su tratamiento preventivo con el fin de reducir la posibilidad de progresión a TB activa.

En la actualidad, tal como se proponía en diferentes trabajos [3, 4], y tal cómo se recoge en el "Documento de consenso sobre la prevención y el tratamiento de la Tuberculosis en pacientes candidatos a tratamiento biológico" [5], la práctica habitual en cuanto a cribado de ITL en estos pacientes consiste en la realización de las dos pruebas: la prueba de la tuberculina (PT) y las técnicas diagnósticas basadas en la liberación de interferón gamma ("*Interferon Gamma Release Assay*" [IGRA]), en serie o en paralelo. Cabe recordar que ambas pruebas son mediciones cuantitativas y que su clasificación en resultados positivos o negativos se define a priori para distintos puntos de corte. Se considera una PT positiva si ≥ 5 mm en pacientes inmunodeprimidos y una prueba IGRA se considera positiva si ≥ 0,35 UI/ml (QuantiFERON®-TB Gold Plus), aunque se han considerado otros puntos de corte como 0,10 o 0,70 UI/ml, para los cuales variaría la sensibilidad de la prueba [6, 7]).

Existe un número muy limitado de estudios que analicen el rendimiento conjunto de ambas pruebas en pacientes con EIMI candidatos a tratamiento IS y que analicen el efecto que pueden tener, tanto la EIMI como el tratamiento IS en el momento del cribado de ITL [6-19]. Existen resultados contradictorios aunque parece que la utilidad diagnóstica de ambas se ve disminuida en este grupo de pacientes comparado con la población sin EIMI [8, 9]. La concordancia de las pruebas es variable y puede verse afectada por la EIMI y el tratamiento IS, así como en determinadas poblaciones (edades extremas, personas vacunadas previamente con BCG) [20].

Por ello, nos proponemos como objetivo principal evaluar la concordancia del IGRA y PT en función de la EIMI y del tratamiento IS recibido. Como objetivos secundarios estudiaremos como varía la concordancia según diferentes puntos de corte de IGRA, según exista o no antecedente de vacunación con BCG, según el paciente sea o no originario de país endémico de TB y en función de la edad.

MATERIAL Y MÉTODOS

Estudio transversal sobre los pacientes con EIMI remitidos a la consulta del Servicio de Medicina Preventiva, de Abril del 2017 a Mayo de 2018 para cribado de ITL, candidatos o en tratamiento con IS. Se excluyen aquellos pacientes que estén en tratamiento con fármacos inmunosupresores en el momento de la valoración y que sean mal cumplimentadores.

Las variables resultados fueron: PT en milímetros y la prueba IGRA en UI/ml. Como variables modificadoras de la concordancia: EIMI, tratamiento IS, edad, sexo, vacunación con BCG previa, originario de área endémica de TB, diagnóstico/tratamiento previo de TB, infección por VIH y diabetes mellitus.

En cada paciente se realizaron extracciones sanguíneas para realizar el test de IGRA QuantiFERON®-TB Gold Plus (QIAGEN, 40724 Hilden, Alemania) y se realizó simultáneamente la PT. Para ello, se inyectó en los pacientes 0,1 ml de tuberculina (2 unidades de tuberculina PPD) (Tuberculina PPD; Evans 2 UT, KREIDYPHARMA, S.L. Madrid, Spain), de acuerdo con las guías de la American Thoracic Society [21]. En cuanto al test de IGRA, se extrajo de cada paciente sangre en 4 tubos y se incubaron entre 16 y 24 horas a 37ºC. Posteriormente se centrifugaron y se separó el plasma para determinar si se había producido IFNγ (mediante enzimoinmunoensayo) como reacción a los antígenos peptídicos.

Hay que recordar que ambas pruebas inmunológicas son mediciones cuantitativas y que su clasificación en resultados positivos o negativos se define a priori para distintos puntos de corte. En inmunodeprimidos la PT se define como positiva si el halo de la induración tras la intradermorreacción es ≥ 5 mm y para el IGRA se considera un resultado positivo si en el tubo TB1 o TB2 se produce una cantidad de IFNγ ≥ 0,35 UI/l, aunque se han considerado otros puntos de corte como 0,10 o 0,70, para los cuales variaría la sensibilidad de la prueba [6, 7].

Se realiza análisis descriptivo de frecuencias con estimación de prevalencias de las distintas variables y se realiza test de Chi-cuadrado para comparar las variables con la positividad de ambas pruebas diagnósticas. Se estima la concordancia entre PT e IGRA como variables categóricas mediante el test Kappa de Cohen. Para ello, estratificamos según: EIMI, tratamiento IS o no y cuál, antecedente de vacunación con BCG previa, según sea originario o no de país endémico de TB y por grupos de edad. También se evalúa la concordancia de ambas pruebas según otros puntos de corte del IGRA (0,10 y 0,70 UI/ml). A tener en cuenta que la concordancia entre pruebas la estimaremos mediante el índice de Kappa (k), considerándose "pobre" si éste es ≤ 0,20; "baja" si $0,20 < k \leq 0,40$, "moderada" si $0,40 < k \leq 0,60$, "sustancial" si $0,60 < k \leq 0,80$ y "óptima" si $k > 0,80$.

El estudio fue aprobado por el Comité de Ética e Investigación Clínica de nuestro centro. Se mantuvo la confidencialidad de los datos obtenidos, se siguieron los principios de la declaración de Helsinki (2013), las reglas de buenas prácticas clínicas y las leyes españolas aplicables a este tipo de estudios.

RESULTADOS

Estudio descriptivo. Se incluyeron en el estudio 146 pacientes, con una edad media de 45 años y 39 eran menores de 35 años (25%). De ellos, 64 (43,9%) eran varones, 33 (22,7%) contaban con el antecedente de vacunación por BCG, 22 (15%) eran originarios de países endémicos de TB, 9 (6,2%)

eran diabéticos y 74 (50,8%) estaban en tratamiento IS. La EIMI más frecuente fue la psoriasis (51), seguida de la artritis reumatoide (23) y la enfermedad de Crohn (21). En cuanto al tratamiento IS empleado, el más empleado fue la monoterapia corticoidea (30) seguido del metotrexato (21) y la asociación corticoides y metotrexato (5).

De los 146 pacientes, 43 presentaron una PT positiva (29,5%), frente a 17 casos de IGRA-positivo (≥0,35 UI/ml) (11,6%). En cambio, si establecemos el punto de corte para considerar una prueba IGRA-positivo en ≥0,10 UI/ml 32 fueron positivos (21,9%) y una prueba IGRA-positiva ≥0,70 UI/ml, 11 fueron positivos (7,5%) (tabla 1).

Tanto el sexo masculino, el ser diabético, tener 35 o más años de edad, contar con el antecedente de vacunación previa con BCG y el ser originario de país endémico se asocian estadísticamente en la muestra con la positividad de la PT. En cuanto a la positividad del IGRA, solo se observó asociación estadísticamente significativa el ser originario de país endémico para punto de corte de 0,35 UI/ml y el sexo masculino para punto de corte de 0,10 UI/ml (tabla 2).

Análisis de la concordancia. En la totalidad de la muestra se obtuvo una concordancia baja entre la PT e IGRA, con un índice de Kappa (k) de 0,338. Según EIMI, se obtuvo concordancia moderada en pacientes con psoriasis (k=0,473), baja en pacientes con artritis reumatoide (k=0,378) y colitis ulcerosa (k=0,308), y pobre en los que sufrían espondilitis anquilopoyética (k=0,186) y enfermedad de Crohn (k=0,125).

En función del tratamiento IS, la concordancia fue moderada en los pacientes que recibían tratamiento con metotrexato (k=0,588) y ésta era baja en aquellos pacientes tratados con corticoides en monoterapia (k=0,222). A destacar que los pacientes que en el momento del cribado de ITL no recibían ningún tratamiento IS, presentaron una concordancia baja (k=0,362) entre ambas pruebas diagnósticas. Para el resto de EIMI y tratamientos IS no se pudo estimar el índice k debido al pequeño tamaño muestral. El análisis de la concordancia detallado queda resumido en la tabla 3.

Además se analizó la concordancia entre ambas pruebas en función de diferentes variables: según si el paciente había recibido o no con anterioridad vacunación con BCG, siendo mayor en los que no (k=0,122 y k=0,483 respectivamente); según el origen del paciente, siendo menor en los que procedían de países endémicos de TB (k=0,128 y k=0,297 respectivamente); según la edad, siendo mayor en los pacientes menores de 35 años (k=0,393 frente a k=0,314 en los mayores de 35 años).

Tabla 1 **Resultados de la prueba tuberculina (PT) y del Interferon Gamma Release Assay (IGRA)**

Test	PT	IGRA ≥ 0,35UI/ml	IGRA ≥ 0,70 UI/ml	IGRA ≥ 0,10 UI/ml
Total positivos, N	43	17	11	32
EIMI, N (%)				
Psoriasis	16 (31,4)	8 (15,7)	5 (9,8)	13 (25,5)
Enfermedad de Crohn	6 (28,6)	2 (9,5)	2 (9,5)	3 (12,3)
Artritis reumatoide	9 (39,2)	3 (13,0)	1 (4,3)	5 (21,7)
Colitis ulcerosa	2 (13,3)	2 (13,3)	2 (13,3)	4 (26,6)
Espondilitis anquilopoyética	6 (42,9)	1 (7,1)	1 (7,1)	3 (21,4)
Lupus eritematoso sistémico	0 (0)	0 (0)	0 (0)	1 (25%)
Hidrosadenitis	2 (33,3)	0 (0)	0 (0)	1 (16,7)
Eritema nodoso	1 (50,0)	1 (50)	0 (0)	1 (50)
Enfermedad de Behçet	0 (0)	0 (0)	0 (0)	1 (100)
Paniculitis	1 (50,0)	0 (0)	0 (0)	0 (0)
Tratamiento inmunosupresor, N (%)				
Sin tratamiento	26 (36,1)	9 (12,5)	7 (9,7)	16 (22,2)
Corticoides	6 (20,0)	5 (16,7)	3 (10,0)	9 (30,0)
Metotrexato	6 (28,6)	3 (14,3)	1 (4,8)	5 (23,8)
Corticoides y metotrexato	2 (40,0)	0 (0)	0 (0)	2 (40,0)
Ustekinumab	3 (75,0)	0 (0)	0 (0)	0 (0)

EIMI: Enfermedad Inflamatoria Mediada por Inmunidad.

Tabla 2 **Asociación de las distintas variables con la positividad de prueba tuberculina (PT) y del Interferon Gamma Release Assay (IGRA)**

Test	PT			IGRA ≥ 0,35 UI/ml			IGRA ≥ 0,70 UI/ml			IGRA ≥ 0,10 UI/ml		
	Positivo, N (%)	Negativo, N (%)	P valor	Positivo, N (%)	Negativo, N (%)	P valor	Positivo, N (%)	Negativo, N (%)	P valor	Positivo, N (%)	Negativo, N (%)	P valor
Sexo masculino	26 (40,6)	38 (59,4)	**0,009**	9 (14,1)	55 (85,9)	0,342	7 (10,9)	57 (89,1)	0,193	20 (30,3)	40 (66,7)	0,031
Edad ≥ 35 años	37 (34,6)	103 (70,5)	**0,024**	15 (14)	91 (85)	0,263	10 (9,4)	95 (89,6)	0,309	28 (27,2)	74 (71,8)	0,158
EIMI			0,687			0,359			0,562			0,321
Tratamiento inmunosupresor			0,373			1			1			0,995
Antecedente de vacunación BCG	20 (60,6)	13 (39,4)	**0,000**	3 (9,1)	30 (90,9)	0,638	3 (9,1)	30 (90,9)	0,700	7 (21,9)	25 (78,1)	0,698
Tto. o diagnóstico previo de TB	0 (0)	1 (100)	0,517	0 (0)	1 (100)	0,927	0 (0)	1 (100)	0,951	1 (100)	0 (0)	0,195
Diabetes mellitus	6 (66,7)	3 (33,3)	**0,011**	1 (91,1)	8 (88,9)	0,933	1 (11,1)	8 (88,9)	0,865	2 (25)	6 (75)	0,936
Originario país endémico	15(68,2)	7 (31,8)	**0,000**	6 (27,3)	16 (72,7)	**0,041**	4 (19)	17 (81)	**0,091**	9 (40,9)	13 (59,1)	**0,098**

EIMI: Enfermedad Inflamatoria, BCG: Bacilo Calmette-Gérin, TB: tuberculosis

Por otra parte, si estableciéramos en la totalidad de la muestra el punto de corte para considerar positivo el IGRA en 0,70 y 0,10 UI/ml, obtendríamos un concordancia inferior en ambos casos (k=0,237 y 0,281 respectivamente).

Por último, realizamos un análisis de la concordancia excluyendo a los pacientes vacunados con BCG. A destacar que_la concordancia entre la PT y el IGRA aumenta en los 113 pacientes que no habían sido vacunados con la BCG, siendo ésta moderada (k=0,483). En todas la situaciones analizadas en este apartado el índice kappa aumenta en relación a la totalidad de la muestra, excepto en los pacientes con enfermedad de Crohn, en los menores de 35 años y en los que tomaban corticoides (tabla 3).

DISCUSIÓN

Siguiendo las recomendaciones actuales propuestas en el año 2016 en el "Documento de consenso sobre la prevención y el tratamiento de la Tuberculosis en pacientes candidatos a tratamiento biológico" [5], en nuestro centro se realiza tanto la PT como el IGRA en paralelo en el cribado de ITL en el paciente con EIMI candidato a tratamiento biológico o ya en tratamiento. Estas directrices surgen con el propósito de aumentar la sensibilidad del cribado de ITL.

Centrándonos en nuestro objetivo principal y a pesar de no poderse estudiar la concordancia para algunas EIMI y tratamientos IS concretos dado el pequeño tamaño muestral, sí que podemos destacar varios resultados interesantes. Por una parte, en la totalidad de la muestra obtenemos una concordancia "baja" entre ambas pruebas, ya observada en anteriores publicaciones [14, 16, 18], y ésta persiste siendo "baja" aún excluyendo a aquellos pacientes que ya han iniciado tratamiento IS, en contra de lo observado en otros trabajos [8]. Además como ya se había comentado en estudios previos, esta concordancia difiere según una serie de variables como son: la EIMI (siendo mucho menor en las enfermedades inflamatorias intestinales) [22], el tratamiento IS empleado (menor con los corticoides), la vacunación previa con BCG [8, 20] y el origen del paciente (menor en originarios de países endémicos de TB).

Según esto, la concordancia entre ambas pruebas diagnósticas parece alterarse por la EIMI y por el régimen terapéutico inmunosupresor que cumple. En este aspecto sería interesante conocer con más exactitud como se comporta ésta según que EIMI sufra o que tratamiento tome el paciente, haciendo necesarios futuros estudios con mayor tamaño muestral.

Como intento de superar este defecto de concordancia, en otros estudios se ha propuesto modificar el punto de corte del IGRA hasta 0,70 o 0,10 UI/ml. Según nuestros resultados, esto empeoraría la concordancia entre ambas pruebas por lo que no parece ser una solución adecuada.

El estudio cuenta con varias limitaciones importantes. En primer lugar, el pequeño tamaño muestral impide analizar la concordancia para cada EIMI y cada tratamiento IS. Sería interesante conocer cómo se comporta la concordancia entre los tests para cada uno de ellos. Por otra parte, este trabajo presenta las propias limitaciones del resto de estudios realizados sobre esta cuestión y que son secundarias al hecho de no existir una prueba de referencia en el diagnóstico de ITL. Por último, el efecto negativo de la vacunación con BCG en la concordancia de ambas pruebas podría ser un factor de confusión, dado que aumenta el número de falsos positivos de la PT [18, 20]. Por ello decidimos además realizar un segundo análisis de la concordancia excluyendo a los pacientes que contaran con dicho antecedente (tabla 3). Aunque en líneas generales mejora la concordancia (de hecho se alcanza el nivel de "moderada" para los 113 pacientes no vacunados), en nuestra opinión sigue sin ser óptima y queda aún por descubrir que otros factores pueden estar influyendo en ello.

Como conclusión, los resultados de este trabajo sugieren

Tabla 3 **Concordancia entre la prueba tuberculina(PT) e Interferon Gamma Release Assay (IGRA)**

	Concordancia		Concordancia excluyendo pacientes vacunados BCG	
	N	k	N	k
Totalidad de la muestra	146	0,338	113	0,483
Según EIMI				
Psoriasis	51	0,473	42	0,634
Artritis reumatoide	23	0,378	15	0,762
Enfermedad de Crohn	21	0,125	17	- 0,970
Colitis ulcerosa	15	0,308	14	0,451
Espondilitis anquilopoyética	14	0,186	10	0,412
Eritema nodoso	2	1	-	-
Según tratamiento IS				
Sin tratamiento IS	72	0,362	55	0,524
Corticoides	30	0,222	26	0,114
Metotrexato	21	0,588	14	1
Según origen				
País no endémico	124	0,297	101	0,371
País endémico	22	0,128	12	0,676
Según edad				
<35 años	39	0,393	34	0,292
≥35 años	107	0,314	79	0,510
Según punto de corte de IGRA				
≥0,70 UI/ml	146	0,237	113	0,311
≥0,10 UI/ml	146	0,281	113	0,395
Según antecedente de BCG				
No	113	0,483	-	-
Sí	33	0,122	-	-

EIMI: Enfermedad Inflamatoria Mediada por inmunidad, IS: inmunosupresor, BCG: Bacilo Calmette-Gérin, k: índice Kappa

que la concordancia entre la PT y el IGRA en el diagnóstico de ITL se ve afectada en pacientes con EIMI y en mayor medida en aquellos sometidos a corticoterapia, padecen alguna enfermedad inflamatoria intestinal, cuentan con antecedente de vacunación con BCG o provienen de áreas endémicas de TB. Dicha concordancia empeoraría al modificar el punto de corte del IGRA de 0,35 a 0,70 o 0,10 UI/ml y mejoraría (aunque no hasta valores óptimos) al excluir a los pacientes vacunados con BCG.

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CONFLICTO DE INTERESES

Los autores declaran no tener ningún conflicto de intereses.

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BIBLIOGRAFÍA

1. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J,Schwieterman WD, et al. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N Engl J Med 2001; 345:1098–1104. DOI:10.1056/NEJMoa011110.
2. Gomez-Reino JJ, Carmona L,Valverde VR, Martin Mola E, Montero MD. Treatment of rheumatoid arthritis with tumor necrosis factor inhibitors may predispose to significant increase in tuberculosis risk. Arthritis Rheum 2003; 48:2122–2127. DOI:10.1002/art.11137
3. Muñoz L, Casas S, Juanola X, Bordas X, Martinez C, Santin M, et al. Prevention of anti–tumor necrosis factor–associated tuberculosis: a 10-year longitudinal cohort study. Clin Infect Dis 2015; 60: 349-356. DOI: 10.1093/cid/ciu796
4. Santin M, García-García JM, Rigau D, Altet N, Anibarro L, Casas I, et al. Sumario ejecutivo de la guía de práctica clínica sobre el uso de las pruebas de liberación de interferón-gamma para el diagnóstico de infección tuberculosa. Arch Bronconeumol 2016; 9: 477-481. DOI: 10.1016/j.arbres.2016.02.020
5. Mir Viladrich I, Daudén Tello E, Solano-López G, López Longo FJ, Taxonera Samso C, Sánchez Martínez P, et al. Documento de consenso sobre la prevención y el tratamiento de la tuberculosis en pacientes candidatos a tratamiento biológico. Arch Bronconeumol 2016; 52: 36-45. DOI: 10.1016/j.arbres.2015.04.016.
6. Maeda T, Banno S, Maeda S, Naniwa T, Hayami Y, Watanabe M, et al. Usefulness and limitations of QuantiFERON-TB Gold in Japanese rheumatoid arthritis patients: proposal to decrease the lower cutoff level for assessing latent tuberculosis infection. Mod Rheumatol 2010; 20:18–23. DOI: 10.1007/s10165-009-0226-5
7. Maeda T, Banno S, Maeda S, Naniwa T, Hayami Y, Watanabe M et al. Comparison of QuantiFERON-TB Gold and the tuberculin skin test for detecting previous tuberculosis infection evaluated by chest CT findings in Japanese rheumatoid arthritis patients. J Infect Chemother 2011; 17:842-848. DOI:10.1007/s10156-011-0250-1
8. Ramos JM, Masiá M, Rodríguez JC, López C, Padilla S, Robledano C et al. Negative effect of immunosuppressive therapy in the performance of the QuantiFERON Gold In-Tube test in patients with immune-mediated inflammatory diseases. Clin Exp Med. 2013;13:177–186. DOI:10.1007/s10238-012-0192-7
9. Sunny H Wong, Quinyan Gao, Kelvin K F Tsoi, William K K Wu, Lai-shan Tam, Nelson Lee et al. Effect of immunosuppressive therapy on interferon γ release assay for latent tuberculosis screening in patients with autoimmune diseases: a systematic review and meta-analysis. Thorax 2015;71:64-72. DOI:10.1136/thoraxjnl-2015-207811.
10. Sellam J, Hamdi H, Roy C, Baron G, Lemann M, Pue´chal X et al. Comparison of in vitro-specific blood tests with tuberculin skin test for diagnosis of latent tuberculosis before anti-TNF therapy. Ann Rheum Dis 2007;66:1610–1615. DOI: 10.1136/ard.2007.069799.
11. Bocchino M, Matarese A, Bellofiore B, Giacomelli P, Santoro G, Balato N et al. Performance of two commercial blood IFN- gamma release assays for the detection of Mycobacterium tuberculosis infection in patient candidates for anti-TNF-alpha treatment. Eur J Clin Microbiol Infect Dis 2008;27:907–913. DOI:10.1007/s10096-008-0519-1.
12. Schoepfer AM, Flogerzi B, Fallegger S, Schaffer T, Mueller S, Nicod L et al. Comparison of interferon-gamma release assay versus tuberculin skin test for tuberculosis screening in inflammatory bowel disease. Am J Gastroenterol 2008;103: 2799–2806. DOI:10.1111/j.1572-0241.2008.02050.x
13. Ponce de Leon D, Acevedo-Vasquez E, Alvizuri S, Gutierrez C, Cucho M et al. Comparison of an interferon-gamma assay with tuberculin skin testing for detection of tuberculosis (TB) infection in patients with rheumatoid arthritis in a TB-endemic population. J Rheumatol 2008;35:776–781. PMID:18398944.
14. Chen DY, Shen GH, Hsieh TY, Hsieh CW, Lan JL.Effectiveness of the combination of a whole-blood interferon-gamma assay and the tuberculin skin test in detecting latent tuberculosis infection in rheumatoid arthritis patients receiving adalimumab therapy. Arthr Rheum 2008;59:800–806. DOI:10.1002/art.23705.
15. Martin J, Walsh C, Gibbs A, McDonnell T, Fearon U, Keane J et al. Comparison of interferon gamma release assays and conventional screening tests before tumour necrosis factor alpha blockade in patients with inflammatory arthritis. Ann Rheum Dis 2010;69:181–185. DOI:10.1136/ard.2008.101857.
16. Inanc N, Aydin SZ, Karakurt S, Atagunduz P, Yavuz S, Direskeneli H et al. Agreement between Quantiferon-TB gold test and tuberculin skin test in the identification of latent tuberculosis infection in patients with rheumatoid arthritis and ankylosing spondylitis. J Rheumatol 2009;36:2675–2681. DOI:10.3899/jrheum.090268.
17. Chiu HY, Hsueh PR, Tsai TF. Clinical experience of QuantiFERON® -TB Gold testing in patients with psoriasis treated with tumour necrosis factor blockers in Taiwan. Br J Dermatol 2011;164:553–559. DOI:10.1111/j.1365-2133.2010.10137.x.
18. Bélard E, Semb S, Ruhwald M, Werlinrud AM, Soborg B, Jensen FK et al. Prednisolone treatment affects the performance of the QuantiFERON gold in-tube test and the tuberculin skin test in patients with autoimmune disorders screened for latent tuberculosis infection. Inflamm Bowel Dis 2011; 17:2340-2349. DOI:10.1002/ibd.21605.
19. Qumseya BJ, Ananthakrishnan AN, Skaros S, Bonner M, Issa M, Zadvornova Y et al. QuantiFERON TB gold testing for tuberculosis screening in an inflammatory bowel disease cohort in the United States. Inflamm Bowel Dis 2011;7:77–83. DOI:10.1002/ibd.21329.
20. Bellido-Blasco JB, Pardo-Serrano F , Romeu-García MA , Moreno-Muñoz MR, Meseguer-Ferrer N, Vizcaíno-Batllés et al. Tuberculina y QuantiFERON©-TB: concordancia, correlación cuantitativa y riesgo de exposición en estudio de contactos de tuberculosis. Enferm Emerg 2017;16:11-22.
21. American Thoracic Society: Centers for Disease Control and Prevention (2000) Diagnostic standards and classification of tuberculosis in adults and children. Am J Respir Crit Car Med 161:1376–1395. DOI:10.1164/ajrccm.161.4.16141.
22. Abreu C, Afonso J, Dias CC, Ruas R, Sarmento A, et al.. Serial Tuberculosis screening in inflamatory bowel disease patients receiving anti-TNF-a therapy. J Crohns Colitis 2017;11:1223-1229. DOI:10.1093/ecco-jcc/jjx080.

