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Sumario

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Cartas al Director

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Review

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ABSTRACT

The incidence and prevalence of sepsis depend on the definitions and records that we use and we may be underestimating their impact. Up to 60% of the cases come from the community and in 30-60% we obtain microbiological information. Sometimes its presentation is ambiguous and there may be a delay in its detection, especially in the fragile population. Procalcitonin is the most validated biomarker for bacterial sepsis and the one that best discriminates the non-infectious cause. Presepsin and pro-adrenomedullin are useful for early diagnosis, risk stratification and prognosis in septic patients. The combination of biomarkers is even more useful to clarify an infectious cause than any isolated biomarker. Resuscitation with artificial colloids has worse results than crystalloids, especially in patients with renal insufficiency. The combination of saline solution and balanced crystalloids is associated with a better prognosis. Albumin is only recommended in patients who require a large volume of fluids. The modern molecular methods on the direct sample or the identification by MALDI-TOF on positive blood culture have helped to shorten the response times in diagnosis, to optimize the antibiotic treatment and to facilitate stewardship programs. The hemodynamic response in neonates and children is different from that in adults. In neonatal sepsis, persistent pulmonary hypertension leads to an increase in right ventricular afterload and heart failure with hepatomegaly. Hypotension, poor cardiac output with elevated systemic vascular resistance (cold shock) is often a terminal sign in septic shock. Developing ultra-fast Point-of-Care tests (less than 30 minutes), implementing technologies based on omics, big data or massive sequencing or restoring "healthy" microbiomes in critical patients after treatment are the main focuses of research in sepsis. The main benefits of establishing a sepsis code are to decrease the time to achieve diagnosis and treatment, improve organization, unify criteria, promote teamwork to achieve common goals, increase participation, motivation and satisfaction among team members, and reduce costs.

Key Words: Sepsis, epidemiology, microbiological diagnosis, resuscitation, biomarkers, stewardship programs, economic evaluation

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Aspectos actuales en el enfoque de la sepsis. Volviendo las aguas al cauce

RESUMEN

La incidencia y la prevalencia de la sepsis dependen de las definiciones y de los registros que empleamos y podemos estar

infravalorando su impacto. Hasta el 60% de los casos proceden de la comunidad y en un 30-60% obtenemos información microbiológica. Su forma de presentación es en ocasiones ambigua y puede haber retraso en su detección, sobre todo en población frágil. La procalcitonina es el biomarcador más validado para la sepsis bacteriana y el que discrimina mejor la causa no infecciosa. La presepsina y la pro-adrenomedulina son útiles para el diagnóstico precoz, la estratificación de riesgo y el pronóstico en pacientes sépticos. La combinación de biomarcadores es aun más útil para esclarecer una causa infecciosa que cualquier biomarcador aislado. La reanimación con coloides artificiales tiene peores resultados que los cristaloides, especialmente en pacientes con insuficiencia renal. La combinación de solución salina y cristaloides equilibrados se asocia con un mejor pronóstico. la albúmina solo se recomienda en aquellos pacientes que requieren un gran volumen de líquidos. Los modernos métodos moleculares sobre la muestra directa o la identificación por MALDI-TOF sobre hemocultivo positivo han ayudado a acortar los tiempos de respuesta en diagnóstico, a optimizar el tratamiento antibiótico y a facilitar los programas de optimización. La respuesta hemodinámica en neonatos y niños es diferente a la de los adultos. En la sepsis neonatal, la hipertensión pulmonar persistente conduce a un aumento de la postcarga del ventrículo derecho y la insuficiencia cardíaca con hepatomegalia. La hipotensión, el gasto cardíaco deficiente con elevadas resistencias vasculares sistémicas (shock frío) son a menudo un signo terminal en el shock séptico. Desarrollar pruebas Point-of-Care ultrarrápidas (menos de 30 minutos), implementar tecnologías basadas en ómicas, big data o secuenciación masiva o restaurar microbiomas "saludables" en pacientes críticos tras el tratamiento son los principales focos de investigación en sepsis. Los principales beneficios de establecer un código de sepsis son disminuir el tiempo para lograr el diagnóstico y tratamiento, mejorar la organización, unificar criterios, promover el trabajo en equipo para lograr objetivos comunes, aumentar la participación, motivación y satisfacción entre los miembros del equipo y reducir costes.

Palabras clave: Sepsis, epidemiología, diagnóstico microbiológico, reanimación, biomarcadores, programas de optimización, evaluación económica

INTRODUCTION

In the last two years, more topics have been written about sepsis than in the former ten. There are new standards in detection and prognosis, microbiological knowledge has been developed obtaining early and reliable results, there are emerging evidences of better initial resuscitation strategies and for the first time there is a greater social awareness in the media. Today, five years after the Declaration of Mallorca, there has been legislation in the European Parliament on Sepsis and each European country, even each region, already has a more or less orchestrated "sepsis code" to attend this process, which results in a higher quality for any health system. For this purpose, the Spanish Society of Chemotherapy (SEQ) has requested experts among the main scientific societies, who attend septic patients (SEMICYUC, SEIMC, SEDAR, AEIP, SEMES, ESCMID, SEDIDA, SEQ, FEPIMCTI), to update the current topics of sepsis, its impact, detection and approach in children and adults. It also includes organizational aspects related to the structure and economic cost of this transversal care process, its inclusion in stewardship programs and current trends in research.

The text has been structured in the following headings: epidemiology of sepsis in the world, evidences in sepsis detection programs, microbiological diagnosis, new evidence in initial resuscitation, usefulness of biomarkers, sepsis in pediatric patients, stewardship programs, new horizons for research, economic evaluation and the importance of sepsis multidisciplinary structure in healthcare.

CURRENT CONTEXT AND EPIDEMIOLOGY OF SEPSIS IN THE WORLD

The actual epidemiology of sepsis is currently unknown and extremely variable, since it will depend on what we are analyzing, from incidence or prevalence to mortality. The incidence of sepsis will depend on the definitions we make of it. Recently, these definitions have changed, with many controversies and we do not have any study that evaluated their impact on incidence [1].

We have to make some considerations when assessing the incidence: when, where, what or how we measure. For example, *when*: the CDC estimated in 1979 that the incidence of sepsis was 73.6 per 100,000 inhabitants and calculated it had increased to 175.9 in 1989, but estimated septicemia and not severe sepsis [2]. Meanwhile, in Germany the incidence of in-hospital sepsis has averaged 5.7% per year from 2007 to 2013, reaching 335 per 100,000 cases per year in 2013 [3]. It is important to assess *where* we measure. Recently, the World Health Organization estimated 30 million cases of sepsis, 19.4 million by severe sepsis and 6 million deaths per year in the world [4]. However, these data were collected from a meta-analysis that analyzed the global incidence of sepsis in 27 studies, and only seven developed countries were included: USA, Germany, Australia, Norway, Sweden, Taiwan and Spain [5]. This is an extremely significant limitation, since about 87% of the world's population was not included. Another aspect is *what* we are analyzing. For example, according to severity, one meta-analysis describes 288 cases of sepsis and 148 cases of severe sepsis per 100,000 inhabitants per year [5]. A reference of that study, including Spanish data, identified 240,939 cases of severe sepsis, 1.1% of all hospitalizations between 2006-2011 [6]. In addition, *what areas* are we measuring? only in ICU, emergency department, hospitalization areas or in the whole Hospital?. Esteban et al described 366 cases per 100,000 inhabitants per year when assessing the entire hospital [7]. These figures go up if we analyze patients in critical areas where there may be 4-6 new cases of sepsis per 100,000 inhabitants per day [2-8]. Another important epidemiological data is to know the *origin* of sepsis, which it is community in most cases, around 60-70% of whole cases [2], followed by hospital-acquired outside ICU in 20-30%, while cases of in-

ICU origin were the least frequent, around 5-9% [1-9]. We obtain microbiological information in 35-60% of cases and bacteremia only in 15-30% [3,5-9]. It is also important to highlight the presence of *organic dysfunction* (OD) that is part of the current definition of sepsis, but with enormous variability according to the study we analyze: 30-50% have one OD, 20-30% two and 20-25% three or more at the time of detection [5,8,9]. Respiratory failure is the most common OD in all studies.

Despite advances in diagnosis, epidemiology still suffers from the enormous variability. Several factors influence, such as poorly classified records of different infectious pathologies and the concept of sepsis in a specific way, poorly or not designed for this purpose, little information at a global and specific level [10-12]. Most of the studies are retrospective, they use the coding of the discharge reports and therefore have a great variability, depending on the capacity of who performs the classification. It is estimated that around 50% of cases of sepsis based on coding are not correctly classified in the USA [2,8,10-12]. Another study by Bouza et al compared using the ICD-9 to directly identify cases of sepsis with another model that combined this plus the identification of OD with a modified code [11]. They obtained an explicit classification of severe sepsis in 62.2% of the cases and the other 37.8% were obtained with the modified code combination. Authors found statistical differences in the incidence, comorbidities, OD and even in mortality between two groups. Other studies are based on the voluntary inclusion of cases, which also generate many problems to extrapolate rates, even in the same region where this incidence or prevalence has been obtained [13]. Regarding mortality this variability is also very marked, and will depend on multiple factors: severity, type of patients, place of analysis, hospital area [2,8,10]. For example, in a recent German study the mortality from severe sepsis and shock was 43.6% and 58.8% [3], respectively. While other series of septic shock has dropped to 22-25% [2-10]

Different aspects from social, economic, political, health (for example, genetic) and even climatological can influence on the epidemiology of sepsis. These factors are extremely dynamic and it's impossible to know or approximate sepsis epidemiology measuring with methods we use today. Solution will be to obtain personalized and high-quality information in an automated way using new technologies, such as Big Data and Artificial Intelligence to reduce variability and generate a precision medicine, properly classifying cases of sepsis.

NEW EVIDENCE IN SEPSIS DETECTION PROGRAMS AND RESULTS

Despite advances, sepsis remains one of the most deadly emergency department (ED) arrival or hospital-acquired conditions [14]. The initial attention of sepsis remains uneven and often slow [15]. There is no one specific test to diagnose sepsis, and a number of different screening tools and biomarkers have been used.

Attention to the pre-hospital phase in patients with sepsis is clearly critical. The initial link in this chain is to increase awareness of sepsis symptoms amongst the public alerting and the importance of seeking medical attention when people display them. Pre-hospital care also plays an important role in recognizing and providing prompt care for patients with sepsis. Approximately 50% of the patients who present to the ED with sepsis will arrive via an Emergency Medical Service (EMS) [16]. Early identification of patients with severe sepsis by EMS providers utilizing a screening tool and a point-of-care venous lactate meter has shown to be feasible [17]. In the ED, there are two main limitations when it comes to optimizing initial sepsis management. Firstly, the difficulty of identifying those patients with this condition, due to the ambiguous nature of the initial manifestations of sepsis, which hinder the diagnosis. This identification is even more complex in elderly and in inmunocompromised patients, more and more often seen in the emergency room. Second, there is variable adherence to the guidelines on the initial management of sepsis by health personnel and early initiation of resuscitation [18].

It is reported a higher mortality rate among ward patients. These populations often have concurrent medical or surgical conditions that confound the diagnosis, making early recognition difficult. Although the causes of this remain little known, many factors play an important role and Schorr et al. [19] have described some of them. First, the diagnosis of severe sepsis may be delayed in ward patients because of physicians or nurses may not identify the progression of sepsis and/or because hospitalized patients may not show obvious systemic manifestations of the process. Second, ward patients may have differences in the timing of their presentation and concurrent conditions confounding the diagnosis. Third, treatment may be delayed once the diagnosis is made on the ward. The Intensive Care Unit and ED are units designed to provide rapid high-acuity care, whereas wards have fewer systems and resources for rapid delivery of care needed for severe sepsis. Finally, some patients on the ward may develop sepsis from nosocomial infection, which can portend a worse prognosis.