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Original

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Historical evolution of the diseases caused by non-pigmented rapidly growing mycobacteria in a University Hospital

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ABSTRACT

Introduction. Non-pigmented rapidly growing mycobacteria (NPRGM) are a group of organisms of increasing interest due to the growing number of potential patients and the difficulties for a proper treatment in many of them. However, the evolution of these diseases in a long period of time and its evolutionary changes has been described only in a scanty number of reports.

Material and methods. We performed a retrospective study between January 1st 2004 and December 31st 2017 in order to evaluate the clinical significance and types of diseases caused by NPRGM. Patients with isolates of NPRGM during this period were selected for the study, and clinical charts were reviewed using a predefined protocol.

Results. During this period we identified 59 patients (76 clinical samples) with isolates of NPRGM, with 12 cases of clinical disease and one patient with doubtful significance (including 6 respiratory tract infections, 2 catheter infections, 1 skin and soft tissue infection, 1 disseminated infection, 1 conjunctivitis, 1 prosthetic joint infection and 1 mastitis). Fifty percent of *M. chelonae* isolates, 37.5% of *M. abscessus* isolates and 23.33% of *M. fortuitum* isolates were clinically significant. None of the isolates of other species were significant.

Conclusions. Most isolates in respiratory samples were contaminants/colonizations. *M. abscessus* was the main etiological agent in respiratory syndromes, whereas *M. chelonae* and *M. fortuitum* were more frequently associated with other infections, especially clinical devices and skin and soft tissue infections.

Keywords: Non-pigmented rapidly growing mycobacteria; *Mycobacterium abscessus*; *Mycobacterium chelonae*; *Mycobacterium fortuitum*; clinical significance; historical evolution; epidemiology.

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Evolución histórica de las enfermedades causadas por micobacterias no pigmentadas de crecimiento rápido en un Hospital Universitario

RESUMEN

Introducción. Las micobacterias no pigmentadas de crecimiento rápido (MNPCR) son un grupo de organismos de interés creciente debido al número cada vez mayor de pacientes potenciales y a las dificultades en el tratamiento. Sin embargo, el número de estudios que analizan la evolución de estos casos a lo largo de un periodo de tiempo largo es escaso.

Material and métodos. Se realizó un estudio retrospectivo entre el 1 de enero de 2004 y el 31 de diciembre de 2017 para evaluar el significado clínico y los tipos de enfermedades causados por MNPCR. Se seleccionaron para ello aquellos pacientes con aislamientos de MNPCR, y se revisaron las historias clínicas mediante un protocolo predefinido.

Resultados. Se identificaron 59 pacientes (76 muestras) con aislamientos de MNPCR, de los cuales 12 presentaron enfermedad y uno tuvo un significado dudoso (incluyendo 6 infecciones respiratorias, 2 infecciones asociadas a catéter, 1 infección de piel y partes blandas, 1 infección diseminada, 1 conjuntivitis, 1 infección de prótesis osteoarticular y 1 mastitis). El 50 % de los aislamientos de *Mycobacterium chelonae*, el 37,5 % de *Mycobacterium abscessus* y el 23,33 % de *Mycobacterium fortuitum* fueron clínicamente significativos. Ninguno de los aislamientos de otras especies fue significativo.

Conclusiones. La mayoría de los aislamientos de muestras respiratorias resultaron ser contaminantes/colonizaciones. *M. abscessus* fue el principal agente etiológico en las infecciones respiratorias, mientras que *M. chelonae* y *M. fortuitum* fueron asociados con mayor frecuencia a otras

infecciones, especialmente infecciones de piel y partes blandas e infecciones asociadas a dispositivos biomédicos.

Palabras clave: micobacterias no pigmentadas de crecimiento rápido; *Mycobacterium abscessus*; *Mycobacterium chelonae*; *Mycobacterium fortuitum*; significado clínico; evolución histórica ; epidemiología.

INTRODUCTION

Non-tuberculous mycobacteria (NTM) are a group of opportunistic pathogens which are being increasingly recognized as a cause of infection [1]. They are also environmental organisms that can be found in many different ecosystems without public health implications [2].

NTM infections are an emerging phenomenon, mainly in the last decade [3]. It has been observed an increasing importance of infections caused by these organisms, both localized and disseminated, including also outbreaks and pseudo-outbreaks [4-5]. Among these, non-pigmented rapidly growing mycobacteria (NPRGM) are ubiquitous in nature and widely distributed in water, soil and animals [2, 6].

The three most important species of this group, regarding their clinical relevance, are *Mycobacterium fortuitum*, *Mycobacterium chelonae* and *Mycobacterium abscessus* [7]. However, there are many other species capable of causing human diseases such as *Mycobacterium mucogenicum*, *Mycobacterium immunogenum*, *Mycobacterium goodii*, *Mycobacterium peregrinum*, *Mycobacterium phocaium*, *Mycobacterium porcinum*, *Mycobacterium smegmatis* or *Mycobacterium wolinskyi* [7-9].

These microorganisms have the ability to form biofilms and this gives these organisms many advantages over the planktonic type of growth, as resistance to environmental aggressions and an increased resistance against disinfectants and antibiotics [10].

M. abscessus is one of the most frequently causative agents of nontuberculous mycobacterial pulmonary disease, often isolated in patients with underlying chronic lung diseases, like old tuberculosis scars, silicosis, bullae and other lung cavities where NTM can develop a biofilm. In recent times, patients with chronic bronchiectasis and cystic fibrosis have been found to be a target for NPRGM infections [11-13].

M. chelonae and *M. fortuitum* are frequently isolated in skin and soft tissue infections [14-15]. However, all these organisms can be isolated in other different clinical samples as a cause of many types of infection.

Here we report our experience with the diseases caused by these organisms isolated in our hospital during a 13-year period in order to compare these results with previous studies regarding these organisms.

MATERIAL AND METHODS

A retrospective study was performed to evaluate the clinical significance of the NPRGM. For this purpose, records dating from January 1st 2004 to December 31st 2017 from the mycobacteriology laboratory of the clinical microbiology department were reviewed. Patients with at least one isolate of NPRGM from clinical samples were selected for clinical charts review.

Sample processing and identification of bacterial isolates was performed following the internationally accepted protocols. The decontamination technique for all samples was

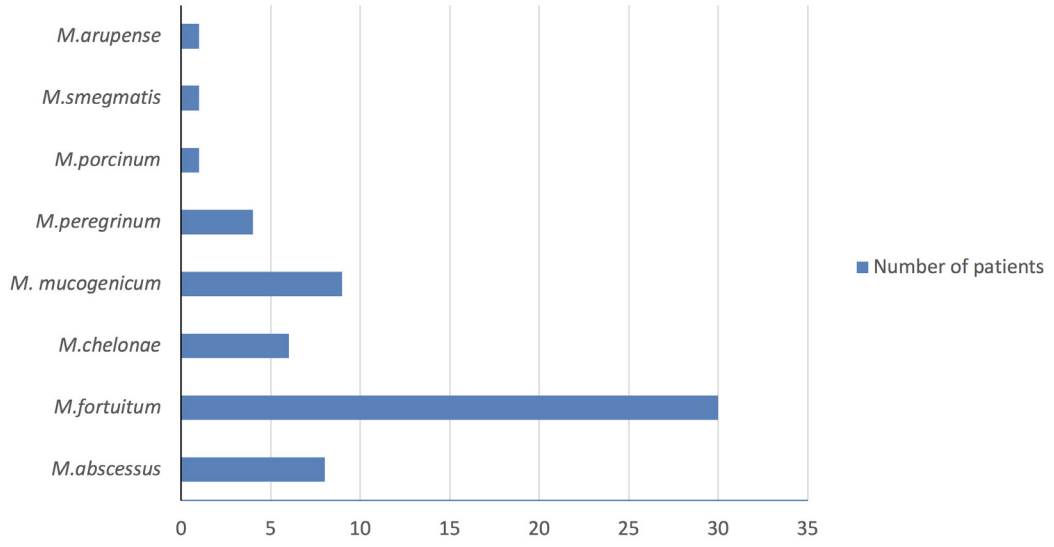

Figure 1 Number of patients with NPRGM

NPRGM: non-pigmented rapidly growing mycobacteria

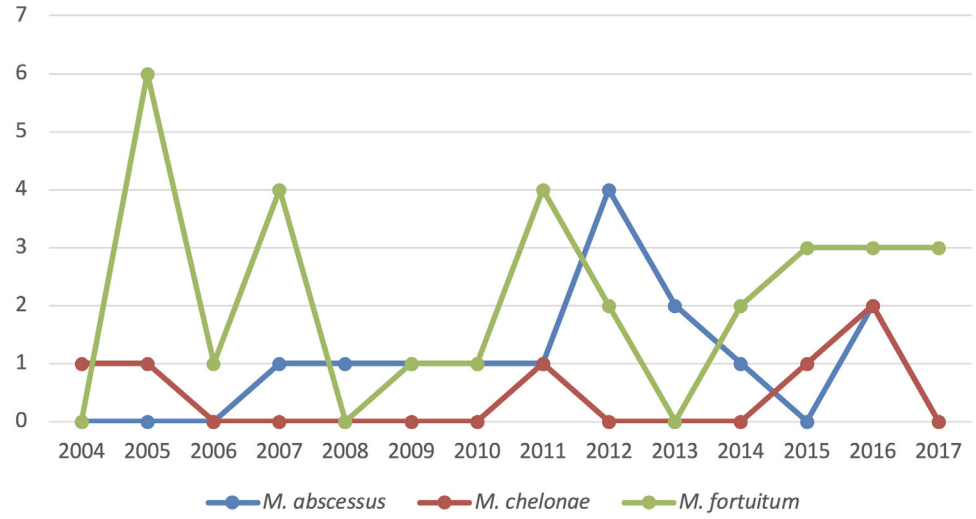

Figure 2 Evolution of cases of infection caused by NPRGM (patients/year)

NPRGM: non-pigmented rapidly growing mycobacteria

the N-acetyl-cysteine-NaOH protocol throughout all these years. After decontamination, all samples were inoculated onto Lowenstein-Jensen and Coletsos solid slants and were inoculated also in a liquid medium (MGIT 960 from 2004 to 2009, from BD, USA, VERSATREK (Biomérieux, France) from 2009 to 2016 and BacT/ALERT 3D (Biomérieux, France) from 2016 to date.

Mycobacterial isolates were identified using a commercial PCR identification test (GenoType CM/AS, Hain, Germany), and those isolates that could not be identified with this technique were sent to the Mycobacteria reference laboratory (Centro Nacional de Microbiología, Majadahonda, Spain). Antimicrobial susceptibility test was performed for all mycobacterial isolates with the broth microdilution reference technique [16].

On one hand, clinical charts were evaluated according to a predefined protocol that includes demographics, evaluation of risk factors (respiratory syndromes, HIV infection, immunosuppressive drug treatment and presence of biomaterials), clinical syndrome, treatment and outcome. Criteria from the ATS [17] for interpretation of an isolate were followed to determine the clinical significance of each case. On the other hand, we considered a case of doubtful clinical significance when it presented with clinical signs of infection in the absence of other possible causes, and showed improvement after treatment with antimicrobial therapy, but did not fulfill the microbiological criteria. The Clinical Research Ethics Committee of our hospital approved the study (registration number EO137-18_FJD)

RESULTS

Growing of NPRGM was observed in 76 clinical samples of 59 patients from January 1st 2004 to December 31st 2017.

Isolated mycobacteria were *Mycobacterium fortuitum* (30 patients), *Mycobacterium abscessus* (8 patients), *Mycobacterium mucogenicum* (9 patients), *Mycobacterium chelonae* (6 patients), *Mycobacterium peregrinum* (4 patients), *Mycobacterium porcinum* (1 patient), *Mycobacterium smegmatis* (1 patient) and *Mycobacterium arupense* (1 patient) (figure 1). One patient had two different NPRGM in two different samples. *M. fortuitum* was the most frequent isolated mycobacterium, with a sharply increase in 2005, 2007 and 2011. *M. abscessus* had a significant increase in 2012. *M. chelonae* was the less isolated mycobacterium with only 0 or 1 isolates per year, except in 2016 when it was isolated three times (figure 2).

Most of the samples were sputum and other respiratory samples (58 samples) followed by wound exudates and skin biopsies (5 samples), urine (3 samples), blood cultures (2 samples) and several other samples (8 samples).

Clinically significant cases appeared in 12 patients (20.3 %). One patient was classified as doubtful, and the rest of them were non-clinically significant cases. Syndromes and treatment of the patients with true or doubtful clinical significance are shown in table 1.

The clinical syndromes related to NPRGM include respiratory tract infections (6 cases), catheter infections (2 cases), skin and soft tissue infection (1 case), disseminated infection (1 case), conjunctivitis (1 case), prosthetic joint infection (1 case) and mastitis (1 case).

Regarding the clinical relevance of each species, 50% of the isolates of *M. chelonae*, 37.5% of *M. abscessus* and 23.33% of *M. fortuitum* were clinically significant. None of the isolates of other species were significant.

Table 1 **Characteristics of the cases of infection caused by NPRGM and the case with doubtful significance.**

Case	Year	Sex	Age	Underlying diseases	Syndrome	Positive samples	Acid-fast stain	Therapy	Species
1	2004	F	36	Chronic bronchopaty	Dysphonia	Laryngeal biopsy	Positive	IS+RI	*M. chelonae*
2	2005	F	45	NO	Mastitis	Skin exudate	Negative	CI	*M. fortuitum*
3	2005	M	55	Multiple myeloma	Catheter infection	Catheter exudate	Positive	AM+CI+CL	*M. chelonae*
4	2006	F	30	Depressive syndrome	Skin and soft tissue infection	Skin biopsy	Negative	CI	*M. fortuitum*
5	2007	F	51	HIV, Burkitt lymphoma	Disseminated infection	Blood cultures	Negative	CL+CO	*M. fortuitum*
6	2007	M	48	Multiple myeloma	Catheter infection	Catheter exudate	Negative	CO	*M. fortuitum*
7	2008-2013	M	45	HIV, Chronic respiratory insufficiency	Bronchiectasias	Sputum	Negative	CL	*M. abscessus*
8	2011	F	86	Lower eyelid myofibroblastic tumor	Conjunctivitis	Conjunctival exudate	Not performed	CL	*M. chelonae*
9	2012-2014	F	56	Chronic obstructive pulmonary disease	Bronchiectasias	Bronchial lavage	Negative	LE	*M. abscessus*
10	2012	F	80	NO	Arthritis	Bone prosthesis	Negative	CI+RI	*M. fortuitum*
11	2012	F	62	Alpha1-antitrypsin deficiency	Bronchiectasias	Sputum	Positive	NO	*M. abscessus*
12	2015	M	71	Chronic obstructive pulmonary disease	Bronchiectasias	Sputum	Negative	CI	*M. fortuitum*
13	2015	F	49	Asthma	Bronchiectasias	Bronchial lavage	Negative	CI+CO	*M. fortuitum*

NPRGM: non-pigmented rapidly growing mycobacteria; M: Male; F: Female; NO: No disease/Not received; AM: Amikacin; CI: Ciprofloxacin; CL: Clarithromycin; CO: Cotrimoxazole; IS: Isoniazid; LE: Levofloxacin; RI: Rifampicin.

When we focus on the underlying diseases in the clinically significant group chronic respiratory disease (6 cases), presence of malignancy (2 cases) and human immunodeficiency virus (HIV) disease (2 cases) were detected. There was 1 case of surgical infection related to shoulder prosthesis.

Acid-fast bacilli were detected in stains from samples in 4 of the significant and doubtful cases (33.33%). There was a clinically significant case (conjunctival exudate) in which the acid-fast stain was not performed. Interestingly, 2 samples of the non-significant group were also acid-fast stain positive.

Regarding the therapeutic actions in the clinically significant group, all the patients were treated with antimicrobial therapy except the doubtful case. There were 8 cases treated with monotherapy regimen (3 cases with ciprofloxacin, 2 cases with clarithromycin, 1 case with levofloxacin and 1 case with cotrimoxazol). Ciprofloxacin and clarithromycin were the mainly used antibiotics, either as monotherapy or in a combination antimicrobial regimen. In the implant-related infection it was necessary to remove the prosthesis in order to cure the infection. All the patients were cured, except 2 cases which are currently being under follow-up/control. One patient died due to other pathology. No resistances during therapy were detected.

DISCUSSION

NPRGM are usually considered environmental opportunistic pathogens. In our series we documented that only 20.34% of the isolates were clinically significant, compared to 30.8 % in a previous study [18]. This fact could be related to the increased number of respiratory tract isolates, probably due to the environmental nature of these bacteria. *M. abscessus*, *M. chelonae* and *M. fortuitum* have been usually associated with human diseases, while other members of the group are environmental isolates that cause human infections in rare cases [18-20]. *M. porcinum* has emerged in the last years as a species clearly related to human diseases, being involved in respiratory infections [21], but our only isolate has no role in the disease of this patient. Among other species, most clinically significant cases of *M. mucogenicum* isolates are involved in catheter-related infections [22]. *M. peregrinum* is a species included in the *Mycobacterium fortuitum complex*, but only a few cases of true infections have been reported, mainly related to surgical site infections and catheter-related infections [23]. *M. arupense* isolates have been related to pulmonary disease and osteoarticular infection [24-25]. In our series, all these species appeared to be colonizing organisms or contaminants, while the clinically relevant isolates belonged to the most common pathogens of this group: *M. fortuitum*, *M. abscessus* and *M. chelonae*.

According to the literature, the isolation of NPRGM has not a clear role in respiratory infection diseases such as chronic obstructive pulmonary disease (COPD) and bronchiectasis [19]. In these patients, the distinction between colonization and infection is a difficult clinical decision in most cases. *M. abscessus* is known to be a pathogen implicated in respiratory syndromes. It account for the majority of pulmonary infection cases in patients with underlying diseases like bronchiectasis,

cystic fibrosis or granulomatous diseases like sarcoidosis [12, 26-28]. Twenty eight percent of cystic fibrosis patients are affected by this species, being associated with increased morbidity and mortality, as well as with a rapid decline in lung function. Although it is not an absolute contraindication for lung transplantation, the pulmonary infection is associated with poor prognosis following this procedure [13, 27, 29].

In our series, most of NPRGM isolations from respiratory samples are not considered to be the major cause implicated in the pathology, but in patients with bronchiectasis, *M. abscessus* was the main isolated pathogen and all cases were treated with monotherapy, except one case that was considered of doubtful significance. This last case had a special clinical situation due to an alpha 1-antitrypsine deficiency, and the isolate was considered colonization because of the lack of symptoms, despite the fact that the organism was isolated from several different samples during a long time period.

The second species more frequently isolated in our series in respiratory samples was *M. fortuitum*. The respiratory infection caused by these mycobacteria is less common than *M. abscessus* disease, but there are cases reported in literature [30-31].

All of the biomaterial-related infections in our series required a combined medical and surgical therapeutic approach. Surgical procedures consisted of implant removal (meshes, catheter, and other prosthesis). *M. fortuitum* was the most frequently isolated mycobacteria from these infections. The ability of rapidly growing mycobacteria to develop biofilms in different surfaces is well known [32-34]. This virulence factor makes almost impossible the eradication of this bacteria using only antimicrobial therapy because of the *in vivo* resistance of sessile organisms against the different antimicrobials [35], so biofilm removal is mandatory in these cases.

In skin and soft tissue infections *M. fortuitum* was the main etiologic agent in our series**.** Acupuncture, infected surgical equipment or tattoos have been established as risk factors to develop a NPRGM skin infection [36-37]. The water used in the sterilising processes seems to be the main source of contamination in many cases**.** Monotherapy regimen was the selected treatment in all cases for these infections.

Due to the fact that NPRGM are resistant to conventional antituberculous drugs, the treatment has to be directed through *in vitro* susceptibility testing [16], being clarithromycin and ciprofloxacin the most frequently selected antibiotics [38]. Despite the previously described development of resistance during monotherapy [39], we have not detected any case of such problem, probably due to the low bacterial load presented in most of the cases, which minimises the probability of selection of resistant mutants.

In conclusion, the major difficulty to evaluate the clinical significance of NPGRM resides in the fact that most of these isolates are regarded as a contamination. However, we observed in our series that the isolation of a specific NPGRM (*M. abscessus, M. fortuitum* and *M. chelonae)*, or in specific samples (respiratory samples, skin, soft tissue, and biomaterials) is almost always related to the clinical syndrome. In order to avoid the failure of the treatment, an adequate microbiological identification and susceptibility test is needed, which would allow to choose a correct antimicrobial therapy and management of patients.

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CONFLICT OF INTEREST

The author(s) declare(s) that they have no conflicts of interest

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REFERENCES

1. Ahmed I, Jabeen K, Hasan R. Identification of non-tuberculous mycobacteria isolated from clinical specimens at a tertiary care hospital: a cross-sectional study. BMC Infect Dis. 2013;13:493. DOI: 10.1186/1471-2334-13-493
2. Falkinham JO, 3rd. Nontuberculous mycobacteria in the environment. Clin Chest Med. 2002;23(3):529-51. PMID 12370991
3. McGrath EE, Blades Z, McCabe J, Jarry H, Anderson PB. Nontuberculous mycobacteria and the lung: from suspicion to treatment. Lung. 2010;188(4):269-82. DOI: 10.1007/s00408-010-9240-9
4. Kressel AB, Kidd F. Pseudo-outbreak of *Mycobacterium chelonae* and *Methylobacterium mesophilicum* caused by contamination of an automated endoscopy washer. Infect Control Hosp Epidemiol. 2001;22(7):414-8. DOI: 10.1086/501926
5. Kohler P, Kuster SP, Bloemberg G, Schulthess B, Frank M, Tanner FC, et al. Healthcare-associated prosthetic heart valve, aortic vascular graft, and disseminated *Mycobacterium chimaera* infections subsequent to open heart surgery. Eur Heart J. 2015;36(40):2745-53. DOI: 10.1093/eurheartj/ehv342
6. Russell CD, Claxton P, Doig C, Seagar AL, Rayner A, Laurenson IF. Non-tuberculous mycobacteria: a retrospective review of Scottish isolates from 2000 to 2010. Thorax. 2014;69(6):593-5. DOI: 10.1136/thoraxjnl-2013-204260
7. Brown-Elliott BA, Wallace RJ, Jr. Clinical and taxonomic status of pathogenic nonpigmented or late-pigmenting rapidly growing mycobacteria. Clin Microbiol Rev. 2002;15(4):716-46. DOI: 10.1128/crm.15.4.716-746.2002
8. Chen YC, Jou R, Huang WL, Huang ST, Liu KC, Lay CJ, et al. Bacteremia caused by *Mycobacterium wolinskyi*. Emerg Infect Dis. 2008;14(11):1818-9. DOI: 10.3201/eid1411.080003
9. Ashraf MS, Swinker M, Augustino KL, Nobles D, Knupp C, Liles D, et al. Outbreak of *Mycobacterium mucogenicum* bloodstream infections among patients with sickle cell disease in an outpatient

setting. Infect Control Hosp Epidemiol. 2012;33(11):1132-6. DOI: 10.1086/668021

10. Esteban J, Garcia-Coca M. *Mycobacterium* Biofilms. Front Microbiol. 2017;8:2651. DOI: 10.3389/fmicb.2017.02651
11. Benwill JL, Wallace RJ, Jr. *Mycobacterium abscessus*: challenges in diagnosis and treatment. Curr Opin Infect Dis. 2014;27(6):506-10. DOI: 10.1097/QCO.0000000000000104
12. Qvist T, Pressler T, Hoiby N, Katzenstein TL. Shifting paradigms of nontuberculous mycobacteria in cystic fibrosis. Respir Res. 2014;15:41. DOI: 10.1186/1465-9921-15-41
13. Floto RA, Olivier KN, Saiman L, Daley CL, Herrmann JL, Nick JA, et al. US Cystic Fibrosis Foundation and European Cystic Fibrosis Society consensus recommendations for the management of non-tuberculous mycobacteria in individuals with cystic fibrosis: executive summary. Thorax. 2016;71(1):88-90. DOI: 10.1136/thoraxjnl-2015-207983
14. Cho SY, Peck KR, Kim J, Ha YE, Kang CI, Chung DR, et al. *Mycobacterium chelonae* infections associated with bee venom acupuncture. Clin Infect Dis. 2014;58(5):e110-3. DOI: 10.1093/cid/cit753
15. Yu JR, Heo ST, Lee KH, Kim J, Sung JK, Kim YR, et al. Skin and Soft Tissue Infection due to Rapidly Growing Mycobacteria: Case Series and Literature Review. Infect Chemother. 2013;45(1):85-93. DOI: 10.3947/ic.2013.45.1.85
16. CLSI. Susceptibility testing of Mycobacteria; Nocardiae and other Aerobic Actinomycetes; Approved Standard. Second ed.; 2011. Second edition. 2011. DOI: 10.1128/9781555816728
17. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med. 2007;175(4):367-416. DOI: 10.1164/rccm.200604-571ST
18. Esteban J, Fernandez Roblas R, Garcia Cia JI, Zamora N, Ortiz A. Clinical significance and epidemiology of non-pigmented rapidly growing mycobacteria in a university hospital. J Infect. 2007;54(2):135-45. DOI: 10.1016/j.jinf.2006.02.017
19. Esteban J, Garcia-Pedrazuela M, Munoz-Egea MC, Alcaide F. Current treatment of nontuberculous mycobacteriosis: an update. Expert Opin Pharmacother. 2012;13(7):967-86. DOI: 10.1517/14656566.2012.677824
20. Vaerewijck MJ, Huys G, Palomino JC, Swings J, Portaels F. Mycobacteria in drinking water distribution systems: ecology and significance for human health. FEMS Microbiol Rev. 2005;29(5):911-34. DOI:10.1016/j.femsre.2005.02.001
21. Brown-Elliott BA, Wallace RJ, Jr., Tichindelean C, Sarria JC, McNulty S, Vasireddy R, et al. Five-year outbreak of community- and hospital-acquired *Mycobacterium porcinum* infections related to public water supplies. J Clin Microbiol. 2011;49(12):4231-8. DOI: 10.1128/JCM.05122-11
22. Adekambi T. *Mycobacterium mucogenicum* group infections: a review. Clin Microbiol Infect. 2009;15(10):911-8. DOI: 10.1111/j.1469-0691.2009.03028.x
23. Nagao M, Sonobe M, Bando T, Saito T, Shirano M, Matsushima A, et al. Surgical site infection due to *Mycobacterium peregrinum*: a case report and literature review. Int J Infect Dis. 2009;13(2):209-11. DOI: 10.1016/j.ijid.2008.06.018
24. Neonakis IK, Gitti Z, Kontos F, Baritaki S, Petinaki E, Baritaki M, et al. *Mycobacterium arupense* pulmonary infection: antibiotic resistance and restriction fragment length polymorphism analysis. Indian J Med Microbiol. 2010;28(2):173-6. DOI: 10.4103/0255-0857.62502
25. Seidl A, Lindeque B. Large joint osteoarticular infection caused by *Mycobacterium arupense*. Orthopedics. 2014;37(9):e848-50. DOI: 10.3928/01477447-20140825-93
26. Do PC, Nussbaum E, Moua J, Chin T, Randhawa I. Clinical significance of respiratory isolates for *Mycobacterium abscessus complex* from pediatric patients. Pediatr Pulmonol. 2013;48(5):470-80. DOI: 10.1002/ppul.22638
27. Esther CR, Jr., Esserman DA, Gilligan P, Kerr A, Noone PG. Chronic *Mycobacterium abscessus* infection and lung function decline in cystic fibrosis. J Cyst Fibros. 2010;9(2):117-23. DOI: 10.1016/j.jcf.2009.12.001
28. Qvist T, Eickhardt S, Kragh KN, Andersen CB, Iversen M, Hoiby N, et al. Chronic pulmonary disease with *Mycobacterium abscessus complex* is a biofilm infection. Eur Respir J. 2015;46(6):1823-6. DOI: 10.1183/13993003.01102-2015
29. Lobo LJ, Chang LC, Esther CR, Jr., Gilligan PH, Tulu Z, Noone PG. Lung transplant outcomes in cystic fibrosis patients with pre-operative *Mycobacterium abscessus* respiratory infections. Clin Transplant. 2013;27(4):523-9. DOI: 10.1111/ctr.12140
30. Cong J, Wang C, Ma L, Zhang S, Wang J. Septicemia and pneumonia due to *Mycobacterium fortuitum* infection in a patient with extronodal NK/T-cell lymphoma, nasal type: A case report. Medicine (Baltimore). 2017;96(18):e6800. DOI: 10.1097/MD.0000000000006800
31. Okamori S, Asakura T, Nishimura T, Tamizu E, Ishii M, Yoshida M, et al. Natural history of *Mycobacterium fortuitum* pulmonary infection presenting with migratory infiltrates: a case report with microbiological analysis. BMC Infect Dis. 2018;18(1):1. DOI: 10.1186/s12879-017-2892-9
32. El Helou G, Hachem R, Viola GM, El Zakhem A, Chaftari AM, Jiang Y, et al. Management of rapidly growing mycobacterial bacteremia in cancer patients. Clin Infect Dis. 2013;56(6):843-6. DOI: 10.1093/cid/cis1032
33. Eid AJ, Berbari EF, Sia IG, Wengenack NL, Osmon DR, Razonable RR. Prosthetic joint infection due to rapidly growing mycobacteria: report of 8 cases and review of the literature. Clin Infect Dis. 2007;45(6):687-94. DOI:10.1086/520982
34. Celdran A, Esteban J, Manas J, Granizo JJ. Wound infections due to *Mycobacterium fortuitum* after polypropylene mesh inguinal hernia repair. J Hosp Infect. 2007;66(4):374-7. DOI: 10.1016/j.jhin.2007.05.006
35. Samimi DB, Bielory BP, Miller D, Johnson TE. Microbiologic trends and biofilm growth on explanted periorbital biomaterials: a 30-year review. Ophthal Plast Reconstr Surg. 2013;29(5):376-81. DOI: 10.1097/IOP.0b013e31829a7313
36. Gracia-Cazana T, Milagro A, Queipo F, Gilaberte Y. *Mycobacterium*

fortuitum infection after acupuncture treatment. Dermatol Online J. 2017;23(9). PMID: 29469732

37. Uzoigwe OF. Contaminated ink might be responsible for *Mycobacterium chelonae* infection after tattooing. BMJ. 2013;346:f122. DOI: 10.1136/bmj.f122
38. Ortiz-Perez A, Martin-de-Hijas N, Alonso-Rodriguez N, Molina-Manso D, Fernandez-Roblas R, Esteban J. Importance of antibiotic penetration in the antimicrobial resistance of biofilm formed by non-pigmented rapidly growing mycobacteria against amikacin, ciprofloxacin and clarithromycin. Enferm Infecc Microbiol Clin. 2011;29(2):79-84. DOI: 10.1016/j.eimc.2010.08.016
39. Wallace RJ, Jr., Meier A, Brown BA, Zhang Y, Sander P, Onyi GO, et al. Genetic basis for clarithromycin resistance among isolates of *Mycobacterium chelonae* and *Mycobacterium abscessus*. Antimicrob Agents Chemother. 1996;40(7):1676-81. PMID: 8807061

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Determination of a cutoff value for medication regimen complexity index to predict polypharmacy in HIV+ older patient

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ABSTRACT

Introduction. HIV+ patients have increased their life expectancy with a parallel increase in age-associated co-morbidities and pharmacotherapeutic complexity. The aim of this study was to determine an optimal cutoff value for Medication regimen complexity index (MRCI) to predict polypharmacy in HIV+ older patients

Patients and methods. A transversal observational single cohort study was conducted at a tertiary Hospital in Spain, between January 1st up to December 31st, 2014. Patients included were HIV patients over 50 years of age on active antiretroviral treatment. Prevalence of polypharmacy and it pattern were analyzed. The pharmacotherapy complexity value was calculated through the MRCI. Receiver operating characteristic curve analyses were used to calculate the area under the curve (AUC) for the MRCI value medications to determine the best cutoff value for identifying outcomes including polypharmacy. Sensitivity and specificity were also calculated.

Results. A total of 223 patients were included. A 56.1% of patients had polypharmacy, being extreme polypharmacy in 9.4% of cases. Regarding the pattern of polypharmacy, 78.0% had a cardio-metabolic pattern, 12.0% depressive-psychogeriatric, 8.0% mixed and 2.0% mechanical-thyroidal. The ROC curve demonstrated that a value of medication complexity index of 11.25 point was the best cutoff for predict polypharmacy (AUC=0.931; sensitivity= 77.6%; specificity= 91.8%).

Conclusions. A cut-off value of 11.25 for MRCI is proposed to determine if a patient reaches the criterion of polypharmacy. In conclusion, the concept of polypharmacy should include not only the number of prescribed drugs but also the complexity of them.

Keywords: HIV, polypharmacy, pharmacotherapy complexity, aging

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Determinación del valor umbral del índice de complejidad de la farmacoterapia para predecir polifarmacia en pacientes VIH+

RESUMEN

Introducción. La esperanza de vida de los pacientes VIH+ se ha incrementado. De forma paralela han aumentado las comorbilidades asociadas a la edad y la complejidad farmacoterapéutica. El objetivo del estudio es estimar el valor umbral del índice de complejidad de la farmacoterapia (MRCI) para la determinación del criterio de polifarmacia en pacientes VIH+ mayores de 50 años.

Métodos. Estudio observacional, trasversal, unicéntrico. Se incluyeron todos los pacientes VIH+ mayores de 50 años, en tratamiento antirretroviral activo entre el 1 enero y 31 diciembre-2015. Se determinó la presencia de polifarmacia y los patrones asociados. La complejidad del tratamiento se calculó con la herramienta MRCI (Universidad de Colorado). Se analizó el índice de complejidad total como marcador cuantitativo de polifarmacia mediante la realización de una curva ROC y el cálculo de su área bajo la curva. Se calculó la sensibilidad y la especificidad de la misma.

Resultados. Se incluyeron 223 pacientes. El 56,1% presentó polifarmacia, siendo extrema en el 9,4% de los casos. En relación con el patrón de polifarmacia, el 78,0% presentaron un patrón cardio-metabólico, el 12,0% psico geriátrico-depresivo, el 8,0% mixto y el 2,0% mecánico tiroideo .Se determinó un valor de área bajo la curva ROC de 0,931 con límites entre (0,901-0,962) y p< 0,001. El valor 11,25 de índice

de complejidad total de la farmacoterapia proporcionó un valor de especificidad del 92% y una sensibilidad del 78%.

Conclusión. El valor de 11,25 de índice de complejidad es un buen indicador para conocer los pacientes con polifarmacia. El concepto de polifarmacia no solo debe incluir el número de fármacos que toma el paciente sino incluir también la complejidad del tratamiento.

Palabra clave: VIH, polifarmacia, complejidad farmacoterapéutica, envejecimiento

INTRODUCTION

Due to the introduction of high activity antiretroviral therapy, nowadays HIV-infected individuals live longer. It is estimated that by 2030 nearly three-quarters of people living with HIV will be 50 years or older [1]. Age-related conditions such as cardiovascular disease, kidney disease, and non-AIDS-defining cancers are likely to continue to increase among HIV-infected patients as their median age also increases [2]. As a result, up to two-thirds of these patients take concomitant medication to mitigate antiretroviral treatment (ART) side effects and/or to treat comorbid conditions [3–6]. People living with HIV (PLWH) often exhibits a higher number of concomitant medication than in the general population. This increase in drugs number has been associated with older age, female gender, obesity, and hepatitis B/C co-infection [4–6]. In addition, HIV-infected individuals may be more vulnerable to age-related conditions [7]. This high prevalence of comorbidities has only exacerbated the polypharmacy problem, which has recently become a clinical concern among providers caring for HIV-infected patients [3–5].

There are different definitions of polypharmacy. In numerical terms, it is most commonly defined as at least five or more prescription drugs, which is also associated to worse health outcomes in older patients such as increased risk for morbidity, non-adherence, drug interactions, and side effects. All these disadvantages have been shown to be more prevalent in PLWH than in the rest of the population [4, 6, 8–11]. In addition, Smit et al. observed an increasing burden of polypharmacy and age-related non-communicable diseases that could cause an increase in complications with first-line antiretroviral treatment [12].

According to the most recent recommendations in our context, the most appropriate number to define polypharmacy is six drugs [13]. However, no definition has included the impact of number of drugs, pill burden, complexity in taking drugs or other important factors including in the MRCI, in elderly patients, both HIV and non-HIV. Moreover, evidence shows that polypharmacy increases with age but is likely under-estimated given that most studies in HIV-infected adults only account for prescribed medicines [4, 10, 12]. Polypharmacy in HIV-infected has a major impact on ART adherence and adverse drug reactions leading to hospitalization [9, 14].

Polypharmacy should be considered the next challenge in clinical follow-up of HIV-infected patients [4, 12]. Strategies to decrease polypharmacy in complex patients with multiple comorbidities should prioritize decreasing the daily pill burden, the risk of toxicity and the drug-drug interactions [15]. New strategies have been developed alongside conventional triple combination ART administered as multi-tablet regimens. They include use of co-formulated, fixed-dose single-tablet regimens (STR) administered once daily, as well as non-preferential less-drug regimens, which reduce the number of compounds administered to either monotherapy or dual combination therapy.

Another critical but less known factor is pharmacotherapy complexity (PC). Martin et al. developed a method for quantifying antiretroviral regimen complexity for HIV patients [16]. This method was the first step toward obtaining a better understanding of the impact of complex ART regimens on adherence and clinical outcomes. Previously, George et al. developed a medication regimen complexity index (MRCI) to estimate complexity of all drugs taken by a patient [17]. This tool has been used in many chronic diseases and most studies showed that an increased regimen complexity is associated with poor clinical outcomes and reduces medication in the general population [18, 19].

There are not published studies addressing the relationship between medication regimen complexity index and polypharmacy.

The aim of this study was to determine an optimal cutoff value for MRCI to predict polypharmacy and to redefine the concept of polypharmacy in older PLWH.

PATIENTS AND METHODS

A cross-sectional, observational single cohort study was conducted at a tertiary Hospital in Spain, from 1 January 2014 to 31 December 2014. Patients enrolled in the study met the following inclusion criteria: HIV patients over 50 years of age on active ART drugs. Participants were given written information about the study and its objectives, and those who agreed to participate provided their written informed consent. Patients participating in another clinical trial or who did not sign the informed consent were excluded.

Data collected from the electronic medical record included demographic data (sex and age) and HIV transmission mode; clinical endpoints: plasma viral load (copies/milliliter [mL]) and CD4+ T-cell count (cells/microliter) and comorbidity related variables (number and type of comorbidities). Pharmacotherapy variables included ART regimen, single treatment regimen (STR) and concomitant medications. ART adherence was measured using the SMAQ questionnaire [20] and hospital dispensing records. A PLWH was considered adherent to antiretroviral treatment if according to hospital pharmacy records adherence was >95% and was not positive in the SMAQ, where positive means that there was a positive response to any of the qualitative questions of the SMAQ, more than two doses missed over the past week, or over 2 days of total non-medication during the past 6 months.

Adherence to concomitant medication was measured using the Morisky-Green questionnaire [21] and electronic pharmacy dispensing records. A PLWH was considered adherent to concomitant medication if according to electronic pharmacy dispensing records adherence was >90% and the Morisky-Green questionnaire scored 4.

To calculate the dispensing record, use the Medication Possession Ratio (MPR), as it measures the percentage of time a patient has access to medication. The formulae to calculate MPR is: MPR Number of days ARV prescribed or dispensed/ number of days in the interval. The follow-up time to the chronic medication adherence was 24 weeks.

The independent variable was polypharmacy, defined as treatment with six or more drugs (including antiretroviral therapy). Major polypharmacy (more than 11 drugs) and excessive polypharmacy (more than 21 drugs) were also considered [13].

Polypharmacy pattern was analyzed according to the Calderón Larrañaga et al study [22], with a non-random association in drug prescription resulting in polypharmacy patterns. Three patterns were applied based on age of participants: cardiovascular, depression-anxiety, and chronic obstructive pulmonary (COPD) disease patterns, with a different prevalence between men and women. A patient was classified into a pattern when at least three drugs of the treatment were in the same pattern. To calculate the corresponding polypharmacy patterns of each patient, drugs were classified according to the Anatomical Therapeutic Chemical Classification System (ATC) using only the first three levels of the classification.

Multimorbidity patterns was analyzed according to the Prados-Torres et al study. Chronic diseases resulted in three multimorbidity patterns: cardiometabolic, depressive-psychogeriatric, and mechanical-thyroidal [23]. Patients were classified into a type if they had two diseases included in a pattern. ART drugs were obtained from a pharmacy-dispensing outpatient program (Dominion-Farmatools©). Non-ART drugs prescribed were provided by an electronic health prescription program of the Andalusian Public Health System. The remaining endpoints were obtained from laboratory tests, microbiology reports, and from the review of the medical history of each patient.

Finally, the pharmacotherapy complexity value was calculated through the MRCI [18]. This validated tool includes 65 items divided into three subgroups: dose forms, dosing frequencies, and additional instructions relevant to drug administration. The calculated value was obtained through the web tool of Colorado University available at http://www.ucdenver.edu/academics/colleges/pharmacy/Research/researchareas/Pages/MRCTool.aspx) [24].

Statistical analyses. Quantitative variables were given as mean and standard deviation or as median and interquartile range (IQR) in case of a skewed distribution. Qualitative variables were given as percentages (%).

Receiver operating characteristic (ROC) curve analyses were used to calculate the area under the curve (AUC) for the MRCI value medications to determine the best cutoff value for identifying outcomes including polypharmacy. The AUC describes the test's overall performance and it can be used to compare different tests. An AUC of 1 indicates perfect discrimination, whereas an AUC of 0.5 indicates discrimination no better than chance. Sensitivity and specificity were calculated. The optimal cutoff point was obtained by using the Youden Index (i.e., sensitivity þ specificity-1), without adjusting for covariates. The Youden Index, a common summary measure of the ROC curve, represents the maximum potential effectiveness of a marker [25]. Logistic regression analysis was performed, and area under ROC curve was calculated for the association of the number of concomitant medications with each of the outcomes. Data are presented as odds ratios with 95% confidence intervals. All models were adjusted for potential covariates including age and medical conditions.

Statistical significance was set at less than 0.05. Data were analyzed using IBM SPSS Statistics version 22.0 software.