One area that offers ongoing promise with regards to the early identification of patients with sepsis is the use of biomarkers. Traditional individual markers of sepsis, such as the total white cell count, neutrophil count, and C-reactive protein, lack the specificity to allow them to discriminate between those patients with an inflammatory response to trauma or surgery, for example, and those with a new infection. In this sense, procalcitonin has shown to have the best accuracy to identify patients with invasive bacterial infection [20]. The Surviving Sepsis Campaign Guidelines endorses the use of procalcitonin.

The Sepsis Code (SC) is a way to provide a tool for standardization in early detection, management, and initiation of therapeutic measures in order to improve the patient´s clinical results. It is based on the structured application of the set of measures proposed by the Surviving Sepsis Campaign, and prioritizing time-adjusted attendance [21]. Several studies have shown how its implantation has improved the results in terms

of mortality in patients attended by sepsis [21, 22]. The cornerstone of a sepsis code program is nurse, who could serve as the initial detector of signs of sepsis, as well as the initiators of evidence-based diagnosis and treatment protocols. The incorporation of the nurse's assessment could be a valuable feature for establishing an alert [19].

The use of automated electronic sepsis alert system to improve sepsis management represents an area of active research [23]. Identifying patients with sepsis in a busy ED may be aided by electronic sepsis alert systems [24, 21], or screening tools, which combines simple clinical characteristics with the use of early lactate measurements [25]. Identifying patients who deteriorate within the hospital secondary to sepsis presents an additional challenge. The widespread introduction of rapid response systems has led to the early identification and the initiation of early intervention to patients within the hospital system [22, 26, 27].

Response program was associated with substantial and sustained decreases in inpatient death rates in patients treated for sepsis [28-30]. The presumed mechanism by which early detection of sepsis reduces in-hospital mortality and reduces the costs of inpatient care is that it stops the progression of sepsis along the trajectory to severe sepsis and septic shock and avoids their attendant morbidity and treatment costs. The four key elements for sepsis early recognition and response program could be summed in organizational commitment, health information technology support bedside, evidence-based screening and response protocols, and nursing taskforce education and training [31].

UPDATE ON MICROBIOLOGICAL DIAGNOSIS IN SEPSIS

A rapid response from the microbiology laboratory is a hallmark in hospital settings as in general terms close to 70% of the clinical decisions for the patient's management are based on laboratory results. This is particularly true in the case of sepsis, for which a very rapid response with regard to patient treatment is critical for patient outcome [32]. Blood culture-based diagnosis is still the gold standard procedure for identification of the microorganism causing bloodstream infection (BSI). However, they are limited when an antibiotic treatment is started before the blood is taken or for fastidious microorganisms. Once the microorganism is isolated and the antibiogram performed, the microbiogical report allows the administration of the adequate antimicrobial treatment. This will permit a reduction in the spectrum of empirical administrated anti-infectious drugs. Such de-escalation reduces the negative impact of combined treatments and/or broad-range antibiotics in term of side effects and in terms of selection pressure on the commensal microbiota, with consequent increase in prevalence of resistant strains. The first 3-6 hours after the clinical suspicion are critical to establish therapeutic measures that improve prognosis, therefore, a microbial diagnosis in less than 6 hours would undoubtedly benefit the optimal management of patients [33].

Microbial diagnosis of sepsis generally starts by blood culture (BC) because of the low quantity of microbes in the blood during such infections. BCs are in continuous optimization in the last years to increase the sensitivity and specificity of microorganism recovery. Nevertheless, in of 50% of cases, BSIs yielded a negative BC, and in sepsis even a higher number of BC occur with negative results, which can delay the introduction of an adequate antimicrobial therapy [34]. This can be due to very low number of circulating microbes (it can reach 1 to 10 CFU/mL or even less), to uncultivable or fastidious microorganisms, or when antibiotic treatment is initiated before blood sampling [35].

However, even in the best scenario and with septic patients with positive blood cultures results, the time required to achieve an etiological diagnosis and some data about the profile of the antimicrobial treatment can range differently. For instance, from a few hours (1-6 hours), if a molecular method (Fluorescence in situ hybridization, Point of care PCR, microarrays) is applied directly to the positive BC, in a few hours more (2-18 hours in the best of cases) we can perform a subculture to identify the pathogen and achieve a profile of antimicrobial sensitivity [36]. Special mention deserves the MALDI-TOF / MS (matrix-assisted laser desorption / ionization time-of-flight) when applied directly from the positive BC, and that allows in <1 hour to achieve in most cases the identification of the pathogen causing the BSI [37]. Noteworthy is the recent application of MALDI-TOF for the determination of antibiotic-resistant bacteria from direct positive BC, achieving in less than 1 hour to identify Gram-negative bacilli producing carbapenemase enzymes [38].

For all these reasons, it is much more interesting to have an etiological diagnosis of sepsis from the patient´s direct blood rather than from positive BC after blood incubation. Most of the current procedures are molecular-based methods. One of the main advantages of working directly from blood is the reduced time to results. First, microorganism detection is independent of enrichment via BC; second, microorganism identification is culture independent as no requires incubation time, and finally, culture independent methods give a snapshot of what is going on in the bloodstream. The low detection limit of specific PCRs can potentially make them more sensitive than BC [35].

Despite the existence of several commercial systems that allow a direct blood diagnosis, none of them has so far, reached a level of development that is sufficiently reliable for its implementation in daily clinical practice in the microbiology laboratory. Reasons for failure may rely on lack of sensitivity due to the intrinsic methodology factors as well as whole blood DNA interference as well as reduced specificity, resulting in false positive results [39, 40]. DNA can bring contamination from the environment or from PCR reagents (carriage of DNA from previous positive results). In addition, false-positive PCR findings can be due to circulating cell-free DNA from dead bacteria or fungal DNA in the absence of infection-DNAemia rather than a true bacteremia or fungemia. Finally, an infection successfully controlled by the immune system or by an

efficient antimicrobial therapy will kill the pathogen, thus releasing pathogenic DNA that can persist several days in the blood [41]. In this scenario, very promising is the new system of diagnosis of sepsis from direct blood. The T2Dx system (T2 Biosystems) represents the first equipment capable of completely automating the diagnosis of circulating pathogens in the blood of patients, and of carrying out the entire process in a turnaround time of three to four hours after obtaining the sample [42, 43]. The T2Dx system applies an innovative approach to the diagnosis of sepsis. The combination of paramagnetic nanoparticle sensors with the detection of them by nuclear magnetic resonance T2, allows the detection of pathogens in blood with a very high sensitivity (> 95%), not reached by the technologies available until now. The T2 system is capable of detecting pathogens at extremely low levels, up to a single cell per milliliter of blood. Cartridges are currently available for the diagnosis of the microorganisms most frequently involved in sepsis, both bacteria and fungi. More studies are however, needed, to confirm the suitability of this system in the diagnosis of sepsis. A summary of the main commercially available systems for identification of microbes directly from blood samples is shown in table 1.

NEW EVIDENCE IN INITIAL RESUSCITATION STRATEGIES

Septic patients suffer from hypovolemia due to two principal mechanisms; relative hypovolemia owing to vascular vasodilatation and rapid fluid loss from vasculature as glycocalyx becomes degraded (both caused by the effect of several inflammatory mediators) [44]. Therefore normalization of volemia is a key issue to achieve blood pressure stabilization (Medium Blood Pressure at least 65 mmHg) [45]. In the early 2000 sepsis resuscitation was guided by searching specific hemodynamic objectives based on the protocol published by Rivers [46]. However, this approach has been challenged following the failure to show a mortality reduction in three subsequent large multicenter studies [47-49]. Moreover, one vast study performed in septic African children showed better results in terms of mortality in the group not receiving fluid bolus in the resuscitation phase [50]. The fact that the study was carried out in children and that most of them had malaria makes it difficult to extrapolate the results to the general population. Truly, no human data has shown that fluid resuscitation reliably improves blood pressure or end-organ perfusion and even some experimental data revealed that organ perfusion could be supranormal in hyperdynamic sepsis and that fluid resuscitation may increase mortality [51,52].

Despite all above, the Surviving Sepsis Campaign keeps recommending the urgent administration of fluid bolus (30 ml/Kg) in the first three hours. Authors encourage initiating this proceeding and further evaluate patient response and clinical characteristics [53]. Fluid therapy is basic in the resuscitation of sepsis but, at the same time, is well known that fluid overload is related to a worse outcome [54]. Recently the existence of 4 phases in sepsis resuscitation has been proposed: salvage, optimization, stabilization and de-escalation [55]; in the first phase, boluses of empirical fluids are administered, in the second stage boluses must be adjusted according to fluid responsiveness parameters and later we must minimize fluidtherapy and even search for negative balance.

Which is the best fluid for my septic patient? The choice of the optimal fluid for the resolution of sepsis remains a matter of debate and the old controversy between colloids and crystalloids continues. Randomized clinical trials of resuscitation with artificial colloids show negative results, especially those of hydroxy-ethyl-starches (HES) (the most studied) with higher incidence of renal failure, need of renal replacement techniques and even higher mortality in patients receiving HES [56-58]. For this reason, current recommendation is to use crystalloids in resuscitation of sepsis and avoid artificial colloids [53]. With regard to crystalloids, several studies have been published in recent years comparing saline 0.9% with balanced crystalloids. Despite there is not enough evidence to recommend its use as the fluid of choice over saline 0.9% [53, 59, 60], it does seem that the combination of both fluids (0.9% saline and balanced crystalloids) is associated with a better prognosis [61]. In the subgroup of septic patients of the SMART study, a better outcome was observed in patients resuscitated with balanced crystalloids [62]. At least, in situations in which metabolic acidosis or hyperchloremia appears during resuscitation, we should use balanced crystalloids. Albumin use as part of fluid resuscitation keeps on being a controversial issue. Although some studies and even meta-analysis have shown beneficial effects in terms of mortality when albumin was compared to other fluids or specifically to crystalloids, more recent trials have failed to demonstrate a clear benefit [53, 63, 64]. Experts have salomonically decided to recommend the administration of albumin only in those patients in whom is expected a wide need of fluids (weak recommendation, low quality of evidence).

USEFULNESS OF BIOMARKERS IN SEPSIS: FROM RESEARCH TO AN EFFICIENT PRACTICE

An ideal sepsis biomarker should have all of the following characteristics: fast and specific increase in sepsis, rapid decrease after effective therapy, short half-life and fast and widely available and reliable method of determination. Unfortunately, none of the current biomarkers exhibits all of these specifications in full.