Ethics approval. The study was approved by the institutional ethics committee of the South Seville area (registration number RAM-VIH-2015-02). Participants were given written information about the study and its objectives, and those who agreed to take part provided their written informed consent.

RESULTS

The sample consisted of 223 patients with a median age of 53.0 years (IQR: 52.0-57.0), 86.5% males, were enrolled into the study. Baseline characteristics of patients are shown in table 1. ART regimens consisted of two nucleoside reverse transcriptase inhibitors (NRTI) plus a non-nucleoside reverse transcriptase inhibitor (NNRTI) in 37.2% of patients; two NRTIs plus a boosted protease inhibitor in 18.8%; two NRTIs plus an integrase strand transfer inhibitor (INSTI) in 12.6%, and other combinations in 31.4% of patients (20% monotherapy and 40% dual antiretroviral therapy). A majority (52%) of patients started antiretroviral therapy before 2002, 14.8% had been on three or more ART regimens and 25.3% had been on STR.

Median number of concomitant drugs prescribed per patient were 3.0 (1.0-5.0). The median was the most frequently prescribed therapeutic drug classes were as follows: psychotropic drugs (35.9%), lipid lowering drugs (29.1%), cardiovascular agents (29.1%), drugs used for treatment for gastroesophageal reflux disease (26.9%), and blood glucose-lowering drugs (11.7%).

The median of comorbidities per patient was 3.0 (IQR: 2.0-4.0). Viral liver diseases were diagnosed in 67.3% of patients, cardiovascular diseases or high blood pressure in 25.0%, and central nervous system diseases in 20.5% of patients. Of the 126 patients who were calculated the multimorbidity pattern, 73.8% were cardiometabolic, 12.7% were mixed, 11.6% were depressive-psychogeriatric and 1.6% mechanical-thyroidal.

Table 1 Baseline demographic, clinic, related to lifestyle and adherence characteristics of the patients.

Characteristics (n=223 patients)		
Demographic Parameters		
Gender (male); n (%)		153 (86.5)
Age (years); (median + IQR)		53.0 (52.0-57.0)
HIV risk factor; n (%)	IDU	121 (54.3)
	Sexual	68 (30.5)
	Unknown	34 (15.2)
Clinic Parameters		
Undetectable Plasmatic Viral Load (<50 cop/mL); n (%)		184 (84.4)
CD4 Levels; n (%)	<200 cells/µL	22 (10)
	≥200 cells/µL	198 (90)
Adherence		
Antiretroviral treatment; n (%)		186 (83.6)
Concomitant drugs; n (%)		84 (37.9)

IQR: interquartile range; IDU: injection drug users.

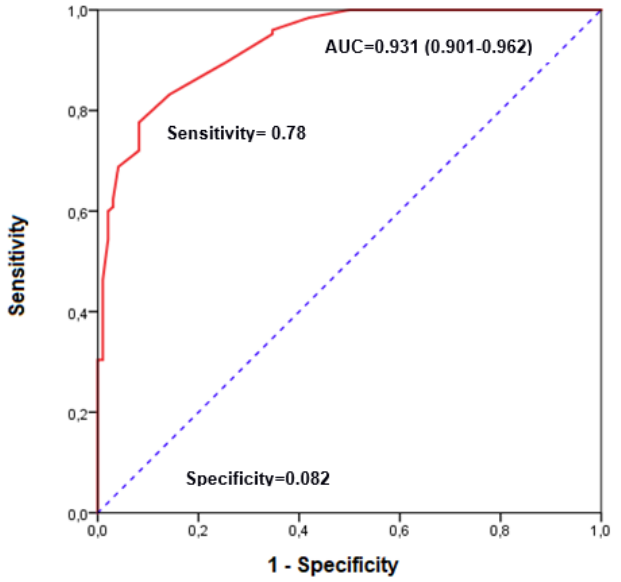

Figure 1 Receiver operating characteristic curves for polypharmacy in relation to the Medication Regimen Complexity Index value.

As regards the main variables, 56.1% of patients had polypharmacy, higher polypharmacy in 9.4% of cases and no patient had excessive polypharmacy. Of the 70 patients who were calculated the polypharmacy pattern, 60.0% were cardiovascular, 27.1% were depression-anxiety, 7.1 were mixed and 5.8 % were COPD.

Presence of polypharmacy was associated to higher PC values. Patients with high PC indices had a 50 times higher chance (p = 0.0001) of polypharmacy than those with low PC values. The PC index significantly correlated with the three index rating sections.

The ROC curve was constructed, and this demonstrated that a value of medication complexity index of 11.25 point was the best cutoff for predict polypharmacy in older HIV-infected patients (area under curve = 0.931; sensitivity of 77.6 % and specificity of 91.8%) (figure 1).

DISCUSSION

We propose a redefinition of the concept of polypharmacy, including not only the quantitative aspect of the number of prescribed drugs but, the medication regimen complexity. A cut-off value of 11.25 for MRCI is proposed to determine if a patient reaches the criterion of polypharmacy.

The cutoff established showed high AUC and specificity and moderate sensitivity which helps identifying more efficiently the presence of polypharmacy in older HIV-infected patients.

According to data published by others author [5, 10–12], our study demonstrates that a half of HIV+ elderly patients currently have polypharmacy. It is particularly important the number of patients who have higher polypharmacy, in our study 9.4%. Regarding the concept of polypharmacy, available literature points to different definitions. Although five medications have been generally a well-accepted criterion, according to most recent recommendations, we suggest six medications [13].

McNicholl et al. suggested that in patients 50 years and older, targeting individuals with 11 or more chronic medications would have the highest yield and greatest impact [26]. Sutton et al. in a retrospective HIV+ cohort studied a different concept, the pill burden. It was associated with poor level of adherence and risk of hospitalization but not included a proposal to analyse higher-risk patient based on this concept [27].

Given the increase in the number of patients older than 50 years expected in the coming years, as well as the increase in the number of patients with polypharmacy, our study indicates polypharmacy should be defined by the number and complexity of prescribed medication.

Additionally, the main contributing factor for a higher MRCI was concomitant medication. These results are in line with data published by Metz et al. [28]. This issue confirms the validity of the proposed cutoff.

Designing ART regimens that do not interact with other chronic medications or exacerbate comorbidities can be

challenging, especially in heavily pretreated patients whose ART options are limited. A recent retrospective study found an association between polypharmacy, and a lower likelihood of using singled-tablet-regimen (STR) [15]. Currently available STR´s may be limited in this aging population by complex drug drug and drug disease interactions, and the desire to make treatment regimens more flexible but other strategies as Less-Drug-Regimen (LDR), including mono or bi-therapies, are becoming more frequent in this type of patients. Manzano et al. [29] demonstrated that the complexity of ART is being reduced mainly by new treatment strategies and the increasing appearance of pharmaceutical coformulations.

In addition, our results indicate that using MRCI scores adds information, particularly for concomitant drug prescribed, extending beyond a simple pill count or pill burden concept. More efforts should optimize to simplify concomitant medication

In this context, according to international guidelines, it will be possible that HIV specialty pharmacists may assist prescribers in reducing polypharmacy and identifying inappropriate prescribing, using Beers or STOPP-START tools [30].

A systematic review in non-HIV-infected patients has showed that although there was heterogeneity regarding the degree of association between complexity and adherence, most studies concluded that an increased regimen complexity reduces medication adherence [19]. In our study, the overall adherence calculated was high for ART, but particularly low for concomitant medication. T-+his suggests a prioritization of patients' medication intake, derived from the patients' beliefs and perceptions regarding medications [31]. These results suggested that, in older HIV patients, it is recommended that all prescribed medication be checked at least every six months in individuals who have more than four medications, and at least once a year for the rest [13]. According to guidelines, it is recommended to carry out a review of the prescribed pharmacotherapy in a systematized way and through a sequential and structured methodology [13]. Additionally, is necessary to go beyond the virologic suppression and ensure adequate control of comorbidities, among other things, improving adherence. Corless et al. raises the prospect that aid providers by guiding their motivational interview to those questions most closely associated with adherence, particularly knowing factors about self-efficacy, depression, stressful life events, and stigma. It is necessary that HIV+ knows pharmacotherapy objectives, not only ART, to be more implicated with this medication [32].

A common limitation of other published studies is that they only include data on medications of official medical prescriptions; they do not include private health system treatments or alternative medicines. However, this is not seen as a very significant limitation in our study; given the universal coverage of the public health system in Spain, with a small number of patients using alternative medications. However, the MRCI is an imperfect tool and faces tradeoffs between sensitivity and specificity, as most clinical measures do. Despite a long list of possible dose formulations, frequencies, and directions, some options are missed, such as once monthly and also missing details, such as how to code 2 once-daily medications that cannot be taken simultaneously. There exist other possibilities to analyse the PC as the antiretroviral regimen complexity Index (ARCI), which had many more such details. As the average complexity score is not significantly different from the MRCI for ART regimens we prefer to choose MRCI for being more studied in the literature, in the recent years [33–35].

Although a finding of multiple medication uses or polypharmacy, defined by a certain cutoff number may be a useful indication for a medication review in older adults, it may not be clinically useful when being associated with adverse outcomes. Instead, exposure to specific pharmacological drug classes, total medication exposure, drug-drug interactions, and medication adherence are important factors that may be used to be considered when evaluating individual's risk for developing adverse outcomes.

Given the characteristics of our population and the pattern of polypharmacy and multimorbidity, coinciding with other published cohorts, our cutoff point offers strength, since it is based on the type of medication commonly used in this type of patient [5, 8, 10, 11, 15, 36].

Our study was not performed to use number of medications to claim that polypharmacy causes different adverse outcomes nor clinical impairment, but simply to determine an optimal discriminating number of medications for polypharmacy. Other important issues as geriatric syndromes, functional outcomes, and mortality in HIV+ older population must be studied, including an analysis using frailty, disability and mortality variables respectively.

It is known that multi-morbidity contributes to further vulnerability and complexity in clinical management in the contemporary ART age. The interest in methods to identify individuals at risk of multi-morbidity is strongest. The concept of frailty must be included routinely in HIV+ older patient because may be useful in discriminating whether it is the morbidities themselves or the toxicity of prescribed treatments that contributes more to adverse outcomes [26].

Given the changing face of the HIV epidemic, providers will be increasingly challenged to effectively manage older, HIV-infected patients with multi-morbidity, polypharmacy and high-level of PC. It is important to increase our knowledge of polypharmacy among the increasing older HIV-infected population in order to be able to develop prevention strategies for the problems inherent in old age and multiple treatments. According with the literature polypharmacy in HIV+ older patient will increase in the coming years [12]. This fact and the progressive physiological deterioration of patients will make it increasingly common and necessary to use not only the classic assessment of actual or potential drug interactions but also other terminology about the use of drugs employed in other types of chronic patients, such as potentially inappropriate medication, cholinergic risk or deprescribing [10–12, 26, 35].

Future researches efforts will focus on include risk assessment, such as that offered by the MRCI Index in our study, to inform the prioritization of medications according to their risks and benefits for each patient. In addition, efforts to promote public health and multidisciplinary initiatives, behavioral changes, and prevention aimed at reducing polypharmacy and MRCI should be investigated.

Additional studies are needed to establish its power and for revealing possible opportunities for clinical intervention to reduce MRCI as a risk factor for nonadherence and its consequence in the use of health resources, including hospitalizations, are proposed.

In conclusion, the concept of polypharmacy should include not only the number of prescribed drugs but also the complexity of them. The findings of this study provide evidence and clarification of the best cutoff value for the MRCI that should be used to identify HIV+ older patient at possible risk of polypharmacy.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest

REFERENCES

1. Tseng A, Szadkowski L, Walmsley S, Salit I, Raboud J. Association of Age With Polypharmacy and Risk of Drug Interactions With Antiretroviral Medications in HIV-Positive Patients. Ann Pharmacother. 2013;47:1429–39. doi:10.1177/1060028013504075.
2. High KP, Brennan-Ing M, Clifford DB, Cohen MH, Currier J, Deeks SG, et al. HIV and Aging. JAIDS J Acquir Immune Defic Syndr. 2012;60:S1–18. doi:10.1097/QAI.0b013e31825a3668.
3. van Sighem AI, Gras LAJ, Reiss P, Brinkman K, de Wolf F, ATHENA national observational cohort study. Life expectancy of recently diagnosed asymptomatic HIV-infected patients approaches that of uninfected individuals. AIDS. 2010;24:1527–35. doi:10.1097/QAD.0b013e32833a3946.
4. Edelman EJ, Gordon KS, Glover J, McNicholl IR, Fiellin DA, Justice AC. The next therapeutic challenge in HIV: polypharmacy. Drugs Aging. 2013;30:613–28. doi:10.1007/s40266-013-0093-9.
5. Gimeno-Gracia M, Crusells-Canales MJ, Javier Armesto-Gómez F, Rabanaque-Hernández MJ. Prevalence of concomitant medications in older HIV+ patients and comparison with general population. HIV Clin Trials. 2015;16:117–24. doi:10.1179/1528433614Z.0000000012.
6. Marzolini C, Elzi L, Gibbons S, Weber R, Fux C, Furrer H, et al. Prevalence of comedications and effect of potential drug-drug interactions in the Swiss HIV Cohort Study. Antivir Ther. 2010;15:413–23. doi:10.3851/IMP1540.
7. Negredo E, Back D, Blanco J-R, Blanco J, Erlandson KM, Garolera M, et al. Aging in HIV-Infected Subjects: A New Scenario and a New View. Biomed Res Int. 2017;2017:5897298. doi:10.1155/2017/5897298.
8. Gnjidic D, Hilmer SN, Blyth FM, Naganathan V, Waite L, Seibel MJ, et al. Polypharmacy cutoff and outcomes: five or more medicines were used to identify community-dwelling older men at risk of different adverse outcomes. J Clin Epidemiol. 2012;65:989–95. doi:10.1016/j.jclinepi.2012.02.018.
9. Marcum ZA, Pugh MJ V., Amuan ME, Aspinall SL, Handler SM, Ruby CM, et al. Prevalence of Potentially Preventable Unplanned Hospitalizations Caused by Therapeutic Failures and Adverse Drug Withdrawal Events Among Older Veterans. Journals Gerontol Ser A Biol Sci Med Sci. 2012;67:867–74. doi:10.1093/gerona/gls001.
10. Moore HN, Mao L, Oramasionwu CU. Factors associated with polypharmacy and the prescription of multiple medications among persons living with HIV (PLWH) compared to non-PLWH. AIDS Care. 2015;27:1443–8. doi:10.1080/09540121.2015.1109583.
11. Gimeno-Gracia M, Crusells-Canales MJ, Armesto-Gomez FJ, Compaired-Turlan V, Rabanaque-Hernandez MJ. Polypharmacy in older adults with human immunodeficiency virus infection compared with the general population. Clin Interv Aging. 2016;Volume 11:1149–57. doi:10.2147/CIA.S108072.
12. Smith JM, Flexner C. The challenge of polypharmacy in an aging population and implications for future antiretroviral therapy development. AIDS. 2017;31 Suppl 2:S173–84. doi:10.1097/QAD.0000000000001401.
13. Grupo de expertos de la Secretaría del Plan Nacional sobre el SIDA (SPNS), Sociedad Española de Geriatría y Gerontología (SEGG). Documento de consenso sobre edad avanzada e infección por el virus de la inmunodeficiencia humana. 2015; Noviembre.
14. Cantudo-Cuenca MR, Jiménez-Galán R, Almeida-González C V., Morillo-Verdugo R. Concurrent Use of Comedications Reduces Adherence to Antiretroviral Therapy Among HIV-Infected Patients. J Manag Care Pharm. 2014;20:844–50. doi:10.18553/jmcp.2014.20.8.844.
15. Guaraldi G, Menozzi M, Zona S, Calcagno A, Silva AR, Santoro A, et al. Impact of polypharmacy on antiretroviral prescription in people living with HIV. J Antimicrob Chemother. 2017;72:511–4. doi:10.1093/jac/dkw437.
16. Martin S, Wolters PL, Calabrese SK, Toledo-Tamula MA, Wood L V, Roby G, et al. The Antiretroviral Regimen Complexity Index. A novel method of quantifying regimen

complexity. J Acquir Immune Defic Syndr. 2007;45:535–44. doi:10.1097/QAI.0b013e31811ed1f1.

17. George J, Phun Y-T, Bailey MJ, Kong DC, Stewart K. Development and Validation of the Medication Regimen Complexity Index. Ann Pharmacother. 2004;38:1369–76. doi:10.1345/aph.1D479.
18. Libby AM, Fish DN, Hosokawa PW, Linnebur SA, Metz KR, Nair K V, et al. Patient-level medication regimen complexity across populations with chronic disease. Clin Ther. 2013;35:385-398.e1. doi:10.1016/j.clinthera.2013.02.019.
19. Pantuzza LL, Ceccato M das GB, Silveira MR, Junqueira LMR, Reis AMM. Association between medication regimen complexity and pharmacotherapy adherence: a systematic review. Eur J Clin Pharmacol. 2017;73:1475–89. doi:10.1007/s00228-017-2315-2.
20. Knobel H, Alonso J, Casado JL, Collazos J, González J, Ruiz I, et al. Validation of a simplified medication adherence questionnaire in a large cohort of HIV-infected patients: the GEEMA Study. AIDS. 2002;16:605–13. PMID: 11873004.
21. Morisky DE, Green LW, Levine DM. Concurrent and predictive validity of a self-reported measure of medication adherence. Med Care. 1986;24:67–74. PMID: 3945130.
22. Calderón-Larrañaga A, Gimeno-Feliu LA, González-Rubio F, Poblador-Plou B, Lairla-San José M, Abad-Díez JM, et al. Polypharmacy patterns: unravelling systematic associations between prescribed medications. PLoS One. 2013;8:e84967. doi:10.1371/journal.pone.0084967.
23. Prados-Torres A, Poblador-Plou B, Calderón-Larrañaga A, Gimeno-Feliu LA, González-Rubio F, Poncel-Falcó A, et al. Multimorbidity Patterns in Primary Care: Interactions among Chronic Diseases Using Factor Analysis. PLoS One. 2012;7:e32190. doi:10.1371/journal.pone.0032190.
24. Universidad de Colorado. Electronic Data Capture and Coding Tool for Medication Regimen Complexity. http://www.ucdenver.edu/academics/colleges/pharmacy/Research/researchareas/Pages/MRCTool.aspx.
25. Ruopp MD, Perkins NJ, Whitcomb BW, Schisterman EF. Youden Index and optimal cut-point estimated from observations affected by a lower limit of detection. Biom J. 2008;50:419–30. doi:10.1002/bimj.200710415.
26. McNicholl IR, Gandhi M, Hare CB, Greene M, Pierluissi E. A Pharmacist-Led Program to Evaluate and Reduce Polypharmacy and Potentially Inappropriate Prescribing in Older HIV-Positive Patients. Pharmacotherapy. 2017;37:1498–506. doi:10.1002/phar.2043.
27. Scott Sutton S, Magagnoli J, Hardin JW. Impact of Pill Burden on Adherence, Risk of Hospitalization, and Viral Suppression in Patients with HIV Infection and AIDS Receiving Antiretroviral Therapy. Pharmacotherapy. 2016;36:385–401. doi:10.1002/phar.1728.
28. Metz KR, Fish DN, Hosokawa PW, Hirsch JD, Libby AM. Patient-Level Medication Regimen Complexity in Patients With HIV. Ann Pharmacother. 2014;48:1129–37. doi:10.1177/1060028014539642.
29. Manzano-García M, Robustillo-Cortés A, Almeida-González C V, Morillo-Verdugo R. [Evolution of the Complexity Index of the antiretroviral therapy in HIV+ patients in a real life clinical practice]. Rev Esp Quimioter. 2017;30:429–35. PMID: 29115368.
30. Schafer JJ, Gill TK, Sherman EM, McNicholl IR. ASHP Guidelines on Pharmacist Involvement in HIV Care. Am J Health Syst Pharm. 2016;73:468–94. doi:10.2146/ajhp150623.
31. Haro Márquez C, Cantudo Cuenca MR, Almeida González CV, Morillo Verdugo R. [Patients'adherence-related beliefs about medicines prescribed for long-term conditions in HIV patients]. Farm Hosp. 2015;39:23–8. doi:10.7399/fh.2015.39.1.8127.
32. Corless IB, Hoyt AJ, Tyer-Viola L, Sefcik E, Kemppainen J, Holzemer WL, et al. 90-90-90-Plus: Maintaining Adherence to Antiretroviral Therapies. AIDS Patient Care STDS. 2017;31:227–36. doi:10.1089/apc.2017.0009.
33. Hirsch JD, Metz KR, Hosokawa PW, Libby AM. Validation of a patient-level medication regimen complexity index as a possible tool to identify patients for medication therapy management intervention. Pharmacotherapy. 2014;34:826–35. doi:10.1002/phar.1452.
34. Wimmer BC, Cross AJ, Jokanovic N, Wiese MD, George J, Johnell K, et al. Clinical Outcomes Associated with Medication Regimen Complexity in Older People: A Systematic Review. J Am Geriatr Soc. 2017;65:747–53. doi:10.1111/jgs.14682.
35. Nozza S, Malagoli A, Maia L, Calcagno A, Focà E, De Socio G, et al. Antiretroviral therapy in geriatric HIV patients: the GEPPO cohort study. J Antimicrob Chemother. 2017. doi:10.1093/jac/dkx169.
36. Guaraldi G, Brothers TD, Zona S, Stentarelli C, Carli F, Malagoli A, et al. A frailty index predicts survival and incident multimorbidity independent of markers of HIV disease severity. AIDS. 2015;29:1633–41. doi:10.1097/QAD.0000000000000753.

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Brief report

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In vitro study of synergy of ampicillin with ceftriaxone against *Listeria monocytogenes*

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ABSTRACT

Objectives. To evaluate if the *in vitro* activity of ampicillin increases when combined with ceftriaxone.

Material and methods. The activity of ampicillin and ceftriaxone was evaluated against six *Listeria monocytogenes* invasive clinical isolates. Ampicillin and ceftriaxone MICs were determined by the broth microdilution method. Synergy was evaluated by checkerboard and time-kill curves methods.

Results. All six *L. monocytogenes* strains were susceptible to ampicillin (MICs 0.25-0.5 mg/L). A bacteriostatic synergy was demonstrated by the FIC index of 0.5 and a 2.5 $\log_{10}$ CFU reduction on the six strains studied for MIC ampicillin plus 16 mg/L ceftriaxone concentrations.

Conclusions. The association of ceftriaxone with ampicillin increases the *in vitro* activity of ampicillin, and therefore could be a valuable option in the treatment of invasive infection by *L. monocytogenes*.

Keywords: ceftriaxone, ampicillin, synergy, Listeria, CNS.

Estudio *in vitro* de la sinergia de ampicilina con ceftriaxona frente a *Listeria monocytogenes*

RESUMEN

Objetivo. Evaluar si la actividad *in vitro* de ampicilina aumenta cuando se combina con ceftriaxona.

Material y métodos. La actividad de la ampicilina y la ceftriaxona se evaluó frente a seis aislados clínicos invasivos de *Listeria monocytogenes*. La CMI de ampicilina y ceftriaxona se determinaron mediante el método de microdilución en caldo. La sinergia se evaluó mediante un ensayo en damero y el método de curvas de tiempo-muerte.

Resultados. Las seis cepas de *L. monocytogenes* fueron sensibles a ampicilina (CMI 0,25-0,5 mg/L). Se demostró una sinergia bacteriostática mediante un índice FIC de 0,5 y una reducción de 2,5 $\log_{10}$ UFC para concentraciones CMI de ampicilina más 16 mg/L de ceftriaxona en las seis cepas estudiadas.

Conclusiones. La asociación de ceftriaxona con ampicilina aumenta la actividad in vitro de ampicilina y, por lo tanto, podría ser una opción valiosa en el tratamiento de la infección invasiva por *L. monocytogenes*.

Palabras clave: ceftriaxona, ampicilina, sinergia, Listeria, SNC.

INTRODUCTION

Invasive infection by *Listeria monocytogenes* presents a high mortality [1], which could be attributed to that the disease usually affects patients who present malignancies or immunosuppressive comorbidities [2, 3], together with that the penicillins have no bactericidal activity against *L. monocytogenes* [4, 5]. Based on the above, the enhancement of the bactericidal effect of ampicillin could play an important role in the success of the antimicrobial treatment, mainly when the infection affects the Central Nervous System (CNS), where ampicillin levels can be very variable and could be close to peri-MIC values along the dose interval [6, 7].

Recent studies have shown the effectiveness of ampicillin-ceftriaxone combination for the treatment of endocarditis due to *Enterococcus faecalis* [8]. *L. monocytogenes* and *E. faecalis* share some characteristics regarding their antibiotic susceptibility, such as the activity of ampicillin is bacteriostatic, and they are both resistant to ceftriaxone. The previous antibiotic combination could be also effective against *L. monocytogenes* improving the bactericidal activity of ampicillin.

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The main objective of this study is to explore the possibility that ampicillin in combination with ceftriaxone could increase its bactericidal activity, which could bring advantages in the treatment of invasive diseases, mainly at the CNS level.

MATERIAL AND METHODS

Bacterial isolates. The activity of ampicillin and ceftriaxone was evaluated against six *L. monocytogenes* invasive clinical isolates belonging to different PFGE types and serotypes: 2 isolates 1/2a (LMP62 y LMP52), 2 isolates 1/2b (LMP22 y LMP42), and 2 isolates 4b (LMP43 y LMP36), which were isolated from CSF samples.

Antimicrobial susceptibility testing. MICs of ampicillin and ceftriaxone were determined by the broth microdilution method in cation-adjusted Mueller Hinton broth with 5% lysed horse blood (CAMHB-LHB) based on The Clinical & Laboratory Standards Institute (CLSI) criteria [9].

Synergy studies. Static synergy was evaluated by the checkerboard assay in CAMHB-LHB in accordance to the American Society for Microbiology recommendations [10]. The assays were performed in duplicate on all 6 strains, twofold serial dilutions of ampicillin (0,015-8 mg/L) and ceftriaxone (0,25-128 mg/L) were individually tested and in all possible combinations of drug concentrations. Checkerboard synergy and non-synergy were defined by the fractional inhibitory concentration index (FICI): FICI ≤0.5 defined as synergy and FICI >0.5 as non-synergy (>0.5 to ≤1: additive and >1 to ≤4: indifference) [11].

The dynamic synergy was studied by time-killing curves in CAMHB-LHB according to CLSI methodology [12]. The assays were performed in duplicate on all 6 strains in the presence of ampicillin and ceftriaxone concentrations, alone and in combination, previously identified as synergistic by the checkerboard assay. Bacterial counts were determined in duplicate at 3, 6 and 24 hours of incubation. Synergy was defined as a 2-$\log_{10}$ decrease in the colony count at 24h with the combination compared to that of the most active single agent [13].

Killing-curves were modeled and studied by GraphPad-Prism 5.0 software (© 2014 GraphPad Software. Inc), using mean (+/- S.D.) values.

RESULTS

All six *L. monocytogenes* strains were susceptible to ampicillin with MICs values ranging from 0.25-0.5 mg/L and the ceftriaxone MIC value was 64 mg/L in all three strains tested (table 1).

A bacteriostatic synergy effect of ampicillin association with ceftriaxone was demonstrated by checkerboard and time-killing curves methods.

-Checkerboard assay: a bacteriostatic synergy was observed with FIC index values of 0.49, was observed when the MIC concentrations of ampicillin are combined with a concentration of 16 mg/L of ceftriaxone. For lower ceftriaxone concentrations, the effect was additive (4-8 mg/L) or indifferent (≤2 mg/L) (table 1).

-Time-kill assay: a bacteriostatic synergy was observed at MIC concentrations of ampicillin plus 16 mg/L concentration of ceftriaxone, demonstrating that the association produces a

Table 1 **Antibiotic susceptibility of *L. monocytogenes* strains to ampicillin (AMP) and ceftriaxone (CAX) by the microdilution and checkerboard methods.**

	Microdilution Method				Checkerboard Assay	
	MIC (mg/L)		MIC (mg/L)		FIC index	
Strains	AMP	AMP+CAX	CAX	CAX+AMP	Value	Interpretation
1/2a LMP22	0.25	0.06	64	16	0.49	Synergism
	0.25	0.125	64	8	0.62	Additive
1/2a LMP42	0.5	0.125	64	16	0.49	Synergism
	0.5	0.25	64	8	0,62	Additive
1/2b LMP53	0.25	0.06	64	16	0.49	Synergism
	0.25	0.125	64	8	0.62	Additive
1/2b LMP62	0.25	0.06	64	16	0.49	Synergism
	0.25	0.125	64	8	0.62	Additive
4b LMP38	0.5	0.125	64	16	0.49	Synergism
	0.5	0.25	64	8	0.62	Additive
4b LMP43	0.5	0.125	64	16	0.49	Synergism
	0.5	0.25	64	8	0.62	Additive

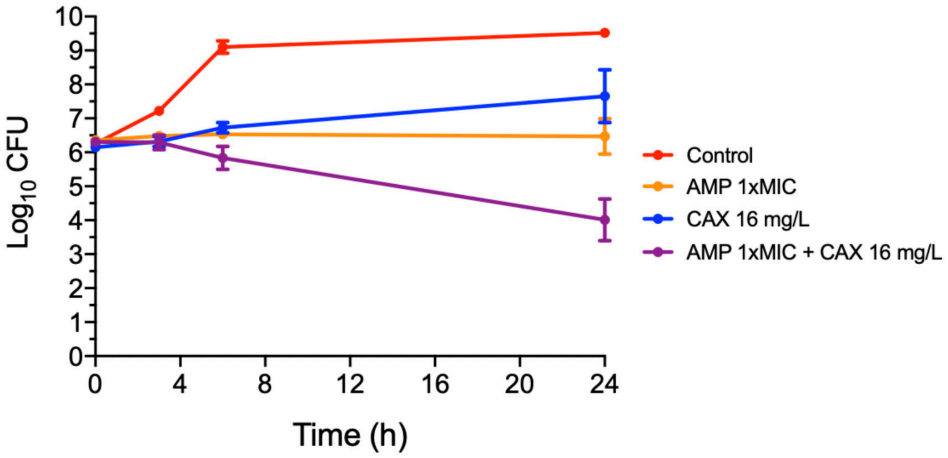

Figure 1 | **Log_{10} reduction (mean and SD) of bacterial growth of *L. monocytogenes* obtained by time-killing curves with: 1xMIC concentration of ampicillin (AMP), 16 mg/L of ceftriaxone (CAX), and the combination of both antibiotics (AMP+CAX) against six strains studied taken as a whole.**

log_{10} CFU reduction of 2.5 for the six strains studied taken as a whole; from 6.5 (95% CI: 6.2-6.8) at ampicillin MIC concentration alone to 4 (95% CI: 3.7-4.3) when combined with ceftriaxone 16 mg/L (figure 1).

DISCUSSION

The results obtained from this in vitro study demonstrated a synergistic effect among ampicillin and 16 mg/L of ceftriaxone against *L. monocytogenes*. This effect could be related to a complementary inhibition of penicillin-binding proteins (PBP) by ceftriaxone, that would enhance the ampicillin killing. In general, cephalosporins are a good inhibitor of PBP1, PBP2 and PBP4 in *L. monocytogenes* [14] and the optimal killing by beta-lactams is achieved only when several of the different PBPs are blocked [6]. A partial synergistic effect has been previously reported using ceftriaxone concentrations of 1-4 mg/L, lower than those of this study of 16 mg/L, which could explain their lack of more conclusive results in these studies [15, 16].

To define the clinical relevance of these *in vitro* results can be difficult. However, from this study derive a series of considerations that could support its use in clinical practice.

This synergistic effect significantly improves the activity of ampicillin, very desirable aspect in the treatment of septic patients with underlying disease.

This combination may have pharmacokinetics advantages over the recommended ampicillin plus gentamicin association in invasive listeriosis [17], since gentamicin does not penetrate the CNS to achieve therapeutically useful concentrations. Based on the above, this combination does not provide a definite clinical advantage over an aminopenicillin alone [18]. In addition, ceftriaxone is one of the cephalosporins with better intracellular penetration within phagocytic cells (30 to 40%), while aminoglycosides although they show an effective and rapid extracellular destruction, are not active intracellularly [19].

Ceftriaxone levels of 16 mg/L can be achieved in CSF. Even though ceftriaxone penetrates poorly into CSF with uninflamed meninges, clinical experience clearly shows that the drug diffuses well into the CSF of patients with bacterial meningitis, after a single 100 mg/kg dose, at two hours after dosing, mean CSF concentrations were 20 mg/L [20]. In another study, in patients with meningitis, the levels ranged from 0.85 to 18.29 mg/L for 4 g/day ceftriaxone dose [21]. Also, the French guideline for meningitis treatment, unlike the American and European guidelines, recommends the prescription of a high concentrations of ceftriaxone (100 mg/kg/day) without limitation of the dose [22]. Although these studies show a certain inter-individual variability, many patients could benefit from this window of high concentration of ceftriaxone, which could suppose a not expected therapeutic advantage in many patients.

The combination is safe from the clinical point of view, since the ceftriaxone plus ampicillin (and vancomycin) association is the recommended empirical treatment of meningitis in people older than 50 years. [23]. In addition, empirical therapy with cephalosporins does not affect the clinical outcome of patients with *Listeria* meningitis when ampicillin was subsequently added to treatment [24].

In conclusion, our results demonstrate that the association of ceftriaxone with ampicillin increases the activity of ampicillin, and therefore could be a valuable option in the treatment of invasive infection by *L. monocytogenes*, especially when the CNS is compromised. Animal models and clinical studies should have to evaluate whether ceftriaxone associated with ampicillin offers a real and successful alternative of listeriosis treatment.

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None to declare.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest

REFERENCES

1. Charlier C, Perrodeau É, Leclercq A, Cazenave B, Pilmis B, Henry B et al. Clinical features and prognostic factors of listeriosis: the MONALISA national prospective cohort study. Lancet Infect Dis. 2017;17:510-519. doi: 10.1016/S1473-3099(16)30521-7.
2. Ramsakal A, Nadaminti H, Field T, Vincent AL, Greene J, Dass VL et al. *Listeria* infections in cancer patients. Infect Med 2004; 21:345-9.
3. Allerberger F, Wagner M. Listeriosis: a resurgent foodborne infection. Clin Microbiol Infect 2010; 16:16–23. doi: 10.1111/j.1469-0691.2009.03109.x.
4. Hof H. Therapeutic activities of antibiotics in listeriosis. Infection 1991;19 (suppl. 4): S229-33.
5. Marget W, Seeliger HPR. *Listeria monocytogenes*: therapeutic possibilities and problems. Infection 1988; 16 (suppl. 2): 175-7.
6. Hof H, Nichterlein T, Kretschmar M. Management of listeriosis. Clin Microbiol Rev 1997; 10:345–57. PMID: 9105758
7. Chanteux H, Mingeot-Leclercq MP, Sonveaux E, Van Bambeke F, Tulkens PM. Intracellular accumulation and activity of ampicillin used as free drug and as its phthalimidomethyl or pivaloyloxymethyl ester (pivampicillin) against *Listeria monocytogenes* in J774 macrophages. J Antimicrob Chemother 2003; 5:610-5. doi: 10.1093/jac/dkg431
8. Gavalda J, Torres C, Tenorio C, López P, Zaragoza M, Capdevila JA et al. Efficacy of ampicillin plus ceftriaxone in treatment of experimental endocarditis due to *Enterococcus faecalis* strains highly resistant to aminoglycosides. Antimicrob Agents Chemother 1999; 43:639-46. PMID: 10049280
9. Clinical and Laboratory Standards Institute. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria-Third Edition: Approved Standard M45. CLSI, Wayne, PA, USA, 2016.
10. Garcia L. Synergism Testing: Broth Microdilution Checkerboard and Broth Macrodilution Methods. In: Leber AL, ed. Clinical Microbiology Procedures Handbook. Washington: ASM Press, 2010;140-62.
11. Odds FC. Synergy, antagonism, and what the chequerboard puts between them. J Antimicrob Chemother 2003; 52:1. doi: 10.1093/jac/dkg301
12. Clinical and Laboratory Standards Institute. Methods for Determining Bactericidal Activity of Antimicrobial Agents. Approved Standard M26-A. CLSI, Wayne, PA, USA, 1999.
13. Eliopoulos GM, Moellering RC. Antimicrobial combinations. In: Lorian V. ed. Antibiotics in Laboratory Medicine. Baltimore: The Williams & Wilkins Co, 1996;330-96.
14. Vicente MF, Perez-Díaz JC, Baquero F Angel de Pedro M, Berenguer J. Penicillin-binding protein 3 of *Listeria monocytogenes* as the primary lethal target for beta-lactams. Antimicrob Agents Chemother 1990; 34:539-42. doi: 10.1128/aac.34.4.539
15. Hoogkamp-Korstanje JA. Activity of cefotaxime and ceftriaxone alone and in combination with penicillin, ampicillin and piperacillin against neonatal meningitis pathogens. J Antimicrob Chemother 1985; 16:327-34. doi: 10.1093/jac/16.3.327
16. Kumaraswamy M, Do C, Sakoulas G, Tseng GW, King H. *Listeria monocytogenes* endocarditis: case report, review of the literature, and laboratory evaluation of potential novel antibiotic synergies. Int J Antimicrob Agents 2018; 51:468-78. doi: 10.1016/j.ijantimicag.2017.12.032.
17. Hof H. An update on the medical management of listeriosis. Expert Opin Pharmacother. 2004; 5:1727-35. doi: 10.1517/14656566.5.8.1727
18. Hof H. Chemotherapy of *Listeria* infections. GMS Infect Dis. 2013; 1:1–10. doi: 10.3205/id000006
19. Kuhn H, Angehrn P, Havas L. Autoradiographic evidence for penetration of 3H-ceftriaxone (Rocephin) into cells of spleen, liver and kidney of mice. Chemotherapy. 1986;32:102-12. doi: 10.1159/000238398
20. Scheld WM, Rocha H, Sande MA, Bryan JP. Rationale for clinical trials evaluating ceftriaxone in the therapy of bacterial meningitis. Am J Med. 1984;77(4C):42-9. PMID: 6093518
21. Navas D, Deslandes G, Dailly E, Caillon A, Chiffoleau G, Potel V et al. Prescription of high dose of ceftriaxone for treatment of meningitis: is therapeutic drug monitoring useful? A cohort study. In: Abstracts of the European Congress of Clinical Microbiology and Infectious Diseases, London, United Kingdom. Abstract P1614, p. 441. Clin Microbiol Infect 2012;18: 114-715.
22. Société de pathologie infectieuse de langue française (SPILF). Practice guidelines for acute bacterial meningitidis (except newborn and nosocomial meningitis). Med Mal Infect 2009; 39:356-67. PMID: 19645087
23. Tunkel AR, Hartman BJ, Kaplan SL, Kaufman BA, Roos KL, Scheld WM et al. Practice guidelines for the management of bacterial meningitis. Clin Infect Dis. 2004;39:1267-84. doi: 10.1086/425368
24. Cherubin CE, Appleman MD, Heseltine PN, Khayr W, Stratton CW. Epidemiological spectrum and current treatment of listeriosis. Rev Infect Dis 1991; 13:1108-14. doi: 10.1093/clinids/13.6.1108

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Carta al Director

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Infecciones profundas por *Gardnerella vaginalis* en el varón. Revisión de la literatura y a propósito de un caso

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Sr. Editor: *Gardnerella vaginalis* es un bacilo gramvariable, anaerobio facultativo e inmóvil, que carece de endosporas y cápsula. Su reservorio principal lo constituyen los genitales femeninos, pudiendo encontrarse en enfermos y portadores sanos. Es un microorganismo que se asocia clínicamente a la vaginosis bacteriana (VB) [1, 2], pero también se ha encontrado en exudados de heridas en cirugías pélvicas, exudados endocervicales en mujeres con rotura prematura de membranas, corioamnionitis, fiebre posparto y bacteriemia en neonatos [2, 3]. Sin embargo, a pesar de su frecuente relación con los genitales femeninos y su patología, se han encontrado otras posibles infecciones por *G. vaginalis*. Así, se han descrito casos de infecciones del tracto urinario, balanitis, uretritis y hasta prostatitis crónica en el hombre [3], y otras infecciones extragenitales, tanto en mujeres como en hombres [4, 5]. La descripción de nuevas infecciones se ve facilitada por la introducción de la espectrometría de masas, MALDI-TOF, en los laboratorios de Microbiología Clínica, ya que permite identificar con facilidad este microorganismo. El tratamiento de las infecciones por *G. vaginalis* no está bien establecido, sobre todo fuera del tracto genial femenino. Se ha demostrado la actividad de metronidazol, siendo el tratamiento de elección, y teniendo como alternativas clindamicina, amoxicilina-clavulánico, vancomicina o ampicilina [6]. Dada la rareza de las infecciones profundas en el varón describimos la participación de *G. vaginalis* como agente productor de infección renal en el hombre y se revisa la patología infecciosa descrita hasta el momento.

Presentamos un caso clínico de infección profunda, relacionado con el aislamiento en nuestro Laboratorio de Microbiología del Hospital Virgen de las Nieves de Granada, mediante el procedimiento normalizado de trabajo para las muestras de abscesos, que incluyó la siembra en medios de cultivo selectivos y no selectivos, en atmósfera de CO_2 y anaerobiosis.

Para el estudio de los procesos clínicos publicados, relacionados con esta bacteria, se localizaron los artículos generados en la base de datos MEDLINE en una búsqueda abierta, hasta diciembre de 2018, con las palabras clave "*Gardnerella vaginalis*". Así se obtuvieron 1.620 publicaciones. Posteriormente, se seleccionaron, tras su revisión manual, los estudios publicados en los que se señaló la acción patógena por esta bacteria en casos de infección profunda en hombres. Esto condujo a la selección de 10 trabajos científicos.

El caso clínico correspondió a un varón de 67 años, con antecedentes personales de trastorno bipolar controlado y resección transuretral, por un tumor urotelial vesical T1, de alto grado, hace años, sin revisiones posteriores por Urología. Acude al servicio de Urgencias porque sufrió 4 días antes un traumatismo en fosa renal izquierda que posteriormente comenzó con dolor en esa localización, hematuria macroscópica intermitente, malestar general con escalofríos y deterioro progresivo. En la exploración, el paciente presentó un estado general deteriorado, con tendencia a la hipotensión y taquicardia, sin fiebre, oliguria, y con una puño-percusión renal izquierda positiva. Analíticamente destacaban una creatinina de 2,53 mg/dL, proteína C reactiva de 215 mg/L, hemoglobina de 10,4 g/dL y 25.740 leucocitos/µL. Se le realizó un TAC sin contraste, que mostraba uretero-hidronefrosis izquierda de grado 4, que podría estar en relación con una estenosis de la unión urétero-vesical izquierda, con contenido ecogénico, dentro del mencionado sistema excretor, que podría corresponder con restos hemáticos o material purulento. Dado el estado del paciente, se inició tratamiento con imipenem y se ingresó a cargo del servicio de Urología para realizar una punción percutánea y colocación de nefrostomía izquierda, que se realizó sin incidencias. En la punción se obtuvo material purulento que se mandó a cultivar. Tras 48 horas de incubación en CO_2 crecieron colonias abundantes y únicas en el medio de agar sangre (Becton-Dickinson, Barcelona, España) que se identificaron co-

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Tabla 1 **Principales manifestaciones clínicas en los pacientes varones con infección profunda por *Gardnerella vaginalis*.**

PACIENTE Nº (CITA/AÑO)	EDAD	FACTORES PREDISPONENTES	MANIFESTACIONES CLÍNICAS	MUESTRA CLÍNICA	TRATAMIENTO	MÉTODO DIAGNÓSTICO MICROBIOLÓGICO
1 ([12]/1981)	37	Trasplante renal	Absceso perinefrítico en riñón trasplantado	Hemocultivo y urocultivo. Biopsia de absceso	Ampicilina	No consta
2 ([13]/1989)	41	Alcoholemia	Absceso pulmonar	Aspiraciones broncoscópicas	Metronidazol. Clindamicina	No consta
3 ([14]/1990)	65	Adenoma de próstata	Bacteriemia	Urocultivo, hemocultivo	Gentamicina Amoxicilina	No consta
4 ([15]/1997)	78	Alcoholemia, Síndrome prostático, infección del tracto urinario inferior, cirugía de neoplasia pulmonar	Bacteriemia	Urocultivo, Hemocultivos	Cefonicid	Vitek NHI
5 ([9]/2005)	50	Fumador	Absceso perinefrítico y empiema pleural derecho	Biopsia de absceso y exudado de empiema	Metronidazol Amoxicilina	API CORYNE
6 ([4]/2008)	41	Ninguno	Bacteriemia	Hemocultivo y urocultivo	Ciprofloxacino	Secuenciación ARNr 16S
7 ([5]/2010)	39	Diabetes mellitus, hipertensión, alcoholemia y promiscuidad	Pielonefritis, endocarditis	Hemocultivos	Metronidazol	Secuenciación ARNr 16S
8 ([16]/2013)	61	Diabetes mellitus, hipertensión, sobrepeso, cirugía digestiva tumoral	Bacteriemia	Coprocultivo y hemocultivos	Metronidazol	MALDI-TOF Rapid ID 32 Strep (BioMérieux)
9 ([17]/2015)	36	Ninguno	Uretritis y Bacteriemia	Urocultivos, hemocultivos	Ciprofloxacino Azitromicina Ceftriaxona	BacT/ALERT 3D System (BioMérieux) MALDI-TOF
10 ([18]/2018)	51	SIDA	Bacteriemia	Hemocultivos	Trimetoprim-Sulfametoxazol Ceftriaxona Metronidazol	MALDI-TOF

rrectamente mediante MALDI-TOF (Bruker Biotyper, Billerica, MA, USA), con un score 2,103, como *G. vaginalis*. Se estudió su sensibilidad mediante el método de E-test (Biomerieux®, Madrid, España) y para la interpretación se siguieron los puntos de corte establecidos por CLSI para anaerobios. Nuestro aislado mostró los siguientes valores de CMI que fueron interpretados como sensible: amoxicilina-ácido clavulánico (0,125 mg/L), clindamicina (0,19 mg/L), metronidazol (8 mg/L) y vancomicina (0,38 mg/L). Ante este resultado se añadió clindamicina al tratamiento, administrándose de forma intravenosa cada 8 horas a dosis de 600 mg.