By far the most studied biomarkers are procalcitonin (PCT) and C reactive protein (CRP). CRP is sensitive but not very specific, being increased in all inflammatory disorders. Despite its limitations, PCT differentiates better between infectious and noninfectious causes of critical illness than CRP [65]. However, different meta-analysis evaluating the ability of PCT to separate sepsis from non-infectious inflammation among critically ill patients showed under-performance of the biomarker, with mean sensitivity and specificity round to 70%, and an area under the summary receiver operator characteristic curve (AUC of the ROC curve) less than 0.80 [66]. For that reason, a careful

Table 1 Summary of the main commercially available systems for identification of microbes directly from blood samples

System	Method	Time to result (hours)	Blood volume (mL)	Microorganism coverage	Resistance and virulence markers	Sensitivity, specificity, and correlation with conventional methods (%)	Comments	Ref
SepsiTest Molzym Bremen,Germany	Broad-range PCR + sequencing	6	1-10	>345 bacteria (Gram+, Gram-) and fungi	0	21-87, 85-96, NR	Pros: can be used in other sterile samples. Cons: variable sensitivity and specificity	35
SeptiFast Roche Molecular System, Basel, Switzerland	Multiple broad-range real-time PCR	3.5-5	1.5	6 Gram+ 8 Gram- 5 fungi	mecA	43-95, 60-100, 43-83	Pros: time to result. Cons: variable sensitivity and specificity, no quantification	35
Magic Plex Seegene, Seoul, Korea	Multiplex PCR + multiplex real-time PCR	3-5	1	21 bacteria (Gram+ and Gram-) at species level 6 fungi	mecA, vanA/B	37-65, 77-92, 73	Pros: fast. Cons: limited number of studies, succession of reaction and device, no quantification	35
VYOO SIRS-Lab, jena Germany	Multiplex PCR + electrophoresis	8	5	14 Gram+, 18 Gram-, 7 fungi	0	NR,NR, 70	Pros: highly sensitive. Cons: limited number of studies, several manual steps	35
PLEX-ID, Abbott Molecular, Carlsbad CA,USA	Multiple broad-range PCR/ESI-MS	6	1.25-5	Up to 800 (Gram+, Gram-, fungi)	mecA, blaKPC, vanA/B	50-91, 98-99, 79-97	Pros: universal, detection of mixed bacterial populations, semiquantitative. Cons: no interventional studies	35
T2 Biosystems Lexington, USA	Multiplex PCR + paramagnetic nanoparticles sensors	3-5	2	5 *Candida* spp. 6 bacteria (2 Gram+, 4 Gram-)	0	91.1, 99.4	Pros: fast, easy to hand, detect 1 CFU/ml. Cons: limited number of pathogens, limited experience	42,43

Adapted from Opota el al. [35]

interpretation of PCT in the clinical context is mandatory [67]. PCT kinetics have also proved to have prognostic value, correlating with disease severity and resolution of illness. Interestingly, PCT serum concentrations could be valuable to monitor clinical response to therapy for sepsis, and have a role in de-escalating antibiotic therapy in the ICU setting [68].

Examples of promising sepsis biomarkers are presepsin, proadrenomedullin and soluble urokinase plasminogen activator receptor (suPAR). Presepsin has demonstrated to be a valuable biomarker for early diagnosis of sepsis, risk stratification, and evaluation of prognosis in septic patients. In a recently published metanalysis presepsin exhibited an area under the summary receiver operator characteristic curve (AUC) of 0.89 for the diagnosis for sepsis [69]. Increasing concentrations of presepsin during the first two days of septic shock presentation predicted higher ICU and 90-day mortality and correlated with the appropriateness of antibiotic therapy [70]. ProADM has demonstrated diagnostic and prognostic role in sepsis [71]. Although its results are comparable to those of classic markers [72], its addition to the latter seems to increase the acuity that these tests show separately [73].

Owing to the complex pathophysiology of sepsis, probably not just looking at one particular biomarker but more likely a combination of readouts will better attain success. Kofoed et al. found that a combination of six biomarkers (sUPAR, soluble triggering receptor expressed on myeloid cells [sTREM]-1, macrophage migration inhibitory factor, CRP, PCT, and neutrophil count) had a significantly greater AUC for an infectious cause of SIRS than did any of the individual markers [74]. On their behalf, Andaluz-Ojeda et al. measured 20 different cytokines concurrently using an automated multiplexed immunoassay approach in 30 patients with severe sepsis. The combined score was more predictive than any one cytokine [75]. Similar results have been observed using different subtypes of immunoglobulins [76].

Research is increasingly focusing on new omics technologies as the future tools associating expression at RNA, protein, and metabolite levels with sepsis diagnosis and prognosis. Several studies have demonstrated different patterns of gene expression able to discriminate between infection and noninfectious acute disease and even between different causative pathogens, as well as differential clinical outcomes, and potentially response to therapeutic interventions [77, 78].

SEPTIC SHOCK IN PEDIATRIC PATIENTS: DIFFERENCES WITH ADULTS

Pediatric sepsis may be defined as a systemic response to infection with the presence of some degree of organ dysfunction [79]. Even though global data are lacking, infection is the leading cause in childhood worldwide (accounting for around 60% of the deaths in children under 5 years) [80]. Physiologically, some main differences between adults and children have to be considered.

Neonatal septic shock with acidosis and hypoxia, often impedes change from fetal circulation pattern (with almost 85% of the fetal circulation by-passing the lungs through the ductus arteriosus and the patent foramen ovale with suprasystemic lung pressures) to the normal neonatal circulation. In neonatal sepsis, persistent pulmonary hypertension (PPH) leads to increased right ventricle afterload and cardiac failure with hepatomegaly, needing pulmonary vasodilatory therapies (nitric oxide, oxygen) that may improve clinical outcome.

The initial clinical presentation of sepsis in children (especially in younger age groups) may be even more difficult to recognize since symptoms and clinical signs are non-specific and often less apparent than in adults. Whereas older children may present with a focus of infection and sepsis typically presents with features of systemic inflammatory response syndrome, including fever, sepsis in newborns often manifests initially as a change in the normal trends of observations for that child, including bradycardic episodes, apneas, or feed intolerance as the first signs. While any infection may precipitate sepsis, grampositive and gramnegative bacteria by far predominate in children. The etiology varies according to host factors, including age, comorbidity, and geographic location. Typical pathogens by patient group are listed in the table 2. Despite adequate microbiological sampling, not uncommonly in children with sepsis the pathogen will not be identified (culture-negative sepsis).

In adults, clinical presentation usually includes a hyperdynamic shock syndrome or warm shock (in more than 90%) with low systemic vascular resistances (SVR) and hypotension but maintaining a normal or even high cardiac output with tachycardia. Usually not lowering central venous oxygen saturations at the beginning, and worsening their myocardial function after fluid resuscitation, with low ejection fractions and ventricular dilatation, with worse outcomes for patients with SVR not amenable to vasopressor therapy [81]. Children often maintain normal blood pressure even in late stages of shock; hypotension is therefore often a terminal sign in septic shock. In spite of these responses to sepsis, pediatric sepsis induces mostly severe hypovolemia, with better response to aggressive fluid management. Almost 50% of the children present vasoconstriction, cold extremities, poor cardiac output and high SVR (cold shock) [81].

Their potential for increasing cardiac output is also more limited than in adults, being even worse in neonates as their resting heart beat rate is already high (120-140 beats per minute) not allowing a high increase of heart rate to relieve diminished cardiac output state (as happens in adults), being vasoconstriction their predominant response. Hypotension is therefore a much later sign in pediatric sepsis, compared to the adult course. This progressive increase in SVR turns detrimental as it may worsen cardiac failure leading to death, so inotropes, vasodilators and Extra-Corporeal Membrane Oxygenation (ECMO) support to cardiac function are appropriate for treating pediatric septic shock. In addition, some vascular accesses are much more common in pediatrics than in adults, using umbilical venous and arterial lines in neonates, and intraosseous accesses in children while central vascular lines are obtained. The prognosis in children is variable depending on the

Table 2 Typical pathogens in neonatal and childhood sepsis

Neonatal sepsis
Early Onset (first 72 hours of life)
Group B streptococci, Gram negative bacilli (especially *E. coli*): most frequent pathogens
Staphylococcus aureus and coagulase negative *staphylococci*, *enterococci* and *Haemophilus influenzae*
Listeria monocytogenes
Late Onset (after 72 hours of life until 1 month)
Coagulase negative *staphylococci* (especially associated to vascular catheters)
Same organisms as early onset
Infants and young children
Diarrhoea and pneumonia are the most common infections in poor resource settings
Streptococcus pneumoniae
Neisseria meningitidis in bimodal age distribution (young children and adolescents)
Staphylococcus aureus and group A *Streptococci*
Haemophilus influenza type b (less in developed countries because of vaccination)
Bordetella pertussis
Infants and children in hospital
Depends on local epidemiology
Coagulase negative *Staphylococci* with vascular catheters
Methicillin resistant *Staphylococcus aureus* (more in USA)
Gram negative organisms (*Pseudomonas aeruginosa*, *Klebsiella species*, *Acinetobacter species*)
Asplenic or functional asplenia
Salmonella species producing sepsis and osteomyelitis in sickle cell disease)
Encapsulated organisms (*Streptococcus pneumoniae*, *Haemophilus influenza*...)
Mosquito-borne disease
Malaria (*Plasmodium falciparum*), dengue virus and *Burkholderia pseudomallei*
Others
Fungal (*Candida species*, *Aspergillus species*) and viral (influenza, respiratory syncytial virus, human metapneumovirus, varicella and herpes simplex virus)

age and predisposing conditions being the overall current mortality around 10% in children, lower than in adults who frequently have associated comorbidities [82].

STEWARDSHIP PROGRAMS IN SEPSIS

Multiple definitions for sepsis have been proposed along the last 10 years. A clinical syndrome that is this hard to define, not surprisingly, is difficult to diagnose. Timely administration of active antimicrobials has been a keystone of sepsis management even before it was included in the original Surviving Sepsis Campaign (SSC) guidelines [83]. However, lack of sepsis diagnostic specificity hampers clinical sepsis pathway implementation and may drive inappropriate antimicrobial use.

The concept antimicrobial stewardship (AS) is often considered to only include efforts to reduce or restrict use of expensive and broad-spectrum antimicrobials. The real exertion of and AS program should be on getting the right antimicrobial in the right dose to the right patient for the right amount of time [84]. So, AS should pursue to achieve optimal clinical outcomes and to diminish drug related toxicity and other adverse events, with the minimum health-care related costs [85]. Enforcement of this concept in sepsis would be to cover all potential involved pathogens with the adequate antimicrobials since the first second. De-escalation will take place days later after the patient has been stabilized or when microbiological results (i.e., pathogen identification and definite antibiogram) are available.

Table 3 **Summary of antimicrobial stewardship interventions in sepsis management**

INTERVENTION	RATIONALE
General interventions	
At admission specifically review: Source of infection Age and renal function Old cultures Antimicrobial allergies Potential drug to drug interactions	Delay in the proper diagnosis and initiation of an adequate treatment has been associated with an increased morbi-mortality
During hospital-course assess in a daily basis: Antimicrobial time-out De-escalate antimicrobials to most narrow spectrum based on culture results Antimicrobial dose, duration, and stop date based on infection site	De-escalation allow to achieve optimal clinical outcomes diminishing drug related toxicity, superinfections and costs
At discharge ensure: Medication reconciliation (i.e., assess necessity for antimicrobials) Counsel patients on taking antimicrobials as prescribe	Antibiotic review and rationalization post sepsis trigger is recommended in sepsis pathways
Specific interventions	
Specific antimicrobial susceptibility maps	Resistance patterns in septic patients may differ from that observed in other populations
Educational and audit/feedback programs	Ensure baseline level of awareness among clinical staff regarding antimicrobial stewardship for sepsis Tailoring individual feedback based on specific cases or practice patterns may encourage behavior change
Standardized care pathways	Assist providers in optimizing the use of antimicrobials using available best practice, evidence-based guidelines
Cultures before antimicrobial therapy	Culture results are a primary tool for antimicrobial stewardship Yield of clinical cultures declines rapidly following antimicrobial therapy
Clinical decision support embedded in an electronic health record	Enhance early detection of sepsis Support compliance with quality measures Assist with optimal antimicrobial selection
Biomarkers and rapid microbiological techniques	Procalcitonin to guide antimicrobial therapy in respiratory tract infections Develop new specific biomarkers Develop rapid and accurate assays to identify etiology.

Adapted and modified from Pulia et al. [94].