Durante los primeros días, tras la colocación de la nefrostomía, el paciente presentó mejoría de su estado general, se mantuvo afebril, recuperó un ritmo de diuresis normal y presentó estabilidad hemodinámica. Se cambió la antibioterapia a amoxicilina con ácido clavulánico, 1000/200 mg por vía intravenosa cada 8 horas. Sin embargo, accidentalmente, pierde el catéter y aparece febrícula en los días posteriores. Se realizó nueva punción renal y colocación de nefrostomía, con nueva salida de material purulento. El cultivo mostró crecimiento de *Candida kefyr* y el antifungigrama, realizado mediante E-test, mostró los siguientes valores de CMI: anfotericina B (0,25 mg/L), caspofungina (0,25 mg/L), fluconazol (1 mg/L), micafungina (0,12 mg/L), voriconazol (0,12 mg/L), siendo interpretados como sensibles anfotericina B y fluconazol. Entonces se añadió al tratamiento fluconazol. A las 48 horas del inicio del tratamiento antifúngico el paciente estaba afebril y evolucionó posteriormente de forma favorable. Se realizó un TAC de control, que informó que persistía la uretero-hidronefrosis izquierda grado 4, con el parénquima renal muy atrofiado, y con el catéter de nefrostomía bien colocado. Se realizó una cistoscopia que descartó una recidiva tumoral vesical. Finalmente, dada la escasa funcionalidad del riñón izquierdo, se decidió realizar una nefrectomía simple izquierda. El paciente no sufrió ninguna otra complicación relacionada con su patología infecciosa, y evolucionó de forma favorable siendo dado de alta diez días tras la cirugía.

La mayoría de las infecciones urinarias son provocadas, en ambos sexos, por microorganismos gramnegativos (*Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomona*

aeruginosa, etc.) y en menor frecuencia por grampositivos (*Enterococcus* spp., *Staphylococcus* spp., etc.) [7]. En mujeres, aunque no es frecuente, *G. vaginalis* se considera un posible uropatógeno, y hay evidencia de la posible transmisión sexual a varones [8]. Este hecho puede justificar un aumento de la probabilidad de aislamientos de esta bacteria en cultivos de orina en varones. Sin embargo, habitualmente, no se investiga su presencia, ya que se ha descrito, clínicamente, en raras ocasiones como causa de infecciones urinarias, aunque puede existir un infradiagnóstico de la misma.

Un estudio [4] describió que un 7-11% de los varones presentan *G. vaginalis* como parte de su flora urogenital y anorrectal, pudiendo, potencialmente provocar infecciones de estas zonas o ser el punto de origen. Aun así, se han descrito muy pocos casos de infecciones urinarias por esta bacteria en varones, y habitualmente se han asociado a factores de riesgo. No obstante, hay algunos casos publicados que describen infecciones más complejas (tabla 1). Entre los casos descritos de infección profunda por *G. vaginalis* en el varón adulto, encontramos endocarditis infecciosa, acompañada de embolia séptica en riñón y cerebro [5]; uretritis [17], bacteriemia [4, 14-16, 18] e, incluso, empiema asociado a absceso perinefrítico [9]. Dichas infecciones que tienen lugar fuera del tracto urogenital se relacionan, generalmente, con factores de riesgo, como el estado de inmunosupresión, anormalidades genitourinarias, alcoholismo [10] y relaciones sexuales no protegidas [11]. En nuestro conocimiento, se han descrito 10 casos previos de infección profunda por *G. vaginalis* en el varón adulto, por lo que estamos ante el décimo primer caso descrito. Actualmente estamos asistiendo a cambios epidemiológicos en la etiología de las infecciones, lo que obligará a una actuación diagnóstica más completa, haciendo peticiones analíticas más amplias y adecuando los procedimientos disponibles a las necesidades clínicas.

La pauta correcta de tratamiento para las infecciones profundas por este microorganismo no está bien establecida. Aunque *G. vaginalis* suele ser sensible a múltiples antibióticos (metronidazol, clindamicina, amoxicilina-clavulánico, vancomicina), actualmente están aumentando las resistencias a metronidazol [6], y se desconoce exactamente la dosis y duración para un tratamiento eficaz. Pensamos que la hidronefrosis debió ser clave en la facilidad para el desarrollo de la pionefrosis y en su agresividad clínica en nuestro paciente.

Como conclusión, se describe un nuevo caso de infección profunda por *G. vaginalis*, consistente en una pionefrosis de un paciente de 67 años, con hidronefrosis secundaria a una resección transuretral, por un tumor urotelial vesical T1 de alto grado. A pesar de su rareza, tras revisar los casos publicados, destacamos la variedad de infecciones en las que ha estado presente esta bacteria, en muchos casos con factores de riesgo asociados, por lo que se debería tener en cuenta a la hora de su investigación, expresa o no en el laboratorio, y su significación clínica, ya que, actualmente, se puede simplificar su identificación mediante la técnica de MALDI-TOF. Además, esta infección justifica una exhaustiva valoración, en determinados casos, de los posibles nuevos agentes microbianos en la orina. También queda patente la escasa experiencia en el manejo de la infección profunda por *G. vaginalis*, lo que nos obligaría a diseñar estudios patogénicos sobre la infección y la actividad de los antibióticos frente a este microorganismo.

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CONFLICTO DE INTERESES

Los autores declaran no tener ningún conflicto de intereses.

BIBLIOGRAFIA

1. Carrillo-Ávila J, Serrano-García M, Fernández-Parra J, Sorlózano-Puerto A, Navarro-Marí J, Stensvold C, et al. Prevalence and genetic diversity of *Trichomonas vaginalis* in the general population of Granada and co-infections with Gardnerella *vaginalis* and Candida species. J Med Microbiol. 2017; 66: 1436-42. doi:10.1099/jmm.0.000603.
2. Sorlózano-Puerto A, Esteban-Sanchís P, Heras-Cañas V, Fernández-Parra J, Navarro-Mari JM, Gutiérrez-Fernández J. Estudio prospectivo de la incidencia de patógenos genitales oportunistas y estrictos que crecen en medios de cultivo artificiales, Rev Lab Clin. 2018; 11:123-30. Doi: /10.1016/j.labcli.2017.11.009.
3. Heras-Cañas V, Gutiérrez-Soto B, Serrano-García ML, Vázquez-Alonso F, Navarro-Marí JM, Gutiérrez-Fernández, J. Prostatitis crónica bacteriana. Estudio clínico y microbiológico de 332 casos. Med Clin. 2016; 147: 144-7. doi: /10.1016/j.medcli.2016.05.018.
4. Lagacé-Wiens PR, Ng B, Reimer A, Burdz T, Wiebe D, Bernard K. *Gardnerella vaginalis* bacteremia in a previously healthy man: case report and characterization of the isolate. J Clin Microbiol. 2008; 46: 804-6. doi: 10.1128/JCM.01545-07.
5. Yoon HJ, Chun J, Kim JH, Kang SS, Na DJ. *Gardnerella vaginalis* septicaemia with pyelonephritis, infective endocarditis and septic emboli in the kidney and brain of an adult male. Int J STD AIDS. 2010; 21:653-7. doi: 10.1258/ijsa.2010.009574.
6. Mensa Pueyo J. Guía de terapéutica antimicrobiana. 2018. 28th ed. Antares, p.382.
7. Sánchez-García JM, Sorlózano-Puerto A, Navarro-Marí JM, Gutiérrez Fernández J. Evolución de la resistencia a antibióticos de microorganismos causantes de infecciones del tracto urinario: un estudio de vigilancia epidemiológica de 4 años en población hospitalaria. Rev Clin Esp. 2019; 219:116-23. doi: 10.1016/j.rce.2018.07.005.
8. Kline KA, Lewis AL. Gram-positive uropathogens, polymicrobial urinary tract infection, and the emerging microbiota of the urinary tract. Microbiol Spectr. 2016; 4(2). doi: 10.1128/microbiolspec.UTI-0012-2012.
9. Calvert LD, Collins M, Bateman JR. Multiple abscesses caused by

Gardnerella vaginalis in an immunocompetent man. J Infect. 2005; 51: E27-9. doi: 10.1016/j.jinf.2004.08.002.

10. Catlin BW. *Gardnerella vaginalis*: characteristics, clinical considerations, and controversies. Clin Microbiol Rev. 1992; 5:213-37. PMID: 1498765.
11. Iser P, Read TH, Tabrizi S, Bradshaw C, Lee D, Horvarth L, et al. Symptoms of non-gonococcal urethritis in heterosexual men: a case control study. Sex Transm Infect. 2005; 81:163-5. doi: 10.1136/sti.2004.010751.
12. Finkelhor RS, Wolinsky E, Kim CH, Tchou P, Frengley JD. *Gardnerella vaginalis* perinephric abscess in a transplanted kidney. N Engl J Med. 1981; 304:846. doi: 10.1056/NEJM198104023041418.
13. Legrand JC, Alewaeters A, Leenaerts L, Gilbert P, Labbe M, Glupczynski Y. *Gardnerella vaginalis* bacteremia from pulmonary abscess in a male alcohol abuser. J Clin Microbiol. 1989; 27:1132-4. PMID: 2787333.
14. Denoyel GA, Drouet EB, De Montclos HP, Schanen A, Michel S. *Gardnerel*la *vaginalis* bacteremia in a man with prostatic adenoma. J Infect Dis 1990; 161: 367–8. PMID: 1688908.
15. Bastida-Vilá MT, López-Onrubia P, Rovira-Lledos J, Martinez-Martinez JA, Expósito-Aguilera M. *Garderella vaginalis* bacteremia in an adult male. Eur J Clin Microbiol Infect Dis 1997; 16: 400–1. PMID: 9228486.
16. Alidjinou EK, Bonnet I, Canis F, Dewulf G, Mazars E, Cattoen C. *Gardnerella vaginalis* bacteremia in a male patient. Med Mal Infect 2013; 43: 434–5. doi: 10.1016/j.medmal.2013.07.007.
17. Babics A, Roussellier P. *Gardnerella vaginalis*: An overlooked pathogen in male patients? Med Mal Infect. 2015; 45: 423-4. Doi: 10.1016/j.medmal.2015.09.007.
18. Bhatia P, Temple J, Kantor M. Not your garden-variety bacteremia: *Gar*dnerella in an immunocompromised man. Clin Infect Dis. 2018; 66: 1458-9. doi: 10.1093/cid/cix1054.

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Carta al Director

Fascitis necrotizante y síndrome del shock tóxico por *Streptococcus pyogenes* tras inyección intramuscular

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Sr. Editor: La fascitis necrotizante (FN) es una infección grave, rápidamente progresiva y de difícil diagnóstico en estadios tempranos. Afecta a la fascia muscular y al tejido celular subcutáneo, produce trombosis de la microcirculación subcutánea, necrosis y se asocia a elevada mortalidad.

Presentamos un caso de FN y shock tóxico por *Streptococcus pyogenes*.

Mujer de 47 años de edad con antecedentes de hipertensión que acudió al Servicio de Urgencias por náuseas, vómitos, sensación distérmica, dolor intenso y tumefacción del glúteo y muslo izquierdo desde que 24 horas antes le fuera administrada una inyección intramuscular de metilprednisolona por faringoamigdalitis. Presentaba eritema (3,5 x 3 cm) en región glútea izquierda, sin fluctuación ni tumefacción, ni datos de repercusión sistémica. Se recomendó hielo local y continuar tratamiento iniciado el día anterior con amoxicilina-clavulánico. Doce horas más tarde acudió de nuevo a Urgencias por aumento del dolor. Se objetivó TA: 73/40 mm Hg, FC: 110 lpm, Tª: 35,5ºC. Se realizó TC observando aumento de tamaño de la zona glútea con pérdida de las interfases y desflecamiento del contorno. Se inició tratamiento con ertapenem y fluidoterapia, mejorando el cuadro hemodinámico. Seis horas después comenzó de nuevo con hipotensión, taquicardia, hiperlactacidemia e hipoglucemia, con progresión de la afectación cutánea en muslo. Se modificó tratamiento a meropenem y daptomicina. Se realizaron fasciotomía y necrosectomía extensas, ingresando en el Servicio de Medicina Intensiva, donde presentó shock refractario a volumen, precisando altas dosis de noradrenalina, signos de coagulación intravascular diseminada y fallo renal con necesidad de hemofiltración venovenosa continua. Mostraba signos de hipoperfusión en manos y pies.

Se remitieron hemocultivos y muestras de tejidos al Servicio de Microbiología. En la tinción de Gram se observaron leucocitos y cocos que tomaban mal la coloración de Gram. A partir de las muestras de tejidos, se realizó inmunoensayo cromatográfico rápido para la detección cualitativa de antígenos de Estreptococo grupo A (Hangzhou AllTest Biotech CO., LTD, China) que fue positivo. Se añadió al tratamiento clindamicina e inmunoglobulina IV. A las 24 horas, se aisló *S. pyogenes* a partir de las muestras de tejido. La cepa se caracterizó como serotipo M1, spe A, spe B, speC, speF, speG, speJ, speZ. Los hemocultivos fueron negativos.

Pese a mejorar el cuadro sistémico y retirar las drogas vasoactivas, presentó gangrena simétrica periférica de manos y pies precisando amputación distal de las cuatro extremidades.

Seis meses después fue dada de alta del hospital, tras cierre completo de las heridas y en tratamiento rehabilitador. Al año del inicio de la patología, la paciente consigue buen acoplamiento a las prótesis biomecánicas de manos y pies, deambulando sin bastones.

S. pyogenes es el agente etiológico más frecuente de FN tipo II, monomicrobiana, que puede afectar a pacientes previamente sanos de cualquier edad sin necesidad de factores de riesgo; si bien, algunos autores establecen relación con inyecciones intramusculares y traumatismo penetrantes [1]. El traumatismo cerrado también podría ser un factor de riesgo por una mayor expresión de vimentina, adhesina principal de *S. pyogenes* a las células musculares lesionadas [2].

Una tercera parte de casos se asocian a síndrome de shock tóxico [1]. La mayoría de las cepas pertenecen a los serotipos M1 y M3, con capacidad de alterar la función fagocítica y la producción de exotoxinas pirogénicas (principalmente la SpeA) que pueden actuar como superantígenos [3]. El serotipo M1 es el más frecuente en la enfermedad invasiva en todo el mundo [4, 5].

En el 50% de casos se desconoce la puerta de entrada. En

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nuestra paciente pudo producirse diseminación hematógena desde orofaringe hasta el microtraumatismo producido por la inyección IM, pero en ausencia de cultivo faríngeo, al igual que reconocen otros autores [1], no se pudo documentar faringoamigdalitis.

La FN es una emergencia quirúrgica, requiere un diagnóstico rápido, manejo quirúrgico inmediato, tratamiento antibiótico y soporte hemodinámico. Las pruebas de imagen se realizarán siempre que no supongan una demora en el tratamiento del paciente. Un retraso en el diagnóstico está asociado a un aumento de la morbimortalidad [3]. Éste se basa en la sospecha clínica, cultivos, marcadores de sepsis y escala de "Laboratory Risk Indicator for Necrotizing Fasciitis" (LRINEC) [6]. Esta escala es una herramienta especialmente útil, una puntuación mayor de 8 indica necesidad de cirugía urgente con desbridamiento de los tejidos, pero en el caso de puntuación entre 6 y 8 no se puede excluir FN, y se necesitarían otras pruebas como RMI, biopsia o finger test.

Algunos autores ante la sospecha de infección invasiva por *S. pyogenes* han utilizado test rápidos diseñados para el diagnóstico de faringoamigdalitis, su uso puede ser relevante ante infecciones graves ayudando a una terapia dirigida [7].

S. *pyogenes* es sensible a los antibióticos β-lactámicos, por lo que se mantienen como tratamiento de primera línea. La Sociedad Americana de Enfermedades Infecciosas recomienda el uso de clindamicina junto a penicilina en el tratamiento de la FN [8]. Clindamicina actúa inhibiendo la síntesis proteica y su papel es esencial ya que inhibe la producción de factores de virulencia como la proteína M o el superantígeno, efecto que en animales de experimentación se ha demostrado incluso en cepas resistentes a clindamicina [9]. Asimismo, linezolid ha demostrado actividad *in vitro* frente a *S. pyogenes,* inhibiendo la síntesis proteica, además de otras endotoxinas estreptocócicas como la proteína M, estreptolisina O, Dnasa, Spe B y proteína F [10]. El uso IV de inmunoglobulina está recomendado en caso de shock tóxico.

Concluimos que el diagnóstico rápido, el manejo quirúrgico inmediato y el tratamiento antibiótico adecuado son esenciales para reducir la mortalidad en estos pacientes.

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CONFLICTO DE INTERESES

Los autores declaran no tener ningún conflicto de intereses.

BIBLIOGRAFÍA

1. Stevens DL, Bryant AE. Necrotizing Soft-Tissue Infections. N Engl J Med. 2017;377(23):2253–65. DOI: 10.1056/NEJMc1800049
2. Bryant AE, Bayer CR, Huntington JD, Stevens DL. Group A streptococcal myonecrosis: increased vimentin expression after skeletal-muscle injury mediates the binding of *Streptococcus pyogenes*. J Infect Dis. 2006; 193(12):1685-92. DOI: 10.1086/504261
3. Baxter F, McChesney J. Severe group A streptococcal infection and streptococcal toxic shock syndrome. Can J Anaesth. 2000;47(11):112940. DOI: 10.1007/BF03027968
4. Steer AC, Law I, Matatolu L, Beall BW, Carapetis JR. Global emm type distribution of group A streptococci: systematic review and implications for vaccine development. Lancet Infect Dis. 2009;9(10):611–6. DOI: 10.1016/S1473-3099(09)70178-1
5. Smit PW, Lindholm L, Lyytikäinen O, Jalava J, Pätäri-Sampo A, Vuopio J. Epidemiology and emm types of invasive group A streptococcal infections in Finland, 2008–2013. Eur J Clin Microbiol Infect Dis. 2015;34(10):2131–6. DOI: 10.1007/s10096-015-2462-2
6. Wong C-H, Khin L-W, Heng K-S, Tan K-C, Low C-O. The LRINEC (Laboratory Risk Indicator for Necrotizing Fasciitis) score: a tool for distinguishing necrotizing fasciitis from other soft tissue infections. Crit Care Med. 2004;32(7):1535–41. DOI: 10.1097/01.CCM.0000129486.35458.7D
7. Gazzano V, Berger A, Benito Y, Freydiere A-M, Tristan A, Boisset S, et al. Reassessment of the Role of Rapid Antigen Detection Tests in Diagnosis of Invasive Group A Streptococcal Infections. J Clin Microbiol. 2016;54(4):994–9. DOI:10.1128/JCM.02516-15
8. Stevens DL, Bisno AL, Chambers HF, Dellinger EP, Goldstein EJC, Gorbach SL, et al. Executive Summary: Practice Guidelines for the Diagnosis and Management of Skin and Soft Tissue Infections: 2014 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2014;59(2):147–59. DOI: 10.1093/cid/ciu444
9. Andreoni F, Zürcher C, Tarnutzer A, Schilcher K, Neff A, Keller N, et al. Clindamycin Affects Group A *Streptococcus* Virulence Factors and Improves Clinical Outcome. J Infect Dis. 2017; 215(2):269-277. DOI: 10.1093/infdis/jiw229
10. Rac H, Bojikian KD, Lucar J, Barber KE. Successful Treatment of Necrotizing Fasciitis and Streptococcal Toxic Shock Syndrome with the Addition of Linezolid. Case Rep Infect Dis. 2017;5720708. DOI: 10.1155/2017/5720708

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Letter to the Editor

Infection of prosthetic material due to *Mycobacterium smegmatis*

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Sir,

Mycobacterium smegmatis is a rapid growth mycobacteria (RGM) classified as group IV Runyon, scotochromogen mycobacterias. It has been found in soil and water and has been considered for many years to be a non-pathogenic environmental microorganism.

There are 3 RGM related to human pathology: *Mycobacterium fortuitum*, *Mycobacterium chelonae/abscessus* and *Mycobacterium smegmatis* (*M. smegmatis* sensu stricto, *wolinskyi* and *goodie* [1]), the latter rarely causing infection; associated with bronchopulmonary illnesses, skin and soft tissue infections [2], surgery and injections, as well as infection of prosthetic material [1-5]. Sporadic cases of bacteremia have been observed [5].