While appropriate antibiotic therapy should be started as prompt as possible (i.e., within 60 minutes) for severe sepsis [32], there is little evidence demonstrating the benefit of early antibiotic administration in uncomplicated sepsis [86]. The combination of inadequate diagnostic criteria for sepsis [1] with the extraordinary time pressure to provide broad-spectrum antimicrobial therapy is troubling from a stewardship perspective [87]. Overuse and/or misuse of antimicrobials may result in selection of multidrug-resistant organisms, high rates of *Clostridium difficile* infections and adverse effects [88]. Some studies have reported a potential benefit on patient outcome by implementing guidelines, bundle care strategies and stewardship programs in clinical practice [89]. However, it is still unclear whether the observed benefit is more due to the effect of the recommended treatments or to a general increase in the awareness of the problem [90].

The SSC guidelines recommend that empiric antimicrobial therapy should be based on likely pathogen and local/hospital resistance patterns [85]. However, it is important to note that hospital antibiograms generated from inpatient may not mirror the septic population [91]. SSC guidelines also recommend obtaining appropriate cultures before administration of anti-

Table 4 **Road map of recommendations and perspectives for sepsis.**

Recommendations
1. The RDT complementing the BC, are very useful tools and efficiency in the diagnosis of sepsis and should be further investigated
2. The combination of RDT and BCs is a strategy that shortens the time to the start of the appropriate antimicrobial therapy.
3. When evaluating RDTs, it is important to focus on the results, including the time for appropriate antimicrobial therapy. Identification of pathogen is important, but knowledge of its susceptibility is the key, so it must have priority.
4. In order to have clinical impact, RDTs must be delivered in real-time decision support, in an automated manner and, ideally, with consultation of specialists in infectious diseases-microbiology and in an antimicrobial administration program.
5. It is important to know the pathophysiological mechanisms that impact on the defence of the host because clinical results depend on them.
6. When looking for new biomarkers for sepsis, it is essential to evaluate their clinical usefulness. They must be easy to obtain, achievable in a limited time and must allow a specific intervention (predictive markers).
7. Molecular signs that allow us to distinguish sterile, non-infectious systemic inflammatory states from systemic infection should be evaluated.
8. Physicians must prescribe antibiotics carefully. Local antimicrobial resistance data should be taken into account as part of good empirical therapy.
9. In patients with septic shock and vasoactive support, it is imperative to start antimicrobials quickly. Delays in treatment should be avoided due to identification or susceptibility of the pathogen.
10. It is essential to educate all health workers for rapid diagnosis, teamwork and personalized management.
Perspectives
1. Detection of pathogens is critical during acute phases of sepsis to optimize empirical antimicrobial therapy. This implies the need to develop ultra-fast POC test (less than 30 minutes), to identify microorganisms and detect resistance profiles.
2. The microbial load is an important parameter that will require more attention. The load predicts the result, the risk of death and the failure of antibiotics when the focus is not drained. The load helps distinguish colonization versus infection by using clinical samples taken from mucosal surfaces. (BAS, BAL)
3. The data on the control of hospitalized patients should be integrated into a continuous assessment of vital signs and oxygen saturation for the early detection of sepsis. An electronic alert should be able to detect the deterioration and demand medical attention from the health workers. This Big Data technology already exists in the intensive care units, but it should also be implemented in the hospitalization rooms.
4. NGS technologies can be the next step of precision medicine in sepsis as it happens in cancer care. That NGS test must be performed in a short period of time, directly from clinical samples, and must be optimized to be faster, easier to use and more cost-effective.
5. New strategies are being evaluated to restore "healthy" microbiomes in critically ill patients through certain strains or next-generation probiotics or by expanding indications for fecal transplantation in these patients.
6. The rapid development of omics-based technologies has changed the focus of traditional biomarkers to the expression profiles of blood genes, proteins and metabolites throughout the genome. Big Data analyzes to identify these profiles will increase the need for the experience of computational biologists in the field of sepsis.
7. The identification of drug response phenotypes is a priority. The development of specific endotypes of sepsis will have a major impact on the future design of clinical trials for the treatment of sepsis.
8. Systematic reviews of the impacts of delays on appropriate therapy for patients with sepsis are required. The ultimate goal is to develop evidence to guide physicians in their early decision making and without ecological impact
9. Bioinformatics should collaborate with physicians in the development of modern Big Data analysis in sepsis to identify associations of clinical parameters with pathogen endotypes, predict responses and recommend interventions
10. It is necessary to develop global records and recommendations on the management of sepsis to better understand its causes and mortality

RDT: rapid diagnosis test, BC: Blood Culture, POC: Point-of-care, BAS: Bronchoaspirate, BAL: Bronchoalveolar lavage, NGS: Next generation sequencing. Adapted and modified from Rello J et al [96].

microbial therapy (without delaying treatment). Although approximately 40% of patients with sepsis are culture-negative, identification of a causative organism is essential to de-escalate antibiotics. There is a great potential for a major innovation in AS for sepsis management within the rapidly advancing field of molecular microbiology diagnostic tools. There is also a great need for biomarkers rapidly produced and easy to measure. The clinical utility of conventional acute phase protein biomarkers (i.e., C-reactive protein, serum lactate and procalcitonin) in the management of sepsis is an area of considerable controversy [92, 93]. The most effective AS intervention for sepsis will likely include a bundle composed of traditional quality improvement strategies (eg., education, audit, and feedback) combined with rapid diagnostic tests and adequate biomarkers (table 3) [94].

NEW HORIZONS FOR RESEARCH IN SEPSIS

Success in oncology argues for precision medicine for sepsis. Identifying drug-response phenotypes by examining interactions between phenotypes and sepsis therapies should be used to optimise clinical trials. Adaptive trials (response-adaptive randomization) should be performed. Precision medicine in advancing the care of sepsis patients is fast approaching and highly anticipated to be a breakthrough in the development of new therapies [95]. We should consider the heterogeneity of septic patients when designing prospective clinical trials. A wide array of diverse subpopulations of subjects exist when we randomly assign them in groups. Variations in the therapy effect size by the identical experimental agent could reasonably be expected regarding the pathogen, infection site; the pre-existing co-morbidities and predisposing factors; the sepsis onset; age and gender; the burden and virulence of the organism; and the state of immune function at the time of randomization [95].

Many other unmeasured host and organism factors play a significant role in determining patients outcome. With the increasing availability of rapid nucleic acid sequencing to interrogate the molecular basis of host variability, the molecular substrates that govern individual host responses are now the focus [96]. This emerging field of genomic medicine has already revolutionized the care of patients with malignancies where genomic signatures have proven to be more reliable as prognostic indicators than traditional staging criteria [97].

The electronic health record should be used to identify endotypes. Replication in multiple data sets require big data with harmonisation across multiple investigator sites. Replicating findings in secondary analyses are required to validate these endotypes. Bio-informaticians and big data analyses to identify (rare) genotypes and associations are expected to play a significant role in sepsis management. Challenges are to establish a proper infrastructure to make optimal use of both clinical and "omics" big data. Data should not only be shared within health institutions, but we must strive towards a system where sharing of big data is beneficial in collaboration to maximize its use [98].

There is consensus that molecular diagnostics will have a major impact on clinical trial design in the future, clinical trial ethics and study execution remain before personalized medicine becomes standard in patients presenting with sepsis [99].

A major unmet medical need is the ability to integrate the functional immune status of each patient with sepsis entering into a clinical trial. It is now possible to segregate patients at the transcriptional level. These critically important immune distinguishing events were not detectable at the bedside using standard variables. Such information will be essential before choosing who should be given an immune inhibitory agent versus an immune adjuvant agent. Other innovative technologies such as rapid HLA haplotype [100] or T cell receptor diversity assays [101] need to become available. Such trials, which can predict benefit or avoid toxicity, will need to be validated by regulatory agencies [96].

Advances in the rapid molecular diagnosis of microbial pathogens will be essential for the further clinical development of highly specific therapeutics such as monoclonal antibodies, novel antibiotics or bacteriophage therapies [102]. Such therapeutics may be limited to a specific, targeted species while others will require even tighter diagnostics such as targeted monoclonal antibodies [103-106]. An overview on rapid diagnostic tests in sepsis has been recently reported [96].

In summary, when applying precision medicine to acute critical illnesses such as sepsis, implementation is difficult due to the high mortality, multisystemic organ dysfunction and the fast evolving physiopathology. A recent ESCMID Position paper [96] identified a Road Map with 10 recommendations and 10 priorities (table 4) to be adopted in future management of sepsis.

ECONOMIC EVALUATION OF HOSPITAL SEPSIS PROGRAMS

Economic analysis is essential to quantify a health problem, estimate its impact, prioritize actions and define its effectiveness. It measures the impact on the health system, health care providers, the patient, their environment and the society. However, the economic studies published have some limitations. In 2010, Porter introduced the concept of value understood as the health outcomes achieved in relation to the costs incurred to achieve them. The concept of health value revolves around the patient and the results obtained [107].

Cost-effectiveness studies analyze the cost overrun by the supplier/payer. They do not measure effects on patients and society (incremental costs of care, dependency, sequelae, loss of productivity, poor quality of life and premature mortality). In contrast, cost-benefit analyses provide information about the real costs of a disease for the payers, patients and society. These include the direct costs of the episode and the indirect costs related to the process [108]. Furthermore, the heterogeneity in the design of the published papers limits the robustness when comparing results. Different tools have been proposed to choose the most appropriate type of analysis and how to record data [109].

Several studies analyze the costs of sepsis. Those with incremental costs [110] use the increase in costs of the hospitalization episode as an independent variable. Others include clinical outcome indicators for the episode [111,112]. Some measure long-term effects such as sequelae (significant impairment of quality of life) or increased late mortality in patients who have survived the acute phase of sepsis [113,114]. These indicators should be systematically included for an accurate assessment of the health impact of sepsis. Our group analyzed the cost-effectiveness of the Surviving Sepsis Campaign (SSC) [53] protocol for sepsis, as compared with usual care of the syndrome, in Spain [115]. The main result of our study was that the reduction in mortality associated with the SSC protocol was accompanied by an increase in costs compared with the standard care for severe sepsis. However, the estimated In-

Table 5 Advantages of management by processes

	Disadvantages of organization by groups	Advantages of management by processes
Hierarchy	Head of department, head of specialists	Head of the multidisciplinary team
Decisions	Decisions by each specialist group	Decisions to achieve the goals
Patient management	Each specialist makes decisions without considering the integral solution for the patient	The team provides integral solutions for patient problems
Focus	The specialist	The patient
Work	Individual work	Teamwork
Communication	Vertical, not horizontal	Horizontal and vertical to unify criteria
Outcome management	Activities of each group are analyzed separately	Collective outcomes may be controlled
Efficiency	Not optimized	Adequate

Adapted from Govindarajan R [130].

cremental cost-effectiveness ratio (ICER), 4,435 euros per life years gained (LYG), was significantly lower than the commonly accepted threshold of 30,000 euros per LYG used in Spain [116]. Moreover, our results are in agreement with a previous study conducted in the U.S. that showed that a protocol similar to the SSC protocol was a cost-effectiveness alternative to the usual care of severe sepsis with an ICER of 11,274 dollars per LYG (8,906 euros per LYG) [117]. Another similar study, also conducted in the U.S., showed even better results as the sepsis protocol both improved mortality and reduced costs [118].

However, all these studies are observational and patients were not randomized to groups. Although this could be considered as an strengths of the study since it better reflects clinical practice [119], there could be unobserved differences between the groups that could not be adjusted. Another limitation is that as patients were not followed after hospital discharge, long-term costs were not included in the analysis. However, other ICU intervention studies suggest that even when long-term costs are included, the ICER remains below the usually accepted thresholds [120]. The NICE guidelines, based on these results and on the epidemiology of sepsis, consider that educational interventions to improve sepsis care are cost-effective and should be implemented [121]. The treatment of sepsis, based in the SSC recommendations, is cost-effective. As performance measures are introduced for improving the management of critically ill patients, it is essential that ongoing evaluations on the impact of these measures on outcomes and costs are rigorously conducted.

IMPORTANCE OF A SEPSIS MULTIDISCIPLINARY STRUCTURE IN HEALTHCARE

Sepsis is increasing its incidence, even exceeding that one of common diseases as stroke, cancer and myocardial infarction [122, 123]. Its mortality rate is very high, from 20% to 50% in case of organ dysfunction and frequently over 50% in septic shock [122-125]. Sepsis is a time-dependent disease, and prognosis may improve if early diagnosis and appropriate treatment is achieved [32,126]. The implementation of the Surviving Sepsis Campaign guidelines has been associated with a significant decrease in mortality and intensive Care Unit (ICU) and hospital length of stay [127]. Notwithstanding, despite important educational efforts to promote bundles for sepsis compliance rates are still low [13]. For all those reasons Sepsis Code (SC) was born, as a tool to standardize and achieve early diagnosis of sepsis and septic shock, early and appropriate antibiotic therapy and resuscitation, and quick infection source control. It is a cross-sectional and multidisciplinary clinical process model.

The Declaration of Mallorca, in November 2012, represented the I Multidisciplinary Sepsis Meeting in Spain, with the implication of 12 scientific societies. In 2015, the Spanish Sepsis Code Consensus Document was published. In that document, the need to involve as many professionals as possible, including nurses, medical staff from different specialties and managers was highlighted [128-129]. An interdisciplinary model for sepsis management is recommendable. All these objectives must be achieved with close, constant and efficient coordination between all physicians and nurses potentially implicated in septic patients management, mostly from the Emergency department, Microbiology, Intensive Care, but also from the ward.

The application of management by processes, opposite to the traditional vision by departments, may improve efficiency and effectiveness in health assistance, and specifically in sepsis management. The heart of the model consists on creation of a mechanism to continually measure, analyze and improve the results. Management by processes focuses on the continuity of care, adequate coordination and implication of all professionals. The main goal is to guarantee the best clinical practice by using unified criteria. Risk and evidence analysis should be employed, in addition to an integrated information management system to measure, analyze and improve each process [130].

Several benefits may be obtained from this model: decrease the time to achieve diagnosis and treatment, improve organization and unify criteria, promote teamwork between health professionals by improving internal communication to achieve common goals, increase participation, motivation and satisfaction among team members, identify and control variability by implementing protocols, assess global efficacy of health services, reduce costs (diagnostic errors, for example may increase the cost) and remove worthless activities. The implementation of management by processes in sepsis by a sepsis code is a key element to obtain all these benefits. In table 5 main advantages of management by processes are summarized. Recent studies have demonstrated the utility of the implementation of Sepsis Code to improve compliance with Surviving Sepsis Campaign recommendations, to reduce intensive care admissions, average hospital stay and even mortality [131-134]. Health professionals are required to work together as a multidisciplinary team to make sepsis code possible.

CONFLICT OF INTERESTS

The opinions expressed here by the authors may not represent the official positioning of the scientific societies to which they belong. The authors declare no conflicts of interest.

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Valoración de escalas de gravedad en pacientes incluidos en un código sepsis en un servicio de urgencias hospitalario

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RESUMEN

Objetivos: El objetivo del estudio es determinar la utilidad de las escalas SOFA (Sequential Organ Failure Assessment), quick SOFA (qSOFA), LODS (Logistic Organ Dysfunction System) y EWS (Early Warning Score) para predecir mortalidad intrahospitalaria entre los pacientes sépticos atendidos en un servicio de urgencias hospitalario. Evaluar los factores de riesgo asociados con la mortalidad y desarrollar un modelo predictivo de la mortalidad intrahospitalaria.

Material y métodos: Estudio, descriptivo, retrospectivo en el que se analizaron los pacientes mayores de 14 años incluidos en el código sepsis del servicio de urgencias de un hospital universitario entre noviembre del 2013 y septiembre del 2015. Se analizaron variables demográficas, hemodinámicas y analíticas, y la mortalidad intrahospitalaria para calcular los resultados de las escalas qSOFA, SOFA, LODS y EWS. Se calculó el área bajo la curva (ABC) de la característica operativa del receptor (COR) de cada una de las escalas. Se utilizó la regresión logística para evaluar la probabilidad de la mortalidad intrahospitalaria.

Resultados: Se analizaron 349 pacientes, edad mediana 72,7 (rango 86), varones: 54,4%. La mortalidad intrahospitalaria fue del 21.8%. ABC obtenidas: LODS: 0,73 (IC95% 0,67-0,80; p<0,001), EWS: 0,73 (IC 95% 0,65-0,81; p<0,001), SOFA: 0,72 (IC 95% 0,65-0,78; p<0,001), qSOFA: 0,67(IC 95% 0,58-0,76; p<0,001). Tras el análisis multivariante los factores asociados con la mortalidad intrahospitalaria fueron: Saturación de oxigeno por pulsiosimetría (SatO2) ≤92%, escala del coma de Glasgow <14, lactato ≥2mmol/L (p<0,05). Se generaron dos modelos pronósticos: MPRO1: edad, SatO2 ≤92% y escala del coma de Glasgow <14, ABC: 0,78 (IC 95% 0,72-0,84; p<0,001) y MPRO2 formado por las anteriores y lactato ≥2mmol/L, ABC: 0,82 (IC 95% 0,76-0,87; 0,001)

Conclusiones: La escala SOFA y los nuevas escalas desarrolladas podrían ser útiles para evaluar el riesgo de la mortalidad hospitalaria entre los pacientes incluidos en el código sepsis.

Palabras clave: Sepsis, departamento de urgencias, pronóstico, escalas.

Assessment of the severity scores in patients included in a sepsis code in an Emergency Departament

ABSTRACT

Objectives. The objective of the study is to determine the usefulness of the SOFA (Sequential Organ Failure Assessment), quick SOFA (qSOFA), LODS (Logistic Organ Dysfunction System) and EWS (Early Warning Score) scores to predict in-hospital mortality among septic patients attended in the emergency department; to evaluate what factors are associated with mortality; and develop a predictive model of in-hospital mortality.

Material and methods. Retrospective study including patients over 14 years of age included in the sepsis code of an Emergency Department of a University Hospital between November 2013 and September 2015. Demographic variables, hemodynamic and analytical variables, and in-hospital mortality were collected to obtain qSOFA, SOFA, LODS, EWS scores. Receiver operating characteristic curves were constructed for each score. Logistic regression was used to evaluate the probability of in-hospital mortality.

Results. A total of 349 patients were analyzed, median age 72.7 (range 86), males: 54.4%. The in-hospital mortality was 21.8%. AUC obtained: LODS: 0.73 (IC 95% 0.67-0.80; p<0.001), EWS: 0.73 (IC 95% 0.65-0.81; p<0.001), SOFA: 0.72 (IC 95% 0.65-0.78; p<0.001), qSOFA: 0.67 (IC 95% 0.58-0.76; p<0.001). After the

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multivariate analysis, these were the independent factors associated with in-hospital mortality: Oxygen saturation ≤92%, Glasgow coma score <14, lactate ≥2mmol/L (p<0.05). Two prognostic models were generated: MPRO1: age, oxygen saturation ≤92% and Glasgow coma score <14, AUC: 0.78 (IC 95% 0.72-0.84; p<0.001) and MPRO2 formed by the previous ones and lactate ≥2mmol/L, AUC: 0.82 (IC 95% 0.76-0.87; p<0.001)

Conclusions. SOFA score and the new developed scores could be useful in asses the risk of in-hospital mortality in patients included in the sepsis code.

Keywords: Sepsis, Emergency departament, Prognosis, scores.

INTRODUCCIÓN

La sepsis es un proceso clínico que se puede observar con relativa frecuencia en los servicios de urgencias hospitalarias (SUH) y que hoy día sigue siendo una de las mayores causas de morbilidad y mortalidad en los países desarrollados. La sepsis es una patología que amenaza la vida, causada por una desregulación de la respuesta del huésped frente a la infección [1]. La detección y el diagnóstico precoz de esta entidad tienen repercusión en el pronóstico y evolución del paciente [2].

La sepsis es un problema de salud del cual se empezó a tener constancia hace mucho tiempo, pero no fue hasta 1991 cuando se realizó una definición duradera en el tiempo y aceptada internacionalmente [3]. Desde entonces se ha seguido avanzando en el estudio de esta patología sin que haya habido grandes modificaciones en las definiciones de sepsis y shock séptico hasta febrero de 2016, en que se publicaron los datos del "Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)" [1,4,5], en el que las definiciones y criterios de esta enfermedad han sido actualizados [1]. Con la introducción de los nuevos criterios Sepsis-3, la clasificación de la sepsis en tres estadios evolutivos desaparece, eliminándose el concepto de sepsis grave por considerarse redundante. Permanecen solamente los conceptos de sepsis y shock séptico [1].

La prevalencia de la sepsis en nuestro país se sitúa hoy día en torno al 6,2% de los pacientes que acuden a un SUH por causa infecciosa. Las causas más frecuentes de sepsis se corresponden con las causas más importantes de infección en general, siendo la etiología más frecuente la infección respiratoria, la infección urinaria y la infección intraabdominal [6]. Además de su elevada incidencia, esta entidad presenta una alta mortalidad, en torno al 10% [1] (mayor que la del Infarto Agudo de Miocardio con elevación del ST que es del 8,1% [7]). Las perspectivas de supervivencia empeoran si se detecta un shock séptico. Actualmente el shock séptico se define como un subgrupo de sepsis en el que la mortalidad es superior al 40% [1]. Uno de los puntos más limitantes en la actuación en los pacientes con sepsis es su reconocimiento. Diferentes entidades y consensos preconizan desde hace tiempo la necesidad de poner en marcha medidas para el diagnóstico precoz de los pacientes con sospecha de sepsis así como la creación de códigos de activación (código sepsis) para su detección temprana que llevan consigo una serie de medidas diagnósticas y terapéuticas asociadas [8-11].

En este momento, en que hay una redefinición de los criterios de sepsis basado en escalas diagnósticas [1], nos planteamos la valoración de las mismas en los servicios de urgencias hospitalarios en aquellos pacientes en los que se sospecha esta entidad. Estas escalas tienen el propósito de estimar de forma rápida la gravedad del paciente para poder centrarnos en aquellos que presenten un mayor compromiso vital. Existen una gran variedad de escalas pronosticas, de ellas las más extendidas son: Early Warning Score (EWS), la escala SOFA (Sequential Organ Failure Assessment), el quick SOFA (qSOFA) y la escala LODS (Logistic Organ Dysfunction System) [1,7,12-15].

El objetivo principal de este trabajo es valorar la utilidad de las escalas pronósticas (qSOFA, SOFA, EWS, LODS) para predecir la mortalidad intrahospitalaria en los pacientes incluidos en un código sepsis en un servicio de urgencias hospitalario. Por otra parte, otro de los objetivos es investigar si se puede generar algún modelo predictivo que supere las escalas analizadas.