We present a case of surgical infection of a wound by *M. smegmatis*. In a 66 year old woman with a history of vertebral fixation due to kyphosis in 2011. In 2018 corrective surgery was carried out with extraction of previous material and installation of new screws (figure 1). The patient returned to Emergency 14 days after surgery due to hyperthermia of unknown cause, and was discharged after having been given paracetamol. Ten days later she was admitted to Emergency with dehiscence of surgical wound with purulent exudate. After cleaning and taking samples of wound exudate for microbiological culture, the patient was treated with clindamycin 600 mg/8h and intravenous linezolid 600 mg/24h. Seven days after taking the sample, the microbiological report indicated the growth of *M. smegmatis*, confirmed by the second sample from the surgical exudate, resulting in changing the treatment to intravenous linezolid 600mg/12h. A month later the patient was discharged and prescribed doxycycline 100mg/12h and levofloxacin 500mg/24h, by mouth for six months. At the end of this period the control samples were negative. Possibly, the origin of the infection was the colonization of the skin by *M. smegmatis*, introduced during the surgical intervention, without confirmation. Microbiological

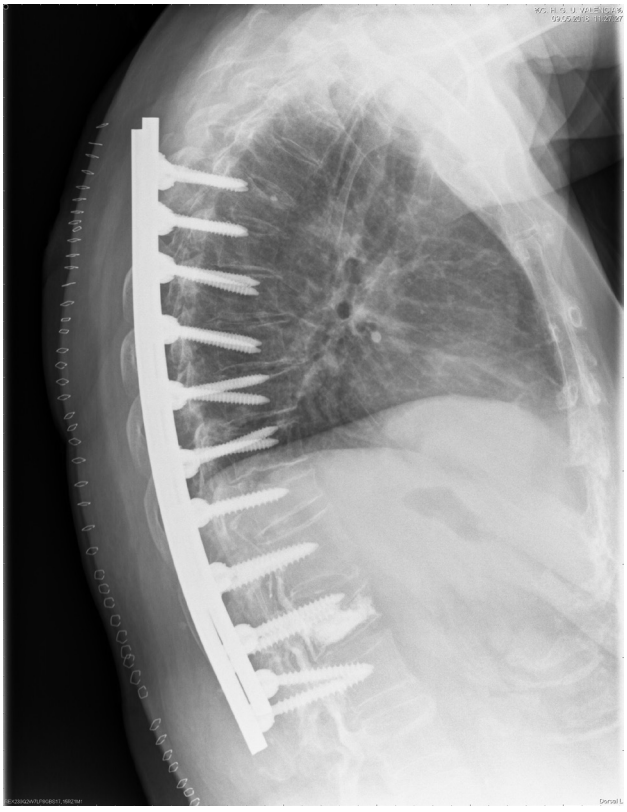

Figure 1 Lateral chest radiograph

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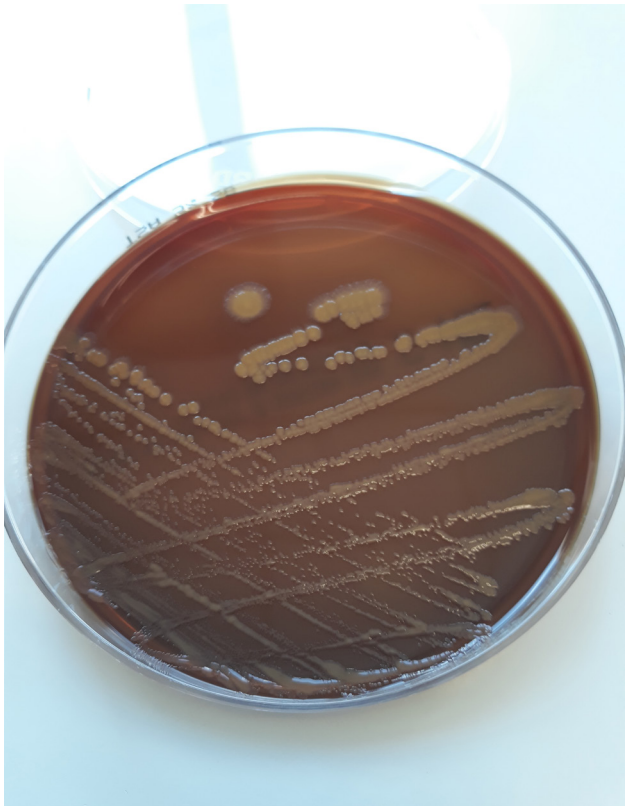

Figure 2 | ***Mycobacterium smegmatis* in chocolate agar**

Processing: The samples were cultivated in aerobic/anaerobic media (blood agar, chocolate agar, MacConkey, Brain Heart Infussion and Thioglycolate). After 6 days of incubation some colonies appeared with yellow-orange pigmentation in the blood agar and chocolate agar media (figure 2), with no growth in MacConkey which showed violet crystal.

In the Ziehl-Neelsen stain acid-alcohol resistant bacilli were observed. Identification was carried out using mass spectrometry (MALDI-TOF Bruker®) profiling as *M. smegmatis,* confirmed by PCR-nesting and genome sequencing (amplification of DNA of the gene rRNA16s) with an approximation of 99%. The sensitivity study was carried out by microdilution, showing sensitivity to cotrimoxazol, doxycycline, linezolid, amikacin, imipenem, ciprofloxacin, intermediate reaction to tobramycin and cefoxitin, and resistance to clarithromycin.

Infections of prosthetic material occur in 1-5% of cases. Occasionally they are produced by RGM [1] and prevention is relatively complicated precisely due to relative infrequency. It may be convenient to include the mycobacteria culture in badly healing wounds. This poses a therapeutic challenge due to limited experience [3] and lack of knowledge of the duration of the treatment; although various authors have coincided in that it should be a combined and long-term treatment directed by sensitivity studies for 6-12 months [5, 6], including the elimination of dead or infected tissue from the wound and the re-opening and extraction of the prosthetic material [1].

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest

REFERENCES

1. Zaid Saffo, DO, Anthony Ognjan, DO, FACP. *Mycobacterium smegmatis* infection of a prosthetic total knee arthroplasty. IDCases 2016; 5: 80–82. DOI:10.1016/j.idcr.2016.07.007
2. Zhe Xu, Di Lu, Xia Zhang,a Haijing Li, Shufang Meng, Yue-Song Pan, Alan S. Boyd, Lin Ma, Yi-Wei Tang. *Mycobacterium smegmatis* in Skin Biopsy Specimens from Patients with Suppurative Granulomatous Inflammation. J Clin Microbiol 2013; 51:1028–1030. DOI:10.1128/JCM.0342112
3. Albert J. Eid, Elie F. Berbari, Irene G. Sia, Nancy L. Wengenack, Douglas R. Osmon, and Raymund R. Razonable. Prosthetic Joint Infection Due to Rapidly Growing Mycobacteria: Report of 8 Cases and Review of the Literature. Clin Infect Dis 2007; 45:687–94. DOI:10.1086/520982
4. Manyoung Kim, Chul-Won Ha, Jae Won Jang , Yong-Beom Park . Rapidly growing non-tuberculous mycobacteria infection of prosthetic knee joints: A report of two cases. Knee 2017;24:869-875. DOI:10.1016/j.knee.2017.04.015
5. Frank Allen Zimba. *Mycobacterium smegmatis* Infection of a Lumbar Spine Instrumented Fusion. Am J Infect Dis 2011;7: 24-27. DOI:10.3844/ajidsp.2011.24.27
6. Mary A. De Groote, and Gwen Huitt. Infections Due to Rapidly Growing Mycobacteria. Clin Infect Dis 2006; 42:1756–63. DOI:10.1086/504381

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Letter to the Editor

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Usefulness of FilmArray Meningitis/Encephalitis panel in the management of an uncommon case of Herpes Simplex Virus type 2 meningitis

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Sir,

Meningitis and encephalitis are serious diseases with high morbi-mortality, especially in bacterial meningitis. Rapid identification of causative pathogens and prompt instauration of an appropriate antimicrobial therapy are crucial to reduce the morbi-mortality, length hospital stay and healthcare costs associated with these syndromes [1, 2]. Differential diagnostic between bacterial and aseptic meningitis is difficult; the former is a medical emergency that requires prompt recognition and treatment, while the latter is a relatively common and often-benign infection mainly caused by viruses [3, 4]. Consequently, cerebrospinal fluid (CSF) indices and microbiological studies are required to identify the etiologic agent [1, 2]. The classic diagnostic is based on a CSF Gram stain with culture and specific viruses PCR, which can delay the diagnostic [2]. Recently, U.S.A Food and Drug Administration (FDA) has approved the FilmArray Meningitis/Encephalitis (ME) panel (bioMérieux, Marcy l'Etoile, France) to detect central nervous system (CNS) infections. The FilmArray ME panel is a multiplex PCR that can identify the most frequent causative agents of CNS infections in an one-step assay, which decreases the diagnostic time [1, 2].

Herein, an uncommon case of aseptic meningitis due to herpes simplex virus type 2 (HSV-2) is presented as well as the usefulness of the FilmArray ME panel in prompt diagnostic, the optimization of antimicrobial therapy and the implementation of infection prevention measures.

A 25-year old man was admitted in our department with a 2-day history of general discomfort, fever, photophobia, nausea and acute headache. One week before admission, he was treated with oral amoxicillin/clavulanic acid for five days, up until 72 hours before hospital admission.

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The admission physical exam showed increased body temperature (37.6ºC), tachycardia and mild neck stiffness. CSF drawn on admission contained 852 cells/µL (100% lymphocytes), elevated protein (143 mg/dL), normal adenosine deaminase level (5.1 U/L) and normal glucose level (55 mg/dL in CSF and 120 mg/dL in serum). The Gram´s stain was negative. Intravenous antimicrobial therapy with acyclovir and ceftriaxone, 750 mg/8h and 2g/12h respectively, were immediately administered and the patient was placed under contact isolation.

The CSF analysis was conducted in the microbiology laboratory as follows: Firstly, CSF was analysed for enteroviruses (EV) detection by targeted testing platform Xpert EV PCR (Cepheid, Sunnyvale, CA, USA), yielding negative results. At this point, a CSF aliquot was sent to a reference center (Hospital Universitario Donostia) in which FilmArray ME assay was performed. A few hours after the patient´s admission, the FilmArray ME assay showed a positive result to HSV-2 being negative to bacterial targets. The patient´s measures of contact isolation and ceftriaxone administration were disrupted after learning these results. Retrospectively, the patient admitted unprotected oral sex with his couple, but he did not remember any initial cutaneous or mucosal lesion. Screening for sexually transmitted infections and urogenital HSV detection of the patient was accomplished (table 1). The patient was discharged without symptoms after 14 days of intravenous acyclovir therapy.

EV is the most frequent agent of viral meningitis, followed by varizela zoster virus and HSV-2 [5]. Nonetheless, the prevalence of HSV-2 meningitis in Spain, is very low (1.2%), with few cases reported in the literature [5–7]. Infection with HSV-2 is mucocutaneous and is acquired vertically as a neonate or as an adult principally through sexual activity [8]. Moreover, HSV-2 is a recognized cause of CNS infection, mainly meningitis and it is also related to benign recurrent meningitis [8]. CNS infection due to HSV-2, usually correspond to the reactivation of a latent virus in sensory ganglia, mostly in young females [8, 9]. As shown in previous reports, only 30% of the

Table 1 Screening of sexually transmitted infections.

Serologic findings	HBV, HCV and HIV	Negative
	Syphilis	Negative
	HSV-2 IgG	Positive
	HSV-2 IgM	Negative
	HSV-1 IgG and IgM	Negative
PCR of HSV 1 and 2 in oral and urethral swabs	Negative	

HSV: Herpes simplex virus; HBV: Hepatitis B virus; Hepatitis C virus; HIV: Human immunodeficiency virus.

patients with HSV-2 meningitis had cutaneous or mucosal lesion at the moment of CNS infection [4, 9]. Although standardized management for HSV-2 meningitis does not exist and it is often-benign infection, long-term neurological sequelae had been reported in some cases, so prompt diagnostic and antiviral treatments are recommended [3].

The recent development of the FilmArray ME assay could help the etiologic diagnostic of meningitis, but unfortunately the high cost of this assay only allows its implementation in reference hospitals. A rational use of this expensive assay could optimize the management of selected patients with CNS infections. Recently Hanson et al., suggested that inmmunocompromised patients and those who had received antimicrobials before the diagnostic lumbar puncture could be potential beneficiaries of this technology [2]. Although this assay has some advantages with respect to classical techniques for viruses CNS infections detection, like in house PCR or cultures, positive results must be interpreted along with entire clinical picture of the patient (symptoms, laboratory findings, cranial imaging...) because other host-virus state, like latency or asymptomatic viral reactivation, can also be detected [10].

Herein, we present an algorithm to the management of aseptic meningitis in a patient with predominance of lymphocytes in CSF and also had antimicrobial therapy before the admission. The implementation of new platforms, such as Xpert EV and FilmArray ME, under the aforementioned algorithm allows, in a relative short time, the identification of causative pathogens of CNS infections to optimize the therapy, avoiding unnecessary antimicrobial use and removing the necessity of contact isolation. The rational use of this new technology can improve the management of CNS infections, and when this assay is developed both under diagnostic algorithms and in selected patients, the FilmArray ME could be cost/effective, although further studies are necessary to assess this fact.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Demogines A, Fouch S, Everhart K, Leber A, Barney T, Daly JA, et al. Multi-Center Clinical Evaluation of a Multiplex Meningitis/Encephalitis PCR Panel for Simultaneous Detection of Bacteria, Yeast, and Viruses in Cerebrospinal Fluid Specimens. J Clin Microbiol 2015;54:2251–61. doi:10.1128/JCM.00730-16.

2. Hanson KE. The First Fully Automated Molecular Diagnostic Panel for Meningitis and Encephalitis: How Well Does It Perform, and When Should It Be Used? J Clin Microbiol 2016;54:2222–4. doi:10.1128/JCM.01255-16.

3. Bodilsen J, Storgaard M, Larsen L, Wiese L, Lebech A, Brandt C, et al. Infectious meningitis and encephalitis in adults in Denmark: a prospective nationwide observational cohort study (DASGIB). Clin Microbiol Infect 2018;24:1102.e1-1102.e5. doi:10.1016/j.cmi.2018.01.016.

4. Jarrin I, Sellier P, Lopes A, Morgand M, Makovec T, Delcey V, et al. Etiologies and Management of Aseptic Meningitis in Patients Admitted to an Internal Medicine Department. Med 2016;95:1–9. doi:10.1097/MD.0000000000002372.

5. Ory F De, Avellón A, Echevarria J, Sánchez-Seco M, Trallero G, Cabrerizo M, et al. Viral Infections of the Central Nervous System in Spain: A Prospective Study. J Med Virol 2013;85:554–62. doi:10.1002/jmv.

6. Reina J, Leyes M, Taboada C. Lymphocytic meningitis caused by herpes simplex virus type 2. Rev Clin Esp 2016;216:290–1. doi:10.1016/j.rce.2016.02.003.

7. Florén-Zabala L, Chamizo-López FJ, Eisman-Maraver A, Pérez-González C, Ory-Marchón F De, Trallero-Maso G, et al. Meningitis aséptica en la población adulta. Etiología y utilidad de las técnicas moleculares en el manejo clínico del paciente. Enferm Infecc Microbiol Clin 2012;30:361–6. doi:10.1016/j.eimc.2012.01.004.

8. Miller S, Mateen FJ, Aksamit AJ. Herpes simplex virus 2 meningitis: a retrospective cohort study. J Nerovirol 2013;19:166–71. doi:10.1007/s13365-013-0158-x.

9. O'Sullivan CE, Aksamit AJ, Harrington JR, Harmsen WS, Mitchell PS, Patel R. Clinical Spectrum and Laboratory Characteristics Associated With Detection of Herpes Simplex Virus DNA in Cerebrospinal Fluid. Mayo Clin Proc 2003;78:1347–52. doi:10.4065/78.11.1347.

10. Green DA, Pereira M, Miko B, Radmard S, Whittier S, Thakur K. Clinical Significance of Human Herpesvirus 6 Positivity on the FilmArray Meningitis/Encephalitis Panel. Clin Infect Dis 2018;67:1125–8. doi:10.1093/cid/ciy288.

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Letter to the Editor

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Male genitourinary infections by *Corynebacterium glucuronolyticum*. A review and clinical experience

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Sir,

Corinebacteria are often considered as part of the commensal microbiota that can be found in the genitourinary tract, however, there is growing evidence that their pathogenic role is being underestimated, even in immunocompetent individuals with no predisposing factors. The species on which this study is focused, *Corynebacterium glucuronolyticum*, is occasionally involved in human infections, so it has an opportunistic pathogen role, presenting some specificity towards the male genitourinary tract [1].

Thus, different research groups have contributed by highlighting its important role in non-gonococcal urethritis in male [1-3]; in chronic bacterial prostatitis (CBP) monomicrobial paucisymptomatic [4]; in the pathogenesis of persistent cystitis in males without predisposing factors [5] and its adverse influence on various laboratory parameters of semen, unknown until very recently [6], has also been investigated.

There are very few bibliographic references abous this species, hardly half a dozen relevant publications focusing on the genitourinary tract. As Mestrovic et al. (2018) points out, one of the main reasons for this fact is that its presence in clinical samples is not usually investigated, as its detection generally requires employing methods that are not part of the basic diagnostic protocols for genitourinary infections [1].

The pathogenic role of typical commensal microorganisms, such as corinebacteria, may come to play in the genitourinary tract has not been fully clarified yet. For this reason, identifying more accurately each species of corinebacteria is essential to move forward in this line of research. In this way, an increasingly precise determination of the pathogenicity of each species may be established, make it posible to differentiate those who behave as harmless commensal bacteria from those usually involved in infections. This precise identification will gradually become easier with the implementation of sequence-based molecular identification diagnostic methods or mass spectrophotometry (MALDI-TOF), fortunately increasingly spreading in laboratories, which are relegating biochemical methods, much less accurate, to identification second level.

Given the rarity of male genitourinary infections in which the participation of *C. glucuronolyticum* as an etiological agent has been reported, in this study we review the cases of genitourinary infectious pathology in which the involvement of this species has been proved so far and those publications in which the bacteria is described as a participant in the pathogenesis of CBP.

The genus *Corynebacterium* groups more than 60 species, many of which were recently described. Most have been isolated from animals and humans, and many of them in samples from the urogenital tract [7]. Some are only commensal species and are part of the normal microbiota, while others behave like opportunistic pathogens, so it is necessary to make an identification of the isolate until reaching the species level [8]. They may be part of the normal microbiota of the skin or upper respiratory tract, digestive or genitourinary apparatus.

A way to group corinebacteria, as it appears in a study led by Funke, et al. (1997), is based on its lipophilic or non-lipophilic character [8]. Lipophilic species, in turn, are classified as fermenters and non-sugar fermenters. The species *C. glucuronolyticum* belongs to the group of fermenting lipophilic bacteria.

It was first described by Funke et al. in 1995 [9], isolating it from samples mostly belonging to males in which there was clinical suspicion of a genitourinary infectious process. During the same year another independent research group, headed by Riegel, also isolated a new species belonging to the genus *Corynebacterium*, from semen cultures from males with a his-

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tory of prostatitis and infertility, which he named *Corynebacterium seminale* [10].

Initially, it was considered that two different species had been described, but soon after it was discovered that both had an identical biochemical profile [11]. This seemed to indicate that the two were, in fact, a single species. This was confirmed, in 1997, by Funke et al., by carrying out genetic tests of the alleged two species, which were eventually called *C. glucuronolyticum* [8]. This thesis received the final confirmation two years later, when Tanner et al. sequenced the 16S subunit of rRNA of the strains of the two alleged species, both being genetically identical, with an homology of 97% [12]. Subsequent investigations by Devriese, et al. [13] confirmed that it was definitely the same species.

Corinebacteria contain catalase and grow better in the presence of oxygen, so they can be considered aerobic or facultative anaerobic. They grow in conventional culture media used in the laboratory, albeit in a variable way depending on the species. They multiply well at the standard incubation temperature (37ºC) [14] and are not overly demanding species in terms of environmental conditions, although they do grow faster in atmospheres with an enriched concentration of CO_2 to 5%. One factor that needs to be taken into account is incubation time. After 24 hours, the colonies generated by *C. glucuronolyticum* are not yet very evident, so it is advisable to wait until a minimum of 48 (figure 1). Regarding their phenotypical characteristics, the colonies are mucous and white-yellow. Its shape is convex and its diameter, small, about 1 mm in length. In addition, they exhibit a characteristic smell that has been called "English caramel"[10].

Microscopic observation of colonies of *C. glucuronolyticum* after undergoing a Gram's stain yields similar results to those of other species of corinebacteria. They are described as elongated grampositive bacilli, between 1 and 3 µm in length [8], which can be grouped, either forming pairs, angular shapes, etc.; or even rather isolated [10].

Concerning the results of biochemical tests, variability between different strains is often found. Many of them are able to hydrolyze sculin, making of *C. glucuronolyticum* one of the few species causing human infections with this capacity. Some strains have marked urease activity and others show little or none urease activity. They all ferment glucose and sucrose, and none of them mannitol. Fermentation of maltose, xylose and ribose, as well as nitrate reduction is variable. As a result of glucose metabolization, they produce propionic acid. Other of the metabolites they release are lactate and succinate. *C. glucuronolyticum* shows a powerful ß-glucuronidase and leucine arilamidase activity. Its colonies are non-hemolytic in blood agar, but an intense CAMP effect does occur when they come into contact with the ß-hemolisin produced by *Staphylococcus aureus* [8, 10, 15].