MATERIAL Y MÉTODOS

Se ha realizado un estudio retrospectivo en el ámbito de la urgencia hospitalaria analizando a los pacientes mayores de 14 años incluidos en el código sepsis del Servicio de Urgencias Hospitalario (SUH) del Hospital Universitario Río Hortega (HURH) de Valladolid desde noviembre del 2013 hasta septiembre 2015. Los criterios para incluir a un paciente en el código sepsis y por tanto formar parte del estudio fueron: pacientes que acudieron al SUH del HURH de Valladolid que presentaron sospecha de infección y además tenían 2 o más de los siguientes criterios: frecuencia cardiaca (Fc) >90 lpm, frecuencia respiratoria (Fr) >20 rpm, saturación de O_2 (SatO2) <90%, temperatura (T^a) >38,5ºC o <36ºC, alteración del nivel de conciencia habitual o signos de mala perfusión, presión arterial media (PAM) <65 mmHg o presión arterial sistólica (PAS) <90mmHg, leucocitosis >12000/mm^3 o leucopenia <4000/mm^3, lactato > 2mmol/L, Procalcitonina >2ng/mL, parámetros de disfunción orgánica de uno o más órganos (plaquetas <100000/mm^3, bilirrubina >2 mg/dL en ausencia de enfermedad hepática conocida, creatinina >1,5 mg/dL en ausencia de insuficiencia renal conocida, INR >1,5 o TTPa >60 segundos en ausencia de tratamiento anticoagulante).

Se ha realizado una revisión de las historias clínicas de los pacientes que cumplieron los criterios de inclusión. Se recogieron variables demográficas (edad y género) y las variables hemodinámicas y analíticas necesarias para realizar el cálculo de las escalas analizadas, qSOFA, SOFA, LODS y EWS. También se ha recogido el valor del lactato sérico. Se incluyó en el registro la primera determinación de todas éstas variables desde la llegada del paciente al SUH. La variable dependiente fue la mortalidad intrahospitalaria (MH).

Todos los datos se almacenaron en una base de datos EXCEL y finalmente se realizó un estudio estadístico mediante los softwares estadísticos SPSS 23.0 para Windows y Matlab R2015 (The Mathworks Inc., Natick, Massachusetts).

Se ha efectuado un estudio descriptivo de las muestras obtenidas. Las variables cuantitativas continuas se describen como media ± desviación estándar (DS) en caso de distribución normal, o como mediana y rango si la distribución no sigue una distribución normal, para ello se ha utilizado la prueba de Kolgomorov-Smirnov. Las variables cualitativas se describen mediante frecuencias absolutas y relativas (%). Para la comparativa de medias de variables cuantitativas se usó la t de Student con los valores distribuidos normalmente y la prueba U de Mann-Whitney si no había una distribución normal. Se utilizó la prueba de chi cuadrado para tablas de contingencia 2x2 y/o contraste de proporciones para estipular la relación asociación o dependencia entre variables cualitativas. Se realizó un análisis univariante observando como variable dependiente la mortalidad durante el ingreso hospitalario y como variables independientes las escalas de gravedad analizadas así como las variables que conforman las distintas escalas y el valor del lactato sérico en mmol/L. Se calculó el área bajo la curva (ABC) de la característica operativa del receptor (COR) de cada una de las escalas, así como la sensibilidad, especificidad, valor predictivo positivo (VPP) y valor predictivo negativo (VPN) para diferentes puntos de corte de cada una de las escalas, con sus respectivos odds ratio (OR): SOFA ≥2; LODS ≥2; qSOFA ≥2 y ≥1; EWS ≥6 y ≥7.

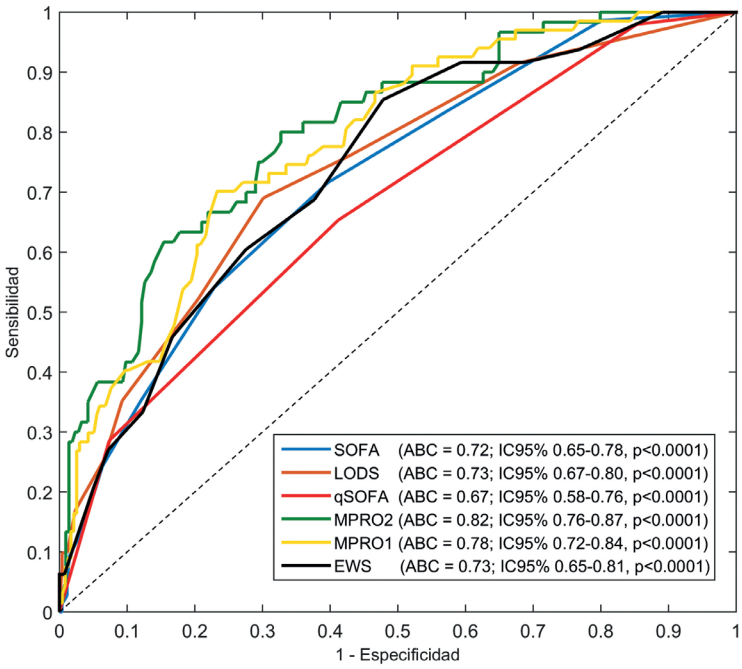

Figura 1 | **Curvas de rendimiento diagnóstico y áreas bajo la curva con su intervalo de confianza al 95%.**

LODS: Logistic Organ Dysfunction System ; SOFA: Sequential Organ Failure Assessment; EWS: Early Warning Score; qSOFA: Quick Sequential Organ Failure Assessment;. MPRO1: Modelo propio 1; MPRO2: Modelo propio 2.

Se realizó un estudio multivariante con las variables independientes asociadas a la mortalidad intrahospitalaria mediante un estudio de regresión logística. Para la generación de los modelos pronósticos se usaron las variables independientes identificadas en el análisis multivariante, además de la edad, por considerarse que ésta podría tener una importante asociación con la mortalidad. Finalmente se procedió a comparar cada una de las ABC obtenidas de todas las escalas y modelos creados mediante test-no paramétricos. En todos los test realizados se ha considerado significativo un nivel de confianza del 95% y un p valor menor de 0.05. El estudio fue aprobado por el comité de ética de investigación del Hospital Universitario Río Hortega de Valladolid.

RESULTADOS

Un total de 349 pacientes fueron incluidos en nuestro estudio: 190 (54,4%) varones y 159 (45,6%) mujeres. La edad mediana fue de 72,71 (Rango 86,00). Durante la hospitalización fallecieron 76 pacientes (21,8%). Del total de los pacientes analizados finalmente 15 de ellos (4,3%) fueron diagnosticados de procesos no infecciosos. En cuanto al foco de infección observado, la gran mayoría de los pacientes se distribuyen entre los focos respiratorio (34,7%) y urinario (34,1%), seguido por este orden de los focos abdominal (12,6%), foco no determinado (7,7%) y otros focos (6,6%)

Se observó que todas las ABC de las escalas analizadas presentaban significación estadística para discriminar la mortalidad hospitalaria (figura 1). Las escalas que mejor ABC obtuvieron fueron la escala LODS y la escala EWS con una ABC de 0,73 para ambas con unos IC 95% de 0,67-0,80 y 0,65-0,81 respectivamente, al comparar ambas no se observaron diferencias estadísticamente significativas (p>0,05). La escala SOFA obtuvo un resultado de 0,72 (IC95% 0,65-78) sin diferencias con la escala LODS (p>0,05) y el EWS (p>0,05). La escala qSOFA fue la que presentó un peor ABC de 0,67 (IC 95% 0,58-0,76) significativamente peor que las otras tres escalas analizadas (tabla 1).

En el análisis de los puntos de corte analizados de las diferentes escalas se comprobó que todas presentan una asociación con la MH, aunque con diferentes OR (tabla 2). En cuanto a la sensibilidad y especificidad de estos puntos, se ha comprobado que para una puntuación de la escala qSOFA mayor o igual a 1 punto la sensibilidad obtenida es de 0,98 (IC 95% 0,89-0,99) pero su especificidad baja al 0,14 (IC 95% 0,09-0,21). Esta mejora si el punto de corte lo situamos en 2, en el que la especificidad sube al 0,58 (IC 95% 0,50-0,66). Las escalas SOFA y LODS presentan una sensibilidad de 0,76 (IC 95% 0,65-0,84) y 0,83 (IC 95% 0,73-0,90) respectivamente, con una mejor especificidad de la escala LODS, que llega al 0,57 (IC 95% 0,51-0,63). En el análisis de la escala EWS se observa que ambos puntos de corte 6 y 7 presentan una buena sensibilidad, 0,91 (IC 95% 0,80-0,96) con una especificidad del 0,31 (IC 95%

Tabla 1 **Comparación de las ABC de las escalas analizadas.**

	LODS	qSOFA	EWS	MPRO 1	MPRO 2
SOFA	NS	<0,05	NS	<0,001	<0,001
LODS		<0,01	NS	<0,01	<0,001
qSOFA			<0,01	<0,001	<0,001
EWS				<0,05	<0,001
MPRO 1					<0,05

Abreviaturas: ABC: Área bajo la curva; LODS: Logistic Organ Dysfunction System; SOFA: Sequential Organ Failure Assessment; EWS: Early Warning Score; qSOFA: Quick Sequential Organ Failure Assessment. NS: No significativo.

Tabla 2 **Análisis de la mortalidad intrahospitalaria según los puntos de corte usados para cada escala y Odds Ratio de las mismas.**

	Exitus Sí n (%)	Exitus No n (%)	Total n (%)	P	OR (IC 95%)	P
LODS ≥ 2						
Sí	54 (32,7)	111 (67,3)	165 (50,0)		4,23	
No	17 (10,3)	148 (89,7)	165 (50,0)	<0,001	(2,32-7,70)	<0,001
SOFA ≥ 2						
Sí	62 (28,7)	154 (71,3)	216 (63,3)		3,79	
No	12 (9,6)	113 (90,4)	125 (36,7)	<0,001	(1,95-7,36)	<0,001
EWS ≥ 6						
Sí	44 (31,9)	94 (68,1)	203 (58,2)		5,03	
No	4 (8,5)	43 (91,5)	146 (41,8)	<0,001	(1,70-14,89)	<0,05
EWS ≥ 7						
Sí	44 (35,2)	81 (64,8)	126 (67,7)		7,60	
No	4 (6,7)	56 (93,3)	60 (32,3)	<0,001	(2,58-22,36)	<0,001
qSOFA ≥ 1						
Sí	48 (29,3)	116 (70,7)	164 (88,6)		8,27	
No	1 (4,8)	20 (95,2)	21 (11,4)	<0,05	(1,08-63,41)	<0,05
qSOFA ≥ 2						
Sí	32 (36,4)	56 (63,6)	88 (47,6)		2,68	
No	17 (17,5)	80 (82,5)	97 (52,4)	<0,05	(1,36-5,30)	<0,05

Abreviaturas: n: número; LODS: Logistic Organ Dysfunction System ; SOFA: Sequential Organ Failure Assessment; EWS: Early Warning Score; qSOFA: Quick Sequential Organ Failure Assessment;. P: significación estadística. OR: Odds Ratio. P: significación estadística.

0,24-0,39) y 0,40 (IC 95% 0,33-0,49) respectivamente. Todas las escalas tienen bajos VPP, que van desde el 0,28 (IC 95% 0,23-0,35) para SOFA ≥2 hasta un 0,36 (IC 95% 0,27-0,46) para qSOFA ≥2. Sin embargo, los VPN son elevados en todas ellas, destacando un 0,95 (IC 95% 0,77-0,99) para qSOFA ≥1 y un 0,93 (IC 95% 0,84-0,97) para EWS ≥7 (tabla 3).