To identify at the species level those strains of corinebacteria that were detected in the laboratory methods such as API Coryne (BioMerieux, France), based on biochemical tests, used to be employed. However, due to the variability of results provided by different strains of the same species, the results of these techniques were not always reliable. Therefore, it is preferable to use methods based on mass spectrometry in order to get the most accurate identification at the species level. Thus, currently, *C. glucuronolyticum* is correctly identified through

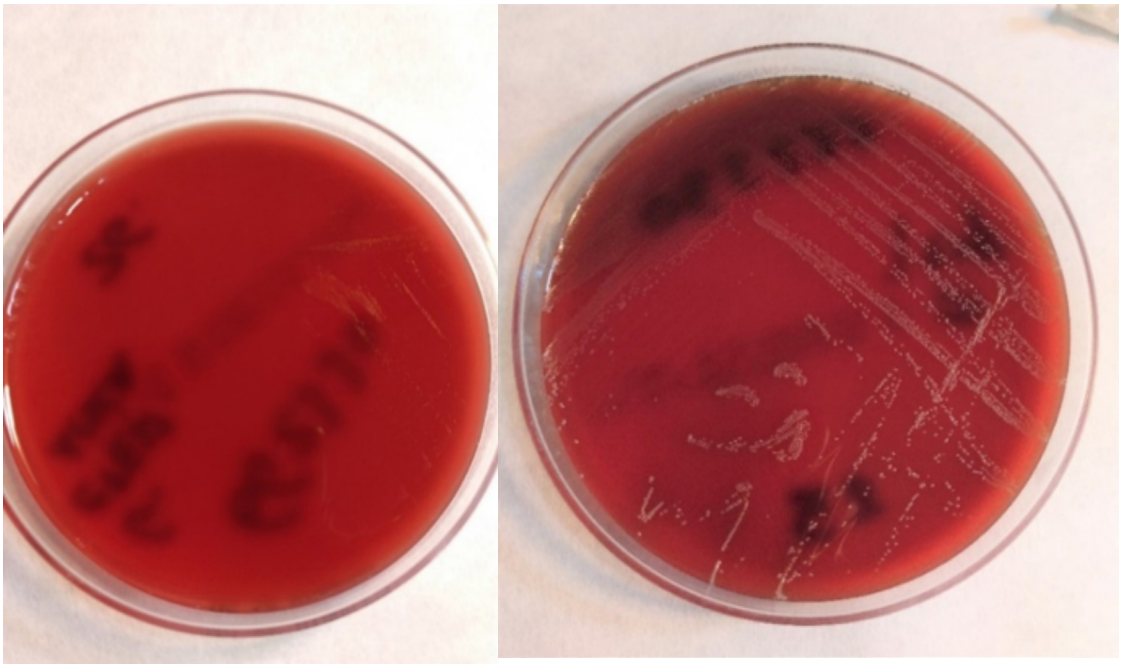

Figure 1 | **Images of the growth of colonies of *C. glucuronolyticum* in lamb blood agar medium at 24 h (left) and 48 h (right), in the presence of CO_2**

Table 1 **Review of reported cases of genitourinary infection caused by *C. glucuronolyticum*.**

Patient number (reference/publication year)	Age/sex	Predisposing factors	Clinical manifestations	Clinical sample	Treatment	Microbiological diagnostic method
1 (13/ 2000)	UNK /M	UNK	Infertility	Semen	UNK	16S rRNA sequencing
2 (13/ 2000)	UNK /M	UNK	Infertility	Semen	UNK	16S rRNA sequencing
3 (13/ 2000)	UNK /M	UNK	Infertility	Semen	UNK	16S rRNA sequencing
4 (13/ 2000)	UNK /M	UNK	Infertility	Semen	UNK	16S rRNA sequencing
5 (13/ 2000)	UNK /M	UNK	Infertility	Semen	UNK	16S rRNA sequencing
6 (13/ 2000)	UNK /M	UNK	Urethritis	Urethral exudate	UNK	16S rRNA sequencing
7 (13/ 2000)	UNK /M	UNK	Urethritis	Urethral exudate	UNK	16S rRNA sequencing
8 (11/ 2002)	46 /M	None	Urethritis	Semen	Trimethoprim-sulfamethoxazole 3 weeks PO	RapID CB Plus system
9 (2/ 2011)	18 /M	Sexual intercourse with multiple partners	Urethritis	Urethral exudate	Ciprofloxacin PO	API Coryne
10 (22/ 2014)	24 /M	None	Urethritis	Urethral exudate and semen	Doxycycline 1 week PO	API Coryne
11 (3/ 2015)	37 /M	None	Urethritis	Urethral exudate, semen and urine	Ciprofloxacin 10 days PO	MALDI-TOF 16S rRNA sequencing
12 (11/ 2015)	57 /M	None	Cystitis	Urine	Vancomycin IV	16S rRNA sequencing
13 (1/ 2018)	28/ M	*Chlamydia trachomatis* urethritis treated with doxycycline (1 week, PO)	Persistent urethritis	Urethral exudate	UNK	API Coryne MALDI-TOF
14 (1/ 2018)	36/ M	*Chlamydia trachomatis* urethritis treated with doxycycline (1 week, PO)	Persistent urethritis	Urethral exudate	UNK	API Coryne MALDI-TOF
15 (1/ 2018)	21/ M	*Chlamydia trachomatis* urethritis treated with doxycycline (1 week, PO)	Persistent urethritis	Urethral exudate	UNK	API Coryne MALDI-TOF
16 (OP/ 2019)	36/ M	None	Chronic prostatitis	Semen	Doxycycline + amoxicillin-clavulanic PO	MALDI-TOF
17 (OP/ 2019)	52/ M	None	Chronic prostatitis	Semen	Levofloxacin 2 weeks PO	MALDI-TOF

UNK: unknown, OP: our patient.

the MALDI-TOF technique with relative ease, providing usually scores above 2 [16].

In order to study the published clinical processes related to this bacteria, an open search of articles in MEDLINE database, with the keywords "*Corynebacterium glucuronolyticum*" was carried out in February 2019. This research made it posible to obtain a total of 15 publications. Subsequently, only published studies showing the pathogenic action of this bacterium at the genitourinary tract were selected by manual reviewing. This led to the final selection of 7 scientific papers (table 1).

In general, CBPs are common infections in males, representing the main cause of prostatic disease in patients under 50 years old and the third cause in patients over 50 years old (only overcome by prostate hyperplasia and prostate cancer) [17]. Although they may be asymptomatic, they usually present with dysuria, tenesm, polaquiuria, nicturia, urinary retention, perineal pain, discomfort during ejaculation, etc. [11]. The most common clinical manifestations in patients with significant microbiological study are perineo-testicular pain, ejaculatory discomfort and hemospermia [17]. Typical urinary pathogens, such as gramnegative bacilli and some enterococci, are the most common cause of bacterial prostatitis. The main etiological agents described in our environment are *Escherichia coli* and *Enterococcus faecalis* [17, 18]. Generally, protocols applied in laboratories are aimed at detecting these etiological agents, making them ineffective in diagnosing other less common agents, such as corinebacteria [11].

To reach the etiological diagnosis of CBP, both urine and semen culture are used in the microbiology laboratory.

The classic method, designed by Meares and Stamey in 1968, which remains the Gold-standard despite its limitations, consists of the conduction of quantitative cultures of pre-ejaculation urine, semen and post-ejaculation urine [19]. Diagnosis of CBP requires a significant count of bacteria in semen and/or post-ejaculation urine, compared to the count obtained in pre-ejaculation urine, although, as suggested by the study conducted by Heras-Cañas et al., the fractional culture for microbiological diagnosis of CBP could be simplified by growing pre-semen urine and semen, without the need to culture samples of post-ejaculation urine. In this series of cases, 96.7% of CBP patients had positive semen cultures, while only 22.95% had positive post-ejaculation urine cultures [17].

However, interpreting the results obtained in segmented cultures is not always an easy task, since, as Nickel concludes, the absence of growth of the microorganism in the culture does not exclude its participation in the pathological process [20]. This, based on the results provided by biopsies of CBP patients, maintains the hypothesis that, through the influence of immune defense and in response to antibiotics, the bacteria can be cantoned in the ducts and prostate acinos forming tiny colonies or biofilms surrounded by a layer of polymers, which protect the bacteria and allow it to persist, even if antibiotics are used to eradicate it.

During the month of June 2018, in the Microbiology Laboratory of the Hospital Virgen de las Nieves in Granada, the species *C. glucuronolyticum* was selectively isolated in counts greater than 10,000 CFU/mL, in two samples of semen from males who were being studied in suspicion of CBP by urologists of the same hospital.

The established protocol for the study of CBP proposed by Heras-Cañas et al., in 2016 [17] was followed. Culture mediums of 5% blood agar and chocolate agar revealed the presence of tiny whitish-yellow colonies convex and circular, with regular edges. Both media were incubated at 37ºC in a CO_2-enriched atmosphere. Bacterial growth could only be clearly detected after 48 hours of incubation, remaining almost undetectable for the first 24 hours. In the two cases described, a CFU number greater than 10,000/mL were obtained; reaching therefore clinically significant values in both cases. The correct identification of the bacteria was carried out through the MALDI-TOF technique.

The first strain isolated corresponded to a 36-year-old patient whose medical history did not report anything relevant except for an alleged allergy to sulfamides. His main complaint when he visited the doctor was a feeling of perineal discomfort that had been present during the previous two weeks; it irradiated to the groin area and was accompanied by a feeling of polaquiuria. In the last 5 days, febricula of up to 37.5ºC, of evening predominance, had appeared. The pre- and post-ejaculation urocultures provided negative results, but *C. glucuronolyticum* could be isolated in semen samples. The isolated strain showed the following MIC values, which were interpreted as susceptible according to the criteria of CLSI 2015 for corinebacteria: trimethoprim/sulfamethoxazole (0.75 mg/L), linezolid (0.064 mg/L), penicillin (0.094 mg/L) and tetracycline (0.0125 mg/L). In addition, the values of MIC were interpreted as resistant following the criteria mentioned above against ciprofloxacin (>32 mg/L), phosphomycin (>256 mg/L) and gentamicin (8 mg/L).

The biochemical analysis and blood count that were requested yielded results within the normal range, without elevation of the acute phase reactants. In terms of treatment, empirical therapy with ciprofloxacin was first dispensed at doses of 750 mg every 12 hours. After verifying that the infecting strain was resistant to quinolones, the administration of trimethoprim/sulfamethoxazole was evaluated and the department of Allergology studied the patient's hypersensitivity to sulfamides. No clinical contraindications were detected and cotrimoxazole was administered at doses of 160/800 mg every 12 hours. A week later, treatment was discontinued because a delayed phase reaction was suspected when a skin rush appeared. Finally, the treatment was changed to a combination of doxycycline (100 mg every 12 hours) and amoxicillin-clavulanic (875/125 mg every 8 hours), for 6 weeks. Within 3 days of starting treatment, febricula subsided and, in the following weeks, the rest of the clinical symptoms disappeared.

The second isolation of *C. glucuronolyticum* was performed in a semen sample from a 52-year-old male, without a medical history of high-interest, who requested a consultation with a specialist of the department of Urology in February 2018. During his visit, he referred symptomatology of a month of evolution consisting of discomfort at the area of the penis, itchy feeling in the urethra, both accompanied by intermittent discomfort in the perineum and during ejaculation. The patient did not describe any febrile syndrome or voiding disfunction.

This second strain isolated showed the following MIC values, which were interpreted as susceptible according to the criteria for corinebacteria in CLSI 2015: trimethoprim/sulfamethoxazole (0.19 mg/L), linezolid (0.094 mg/L), tetracycline (0.094 mg/L), ciprofloxacin (0.047 mg/L) and rifampicin (<0.016 mg/L). In addition, the MIC values were interpreted as resistant according to the criteria mentioned above against clindamycin (>256 mg/L) and erythromycin (>256 mg/L).

Previously, the patient had gone to the emergency room where he was prescribed phosphomycin and tamsulosin. Despite some improvement with this treatment, no total remission of the symptoms had occurred. The examination of the penis and testicles was normal, with no herniary points. The appearance of the urethral meate was also normal. His family doctor, who referred him to the urologist, had requested blood count, biochemistry and urine sediment, which were all normal. PSA (<4) was within the normal range and the uroculture result was also negative. Upon suspicion of CBP, the urologist requested an abdominal and pelvic ultrasound, in which no pathological findings were detected, and the measurement of post-mictional residue that was also normal. In addition, urethral exudate, post-ejaculated urine and semen cultures were requested. The treatment was modified, adding Permixon (1 tab/12h for 1 month) and provisionally suspending tamsulosin.

After receiving the report from the Microbiology laboratory, with culture of semen positive to *C. glucuronolyticum* levofloxacin (1 tab/24h for 6 weeks) was prescribed, with disappearance of the clinical symptomatology of the patient.

In both cases, other etiological agents such as *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Ureaplasma parvum* and *Ureaplasma urealyticum*, which are common causes of genitourinary infections were excluded by using molecular PCR techniques (Becton Dickinson, Sparks, USA).

The study of antibiotic susceptibility is usually carried out by performing E-tests on isolates in lamb blood agar and in HTM in the case of trimethoprim-sulfamethoxazole. The plates are incubated according to the standard procedure, without the need for CO_2-enriched atmosphere, for a period of 24-48 hours; and the MICs are interpreted according to the updated criteria of the CLSI or the EUCAST.

Quinolones are one of the antibiotics that belong to the first line treatment in case of genitourinary tract infections. Recent studies support the success of ciprofloxacin, when it is indicated according to the antibiogram, in the treatment of genitourinary infections caused by *C. glucuronolyticum*, reaching its eradication and a total remission of symptoms [2, 3].

However, in recent years, there has been an increase in in-vitro resistances manifested by this species to different antibiotics, including ciprofloxacin [1, 6, 11, 22, 23]. Proof of this is that one of the isolated strains in the laboratory gave a MIC interpretable as resistant to ciprofloxacin (>32 mg/L). The mechanism used by *C. glucuronolyticum* to acquire such resistance could consist of a mutation in the *gyrA* gene. This can be drawn from the fact that it has been confirmed that other species of corinebacteria have acquire resistance to ciprofloxacin by using a similar mechanism [14].

Tetracyclines are one of the groups of antibiotics most commonly used in the treatment of non-gonococcal urethritis. However, there have been numerous cases in which isolates of the species *C. glucuronolyticum* have turned out to be resistant to tetracyclines [1, 3, 11, 22]. Despite this fact, the two isolated strains in the laboratory of this hospital gave MIC values corresponding to the susceptible category (0.094 – 0.125 mg/L). In addition, doxycycline (at 100 mg doses for 7 days) has proven to be effective in treating urethritis caused by *C. glucuronolyticum* [23].

Azithromycin is used, associated with ceftriaxone, in the empirical treatment of urethritis. But, resistance to both azithromycin and erythromycin [2, 3, 11] is common. This is usually due to the presence of the methylase *ermX* gene, which is associated with the macrolide-resistant phenotype – lincosamide – streptogramine B (MLSb). The expression of erythromycin resistance seems to be linked to clindamycin resistance [22]. The isolate in which erythromycin was tested in this laboratory proved to be resistant, with a MIC of >256 mg/L. It was also resistant to clindamycin, obtaining MIC >256 mg/L.

Phosphomycin, indicated in the treatment of uncomplicated low-tract urinary tract infections, was tested in one of the isolated strains in this laboratory, showing it resistance to this antibiotic (MIC >256 mg/L). Conversely, the two isolated strains of *C. glucuronolyticum* turned out to be susceptible to trimethoprim-sulfamethoxazole, obtaining MICs of 0.19 and 0.75 mg/L. In addition, this antibiotic has an excellent penetration capacity in prostatic tissue.

In general, *C. glucuronolyticum* is susceptible to beta-lactamic antibiotics providing relatively low MIC values [11, 22]. Penicillins are, within this group, the most useful antibiotics to provide outpatient treatment to patients with urinary tract infections caused by grampositive bacilli, but they lack clinical efficacy, due to the difficulty they find to penetrate the prostate. Previous studies show that *C. glucuronolyticum* is susceptible to penicillin [1-3, 11, 22, 23], amoxicillin-clavulanic [22, 23] and piperacillin-tazobactam [23]. Penicillin was tested in one of the isolated strains in the laboratory and a MIC value of 0.094 mg/L was obtained, corresponding to the range of susceptible.

The use of gentamicin, despite being restricted by its toxicity and difficulty in entering the prostatic tissue, may be indicated in the treatment of other genital infections in male, since *C. glucuronolyticum* is usually susceptible to it [1, 11, 22, 23]. However, genemicin-resistant strains [3] have been detected, as sustains one of the results obtained in our hospital (MIC 8 mg/L).

To date, no resistance to linezolid has been reported [3, 22]. This is a useful antibiotic versus grampositive bacteria, representing an effective alternative for the treatment of CBPs. The two isolated strains in this laboratory were susceptible to it (MIC 0.047-0.094 mg/L). Rifampicin was also very active against this species [1-3, 11, 22], as corroborates the MIC values obtained by this laboratory, which was <0.016 mg/L in the two strains.

More recently [24] we have reviewed the antibiotic susceptibility reported by all the strains of *C. glucuronolyticum* that were isolated in the genitourinary samples that our center received during the years 2017 and 2018. In this research, a series of 7 microbiologically significant isolates of *C. glucuronolyticum*, all from males (1 urine, semen 4 and 2 of urethral exudates) were reviewed retrospectively. Linezolid, rifampicin, trimethoprim-sulfamethoxazole and vancomycin were active in all strains tested; but 83% of the strains were susceptible to tetracycline, 50% to penicillin, 43% to ciprofloxacin, 25% to gentamicin and erythromycin, and 20% to clindamycin.

Although *C. glucuronolyticum* is not frequently isolated in clinical genitourinary samples, its potential pathogenicity has been demonstrated and, therefore, the necessary procedures to detect this species should be included in the diagnostic algorithm of these infections.

To identify some of the less common ethological agents causing prostatitis, such as *C. glucuronolyticum*, some methods differing from the traditional growing in culture media need to be employed. These methods are neccessary but they are not included in the standard protocols of the laboratories.

This fact suggests that there might be an unacceptable number of false negatives. Lack of awareness means that many cases of chronic prostatitis fall directly into the category of non-infectious by providing the culture of samples with a falsely negative result.

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None to declare

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Mestrovic T. A microbial game of whack-a-mole: clinical case series of urethral uncloaking phenomenon caused by *Corynebacterium glucuronolyticum* in men treated for *Chlamydia trachomatis* urethritis. Infection. 2018; 47; 1:121-4. doi: 10.1007/s15010-018-1211-8.
2. Galán Sánchez F, Aznar Marín P, Marín Casanova P, García Martos P, Rodríguez Iglesias M. Urethritis due to *Corynebacterium glucuronolyticum*. J Infect Chemother 2011; 17:720–1. doi: 10.1007/s10156-011-0237-y.
3. Gherardi G, di Bonaventura G, Pompilio A, Savini V. *Corynebacterium glucuronolyticum* causing genitourinary tract infection: Case report and review of the literature. IDCases. 2015; 2:56-8. doi: 10.1016/j.idcr.2015.03.001.
4. Novo-Veleiro I, Hernández-Cabrera M, Cañas-Hernández F, Pisos-Álamo E, Francés-Urmeneta A, Delgado-Yagüe M, et al. Paucisymptomatic infectious prostatitis as a cause of fever without an apparent origin. A series of 19 patients. Eur J Clin Microbiol Infect Dis. 2013; 32:263-8. doi: 10.1007/s10096-012-1738-z.
5. Curry CR, Saluja K, Das S, Thakral B, Dangle P, Keeler TC, et al. Encrusted Cystitis Secondary to *Corynebacterium glucuronolyticum* in a 57-Year-Old Man Without Predisposing Factors. Lab Med. 2015; 46:136-9. doi: 10.1309/LMXQP557EINXBXIF.
6. Meštrović T, Bedenić B, Wilson J, Ljubin-Sternak S, Sviben M, Neuberg M, et al. The impact of *Corynebacterium glucuronolyticum* on semen parameters: a prospective pre-post-treatment study. Andrology. 2018; 6:223-9. doi: 10.1111/andr.12453.
7. Khamis A, Raoult D, La Scola B. Comparison between rpoB and 16S rRNA gene sequencing for molecular identification of 168 clinical isolates of *Corynebacterium*. J Clin Microbiol. 2005; 43:1934-6. PMID: 15815024.
8. Funke G, von Graevenitz A, Clarridge JE 3rd, Bernard KA. Clinical microbiology of coryneform bacteria. Clin Microbiol Rev. 1997; 10:125-59. PMID: 8993861.
9. Funke G, Bernard KA, Bucher C, Pfyffer GE, Collins MD. *Corynebacterium glucuronolyticum* sp. nov. isolated from male patients with genitourinary infections. Med Microbiol Lett. 1995; 4:204-15.
10. Riegel P, Ruimy R, de Briel D, Prévost G, Jehl F, Bimet F, et al. *Corynebacterium seminale* sp. nov., a new species associated with genital infections in male patients. J Clin Microbiol. 1995; 33:2244-9. PMID: 7494009.
11. Netzel TH, Rand KH. Prostatitis associated with *Corynebacterium glucuronolyticum*. Clin Microbiol Newslett. 2002; 24:94–5. doi: 10.1016/S0196-4399(02)80023-X.
12. Tanner MA, Shoskes D, Shahed A, Pace NR. Prevalence of corynebacterial 16S rRNA sequences in patients with bacterial and "nonbacterial" prostatitis. J Clin Microbiol. 1999; 37:1863-70. PMID: 10325338.
13. Devriese LA, Riegel P, Hommez J, Vaneechoutte M, de Baere T, Haesebrouck F. Identification of *Corynebacterium glucuronolyticum* strains from the urogenital tract of humans and pigs. J Clin Microbiol. 2000; 38:4657-9. PMID: 11101617.
14. Bernard K. The genus *Corynebacterium* and other medically relevant coryneform-like bacteria. J Clin Microbiol. 2012; 50:3152-8. PMID: 22837327.
15. Riegel P, Ruimy R, Christen R, Monteil H. Species identities and antimicrobial susceptibilities of corynebacteria isolated from various clinical sources. Eur J Clin Microbiol Infect Dis. 1996; 15:657-62. PMID: 8894575.
16. Alatoom AA, Cazanave CJ, Cunningham SA, Ihde SM, Patel R. Identification of non-diphtheriae *Corynebacterium* by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2012; 50:160-3. doi: 10.1128/JCM.05889-11.
17. Heras-Cañas V, Gutiérrez-Soto B, Serrano-García ML, Vázquez-Alonso F, Navarro-Marí JM, Gutiérrez-Fernández J. Prostatitis crónica bacteriana. Estudio clínico y microbiológico de 332 casos. Med Clin. 2016; 147:144-7. doi: 10.1016/j.medcli.2016.05.018.
18. Sorlózano-Puerto A, Esteban-Sanchís P, Heras-Cañas V, Fernández-Parra J, Navarro-Mari JM, Gutiérrez-Fernández J. Estudio prospectivo de la incidencia de patógenos genitales oportunistas y estrictos que crecen en medios de cultivo artificiales. Rev Lab Clín. 2018; 11: 123-30. doi: 10.1016/j.labcli.2017.11.009.
19. Meares EM, Stamey TA. Bacteriologic localization patterns in bacterial prostatitis and urethritis. Invest Urol. 1968; 5:492-518. PMID: 4870505.
20. Nickel JC. Prostatitis: myths and realities. Urology. 1998; 51:362-6. PMID: 9510337.
21. Krieger JN, Riley DE, Roberts MC, Berger RE. Prokaryotic DNA sequences in patients with chronic idiopathic prostatitis. J Clin Microbiol. 1996; 34:3120-8. PMID: 8940458.
22. Funke G, Pünter V, von Graevenitz A. Antimicrobial susceptibility patterns of some recently established coryneform bacteria. Antimicrob Agents Chemother. 1996; 40:2874-8. PMID: 9124857.
23. Mestrovic T, Bedenic B, Ljubin-Sternak S, Sviben M, Profozic Z. Ciprofloxacin resistant *Corynebacterium glucuronolyticum* as a cause of male urethritis syndrome. JMM Case Rep. 2014; 2:7. DOI 10.1099/jmmcr.0.000208.
24. Casanovas-Moreno-Torres I, Borrego-Jiménez J, Calatrava-Hernández E, Foronda- Garcia-Hidalgo C, Jimémenz-Guerra G, et al. Estudio de la sensibilidad antibiótica de *Corynebacterium glucuronolyticum* y *Aerococcus* spp. en muestras genitourinarias durante los años 2017 y 2018. AMPAC. 2018;1:114-5. http://ampac.sampac.es/sites/ampac.sampac.es/files/AMPAC_VOL1_NUM2_0.pdf