En el análisis univariante de las variables dependientes analizadas se observó que la edad, el género femenino, así como presentar una Fr ≥22 rpm, una PAS ≤100 mmHg, una PAM ≤65 mmHg, una SatO2 ≤92%, una puntuación en la escala de coma de Glasgow (ECG) <14 puntos, y un lactato ≥2 mmol/L, presentaban una asociación estadísticamente significativa con el éxitus hospitalario de los pacientes analizados (tabla 4). En el análisis multivariante se identificaron como factores asociados de manera independiente a la mortalidad intrahospitalaria la SatO2 ≤92%, la ECG <14, y un lactato ≥2 mmol/L. Finalmente se crearon dos modelos pronósticos denominados modelo pronóstico propio 1 (MPRO1) y modelo pronóstico propio 2 (MPRO2) (tabla 5). El MPRO1 incluye las siguientes variables: la edad, una SatO2 ≤92%, y una puntuación en la ECG<14. El modelo MPRO2 incluye las variables del modelo MPRO1 y el nivel de lactacto ≥2mmol/L. Las ABC para los modelos MPRO1 y MPRO2 son respectivamente 0,78 (IC95% 0,72-0,84; p<0,001) y 0,82 (IC95% 0,76-0,87; p<0,05) (figura 1). Al comparar las ABC de estos dos modelos pronósticos se observaron diferencias estadísticamente significativas entre ambas curvas (tabla 1).

DISCUSIÓN

Con los datos obtenidos se observa que todas las escalas analizadas presentan una moderada capacidad para pronosticar la mortalidad intrahospitalaria entre los pacientes atendidos dentro del código sepsis, aunque lo hacen con distinta precisión. Los modelos propuestos mejoran la capacidad para identificar a los pacientes en riesgo de malos resultados a corto plazo frente a las escalas ya conocidas.

En relación con la escala qSOFA, ésta es la que parece que se comporta peor entre las que hemos analizado, con diferencias significativas con todas las demás. Si comparamos nuestros resultados con los obtenidos por otros autores, se comprueba que el ABC de la escala qSOFA que presenta nuestra serie es sensiblemente inferior al descrito en el artículo original de Seymour et al. o en otros trabajos más recientes [4,16]. Esto puede justificarse por el hecho de que nuestros pacientes, a diferencia de otros estudios, no sólo presentan sospecha de infección, sino que ya han sido incluidos en el código sepsis del hospital por cumplir los criterios definidos en Sepsis-2. Es decir nuestros pacientes podrían presentar una mayor gravedad que los analizados en otras series.

Sin embargo, en el resto de las escalas analizadas los resultados obtenidos son muy similares a los de la bibliografía revisada, y destaca que el ABC para SOFA que se ha obtenido en otros estudios (0,72) es igual que el obtenido en nuestro trabajo (0,72) [4]. Algo similar sucede con la escala LODS, que es una escala que se comporta mejor que SOFA aunque

Tabla 3 **Análisis de sensibilidad, especificidad y valores predictivos para cada uno de los puntos de corte de las escalas analizadas.**

	S (95% IC)	E (95% IC)	VPP (95% IC)	VPN (95% IC)
LODS ≥ 2	0,76 (0,65-0,84)	0,57 (0,51-0,63)	0,32 (0,26-0,40)	0,89 (0,84-0,93)
SOFA ≥ 2	0,83 (0,73-0,90)	0,42 (0,36-0,48)	0,28 (0,23-0,35)	0,90 (0,84-0,94)
EWS ≥ 6	0,91 (0,80-0,96)	0,31 (0,24-0,39)	0,31 (0,24-0,40)	0,91 (0,80-0,96)
EWS ≥ 7	0,91,7 (0,80-0,96)	0,40,9 (0,33-0,49)	0,35,2 (0,27-0,43)	0,93,3 (0,84-0,97)
qSOFA ≥ 1	0,98 (0,89-0,99)	0,14 (0,09-0,21)	0,29 (0,22-0,36)	0,95 (0,77-0,99)
qSOFA ≥ 2	0,65 (0,51-0,77)	0,58 (0,50-0,66)	0,36 (0,27-0,46)	0,82 (0,73-0,88)

Abreviaturas: LODS: Logistic Organ Dysfunction System; SOFA: Sequential Organ Failure Assessment; EWS: Early Warning Score; qSOFA: Quick Sequential Organ Failure Assessment S: Sensibilidad; E: Especificidad; VPP: Valor Predictivo Positivo; VPN: Valor Predictivo Negativo. IC: Intervalo de confianza

sin diferencias significativas entre ambas [4]. Estas dos escalas, tanto SOFA como LODS, contienen parámetros analíticos, y tienen la limitación de que no se pueden usar en entornos extrahospitalarios, por lo cual su validez para el diagnóstico precoz se ve disminuida. Si nos centramos en la escala EWS, se comprueba que esta escala tiene una aceptable potencia discriminativa y además sólo incluye parámetros clínicos, por lo tanto puede realizarse tanto en el ámbito extra-hospitalario como a la llegada de los pacientes a los SUH. En la comparación con la otra escala que sólo maneja parámetros clínicos (qSOFA) se observa que el ABC obtenido para EWS es significativamente mejor que el obtenido para qSOFA. Esto se ve reforzado por otros trabajos como el de Williams et al, en el que se ratifica la validez de EWS para la detección precoz de patologías graves en el ámbito extra-hospitalario [13]. En este trabajo los resultados obtenidos fueron algo superiores a los nuestros: el ABC para EWS fue de 0,78 mientras que en nuestra serie el ABC obtenido fue del 0,73. En estudios más recientes se comprueba que escalas derivadas de EWS como National Early Warning Score (NEWS) o Modificated Early Warning Score (MEWS) presentan mejores resultados en cuanto a predicción de gravedad y mortalidad entre pacientes con sospecha de infección que la escala qSOFA, [17] lo que coincide con los resultados obtenidos en nuestro trabajo.

Una de las críticas que se han realizado a los nuevos criterios diagnósticos de sepsis basados en las escalas SOFA y qSOFA es la baja sensibilidad que presentan frente al clásico síndrome de respuesta inflamatorio (SIRS) [18], lo que no permitiría detectar pacientes sépticos con la suficiente antelación. Nuestros resultados indican que una puntuación igual o mayor a dos puntos para la escala SOFA presenta una buena sensibilidad. Con respecto a la escala qSOFA, una de las opciones para mejorar su sensibilidad en entornos fuera de unidades de cuidados intensivos, como son los SUH o los servicios de urgencias y emergencias extrahospitalarios, es considerar como positivo qSOFA ≥1 punto, lo que hace que la sensibilidad de la escala aumente de forma considerable, a expensas de bajar la especificidad de la escala, y a un aumento de los falsos positivos, no obstante, para valorar adecuadamente estos resultados debemos tener en cuenta lo mencionado previamente, y es que la población de nuestro estudio ya está seleccionada por los criterios diagnósticos de Sepsis-2. En este contexto se ha propuesto también el uso de otras escalas como NEWS que presentan una sensibilidad superior a qSOFA, con una capacidad de predicción mayor [17]. A pesar de que la escala qSOFA presenta peores resultados, ésta es una escala rápida, muy sencilla de realizar en cualquier ámbito, de fácil aprendizaje, mucho más sencilla que EWS, y que con valores mayores o iguales a 2 presenta una sensibilidad aceptable, una buena especificidad y, lo que es más importante en este tipo de entidades graves, presenta unos buenos valores predictivos negativos, similares a las otras escalas analizadas, lo que la hace ideal para el cribado rápido de sepsis en los SUH [19]. Sí parece evidente que el resto de las escalas, entre las que se incluye SOFA, tienen una mejor capacidad pronóstica que qSOFA entre estos pacientes en los que sospechamos sepsis, confirmando su utilidad y su uso en entornos de urgencias. [1]

Por otra parte, además del análisis de las escalas clásicas, en el estudio multivariante hemos encontrado que hay una serie de variables que en nuestros pacientes se asocian de forma independiente con la mortalidad hospitalaria, y que son, la SatO2 ≤92%, la alteración del nivel de conciencia y un nivel de lactato sérico ≥2mmol/L. Usando estas variables y la edad se han obtenido dos modelos pronósticos, uno basado solo en variables clínicas (MPRO1), y el otro, en el que se añadió una variable analítica como es el lactato (MPRO2). Ambos modelos se componen de parámetros sencillos de calcular, y el único parámetro analítico, empleado en la MPRO2 es el nivel de lactato sérico; hay que destacar que un lactato ≥2mmol/L es uno de los criterios de diagnóstico de shock séptico [1] y se ha propuesto como complemento en el cribado de sepsis, al ser además un valor analítico cuyo resultado puede obtenerse en pocos minutos [18]. Comparando las ABC de ambos modelos, se comprueba que el ABC de MPRO2 es significativamente mejor que MPRO1 y ambos modelos presentan ABC significativamente superiores a todas las escalas que se han analizado.

Aunque sería necesario realizar estudios de validación de estos modelos pronósticos (MPRO1 y MPRO2), la obtención de los mismos pone de manifiesto que es muy importante seguir investigando qué variables están relacionadas de forma

Tabla 4 **Variables analizadas significativas según mortalidad intrahospitalaria. Estudio Univariante y Multivariante.**

	Éxitus Sí Mediana (Rango)	Éxitus No Mediana (Rango)	Total Mediana (Rango)	p[a]	OR (IC 95%)	p
Edad	83,50 (44,00)	75,00 (86,00)	72,71 (86,00)	0,0001	1,00 (0,99-1,09)	0,075
	Éxitus Sí n (%)	Éxitus No n (%)	Total n (%)	p[b]	OR (IC 95%)	p
Sexo						
Hombre	33 (43,4)	157 (57,5)	190 (54,4)		2,78	
Mujer	43 (56,6)	106 (42,5)	149 (45,6)	0,029	(1,00-7,73)	0,050
FR						
< 22	5 (10,9)	44 (32,1)	49 (26,8)		2,07	
≥ 22	41 (89,1)	93 (67,9)	134 (73,2)	0,005	(0,49-8,66)	0,317
PAS						
≤ 100	44 (57,9)	115 (42,3)	159 (45,7)		0,70	
>100	32 (42,1)	157 (57,7)	189 (54,3)	0,016	(0,20-2,41)	0,574
SatO2						
≤ 92	50 (67,6)	95 (36,7)	145 (43,5)		0,26	
> 92	24 (32,4)	164 (63,3)	188 (56,5)	0,000	(0,95-0,72)	0,010
ECG						
3-13	18 (26,1)	12 (4,8)	30 (9,4)		0,10	
14-15	51 (73,9)	237 (95,2)	288 (90,6)	0,000	(0,22-0,41)	0,002
Lactato						
< 2	10 (14,9)	112 (45.3)	122 (38,9)		4,00	
≥ 2	57 (85,1)	135 (54,7)	192 (61,1)	0,000	(1,20-13,31)	0,024
PAM						
≤ 65	33 (44)	69 (25,7)	102 (29,7)		1,12	
> 65	42 (56)	199 (74,7)	241 (70,3)	0,002	(0,30-4,05)	0,863

n: Número; P: Significación estadística; OR: Odds ratio; IC: Intervalo de confianza; n: número de pacientes; FR: Frecuencia respiratoria; PAS: Presión arterial sistólica; Fr: Frecuencia respiratoria; SatO2: Saturación de oxigeno; ECG: Escala del Coma de Glasgow; PAM: Presión arterial media. [a]U de Mann-Witnney. [b]Chi-cuadrado

Tabla 5 **Odds Ratio de las variables que forman parte de los modelos: MPRO1 y MPRO2**

MPRO1		
Variables	OR (IC95%)	p
Edad	1,05 (1,02-1,08)	0,000
SatO2 ≤ 92%	0,16 (0,07-0,39)	0,000
ECG<14	0,35 (0,18-0,65)	0,001
MPRO2		
Variables	OR (IC 95%)	p
Edad	1,05 (1,02-1,09)	0,001
SatO2 ≤ 92%	0,33 (0,17-0,65)	0,001
ECG<14	0,18 (0,07-0,46)	0,000
Lactato ≥ 2mmol/l	4,18 (1,18-9,72)	0,001

P: significación estadística; OR: Odds ratio; IC: Intervalo de confianza; Sat O2: Saturación de oxigeno; MPRO1: Modelo propio 1; MPRO2: Modelo propio 2; ECG: Escala del coma de Glasgow.

independiente con la mortalidad entre los pacientes sépticos. Variables que pueden modificarse según el tipo de población. Este hecho se corrobora con el desarrollo de otras escalas específicas para algún grupo de población en concreto. Un ejemplo es el desarrollo de la escala GYM validada para población anciana mayor de 75 años y con sospecha de infección. Esta escala compuesta de tres parámetros que son el nivel de conciencia, la frecuencia respiratoria y la comorbilidad según el Índice de Charlson, presenta una capacidad pronóstica de mortalidad a los 30 días

La mayor limitación de nuestro estudio es que es un análisis retrospectivo mediante revisión de historias clínicas, y llevado a cabo en un único servicio de urgencias. Habría que plantear más trabajos sobre otros grupos de pacientes para poder verificar y validar los datos obtenidos.

En conclusión, los resultados muestran que la escala SOFA y los nuevos modelos propuestos presentan una adecuada capacidad para identificar pacientes atendidos por sospecha de sepsis que tienen un riesgo incrementado de muerte intrahospitalaria.

FINANCIACIÓN

Los autores declaran no haber recibido financiación para la realización de este estudio.

CONFLICTO DE INTERESES

Los autores declaran no tener ningún conflicto de intereses.

RESPONSABILIDADES ÉTICAS

Este trabajo cumple con los requisitos establecidos en la legislación vigente en materia de investigación biomédica, protección de datos de carácter personal y bioética. Se solicitó el permiso al Comité Ético de Investigación Clínica del Hospital Universitario Río Hortega de Valladolid, que tras su pertinente evaluación emitió un informe favorable (número PI 3/15).

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Candiduria en pacientes hospitalizados: etiología, sensibilidad a los fármacos antifúngicos y factores de riesgo

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RESUMEN

Introducción. *Candida* puede llegar a ser el segundo microorganismo responsable de la infección del tracto urinario nosocomial. Aunque sigue destacando *Candida albicans* entre todas las especies, otras han surgido como patógenos emergentes. En este trabajo se analizó la presencia de candiduria en pacientes hospitalizados.

Material y Métodos. Se estudiaron, de forma retrospectiva, todos los aislamientos de *Candida* procedentes de los urocultivos en pacientes adultos hospitalizados durante un periodo de 5 años. Se recogieron los datos referentes a la especie, sensibilidad frente a los fármacos antifúngicos fluconazol, anfotericina B y voriconazol (Vitek2, BioMerieux), presencia de sonda urinaria, servicio de procedencia, edad y sexo del paciente.

Resultados. Se detectaron 289 episodios de candidurias, con un incremento anual. En 134 (46,4%), fueron levaduras no *C. albicans*, siendo 57 (19,7%) *Candida glabrata*, 37 (12,8%) *Candida tropicalis*, 25 (8,6%) *Candida parapsilosis* y 10 (3,5%) *Candida lusitaniae*. La mayoría provenían de pacientes sondados (240; 83,0%) y hospitalizados en el Servicio de Medicina Interna (118; 40,8%). Los aislados procedentes de varones fueron 152 (52,6%) y la edad media superior a los 65 años. La sensibilidad a los fármacos antifúngicos fue superior al 85%.

Conclusiones. Los estudios de urocultivos en pacientes hospitalizados deben incluir la posible presencia de candiduria, que es más prevalente en los procedentes de los Servicios de Medicina Interna, portadores de sonda urinaria y con una edad superior a los 65 años. Dada la elevada prevalencia de levaduras no *C. albicans*, se recomienda incorporar estudios de identificación de especie.

Palabras clave: *Candida albicans*, levaduras no *Candida albicans*, fármacos antifúngicos, orina, sonda urinaria.

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Inpatient candiduria: etiology, susceptibility to antifungal drugs and risk factors

ABSTRACT

Introduction. *Candida* could become the second most frequent cause of nosocomial urinary tract infection. Although *Candida albicans* is the most important species, others have arisen as emerging pathogens. The aim of this study was to analyze the presence of candiduria in inpatients.

Material and methods. We performed a retrospective study of *Candida* isolates from adult inpatient urocultures over five years, gathering and tabulating data on: the species; susceptibility to fluconazole, amphotericin B, and voriconazole (Vitek2, BioMerieux); presence of catheter; hospital department of origin; and patient age and sex.

Results. We detected 289 yeast episodes, observing an annual increase: 134 (46.4%) were non-*C. albicans* yeasts, with 57 (19.7%) being *Candida glabrata*, 37 (12.8%) *Candida tropicalis*, 25 (8.6%) *Candida parapsilosis*, and 10 (3.5%) *Candida lusitaniae*. Most isolates derived from catheterized (240, 83.0%) and Internal Medicine Department (118, 40.8%) patients, observing an annual increase; 152 (52.6%) isolates were from males, and the mean age was >65 years. Susceptibility to antifungals was >85%.

Conclusions. Inpatient urocultures should include data on the presence of *Candida*, which is more prevalent in Internal Medicine Department inpatients, in those with urinary catheter, and in over 65-year-olds. Almost half of the isolates were non-*C. albicans* yeasts, and we recommend complete identification of the species involved.

Key words: *Candida albicans*, no *Candida albicans* yeasts, antifungal drugs, urine, urinary catheter.

INTRODUCCIÓN

Las especies de *Candida* son la causa más común de infecciones por hongos, siendo entre ellas *Candida albicans* la más frecuente. Se trata de levaduras comensales, que forman parte de la microbiota de la piel, gastrointestinal y genital. Otras especies del género *Candida* han surgido como patógenos emergentes [1] y también pueden formar parte de la microbiota mucocutánea. La patogenicidad de este grupo de microrganismos está muy influenciada por el estado inmune y la enfermedad de base del hospedador.

La presencia de candiduria en pacientes hospitalizados es un hallazgo frecuente, con particular importancia en aquellos pacientes ingresados en la Unidad de Cuidados Intensivos y en los pacientes sondados [2]. La candiduria hay que valorarla cuidadosamente ya que puede corresponder a una colonización. La utilización de criterios estrictos, como recuentos superiores a 100.000 UFC/ml y el carácter monomicrobiano, ayudan a excluir esta situación [3]. Además, la presencia de levaduras en orina está claramente relacionado con un aumento de la morbilidad y mortalidad en pacientes con enfermedades de base, de ahí la importancia de valoración correcta [4]. Se considera que *Candida* pueden ser los segundos patógenos responsables de las infecciones del tracto urinario (ITU) nosocomiales tras *Escherichia coli* [2]. En la comunidad, menos de 1% de las muestras de orina contienen una cantidad considerable de *Candida* [5].

Se han descrito diferentes factores de riesgo para la candiduria [6]: inmunodepresión, diabetes mellitus, ingreso hospitalario prolongado, presencia de sonda urinaria, antibioterapia de amplio espectro, sexo femenino y edad superior a los 65 años. Finalmente, la decisión de tratar la candiduria puede ser controvertida, si no se descarta un estado de colonización. La recomendación actual es administrar tratamiento antifúngico si el paciente tiene un riesgo de desarrollar candidemia, como ocurre en trasplantados renales, recién nacidos prematuros, inmunodeprimidos y pacientes sometidos a instrumentación quirúrgica urinaria. En estos casos el tratamiento de elección ha sido fluconazol [5].

En los estudios realizados, la principal especie aislada en la candiduria es *C. albicans*, seguida por especies del mismo género: *Candida tropicalis*, *Candida parapsilosis* y *Candida glabrata*. Estas especies representan un posible mayor riesgo de resistencia a los fármacos antifúngicos, sobre todo a fluconazol [1, 7].

El objetivo de este trabajo fue la descripción de los episodios de candidurias en adultos hospitalizados registrados en un Hospital Regional, su perfil de sensibilidad a los fármacos antifúngicos, y su relación con la presencia de sonda urinaria, servicio de procedencia, edad y sexo del paciente.

MATERIAL Y MÉTODOS

Se analizaron de forma retrospectiva los episodios de candiduria, ocurridos en el Hospital Universitario Virgen de las Nieves de Granada en adultos, durante un periodo de 5 años (enero de 2011-diciembre de 2015). Este Hospital es un centro de tercer nivel que, además, atiende a una población de unas 440.000 personas en la provincia de Granada (Andalucía, España).

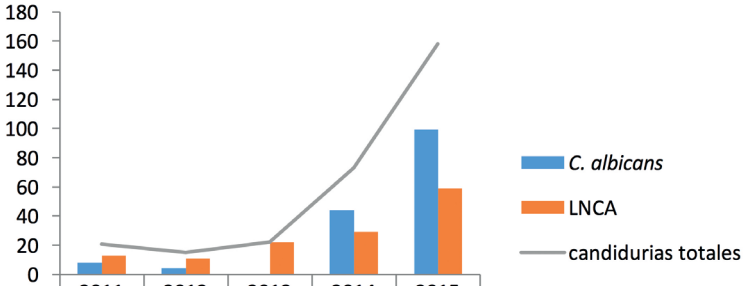
Se incluyeron los episodios de ITU con urinocultivo positivo identificados desde las Salas de Hospitalización, excluyendo los procedentes de las Unidades de Urgencias o consultas externas del hospital. De la revisión se excluyeron los episodios detectados en los 30 días posteriores tras la primera detección, incluyendo sólo el primer episodio de ITU para el estudio. La detección de levaduras en las muestras de orina se hizo según el procedimiento habitual [8]. Diez colonias de aspecto compatible con levaduras en el medio cromogénico del urocultivo se subcultivaron en medio cromogénico para levaduras para demostrar su carácter monomicrobiano. La identificación definitiva de las especies, *C. albicans* y levaduras no *C. albicans* (LNCA), y la sensibilidad a los fármacos antifúngicos se realizó mediante el sistema Vitek2 (bioMerieux, Madrid, España) según protocolo del fabricante, que investiga la sensibilidad a micafungina, caspofungina, voriconazol, fluconazol y anfotericina B [9], pero sólo los resultados frente a estos tres últimos fueron analizados. En caso de resultado indeterminado en la identificación se usó espectrometría de masas (MALDI-TOF Byotiper, Brucker-Daltonics, Billerica, EEUU) desde 2012. La sensibilidad a los fármacos antifúngicos se interpretó según los criterios del EUCAST [10]. Se consideró un aislado sensible cuando los valores de CMI fueron iguales o inferiores a 1mg/L para anfotericina B; a 2 mg/L para fluconazol en los aislados de *C. albicans, C. parapsilosis* y *C. tropicalis*; a 0,002 mg/L para fluconazol en los aislados de *C. glabrata*; y 0,125 mg/L para voriconazol, sin interpretación para *C. glabrata*.

Los datos se analizaron con el paquete de programa estadístico SPSS por MS Windows versión 17.0 (Chicago, IL, EE. UU.). Las variables cuantitativas se describen como la frecuencia de distribución de cada una de las categorías.

Consideraciones éticas. El protocolo del estudio se llevó a cabo con arreglo a la Declaración de Helsinki. Este fue un estudio no intervencionista, con ninguna investigación adicional a los procedimientos rutinarios. El material biológico se utilizó sólo para el diagnóstico estándar de ITU, siguiendo las prescripciones de los médicos. No se realizó muestreo adicional ni modificación del protocolo de rutina. Se hicieron los análisis de datos utilizando una base de datos anónima. Por lo tanto, la aprobación fue considerada innecesaria según las pautas de nuestro país. La entidad que concedió el permiso para acceder y utilizar los datos fue la «Unidad de Gestión Clínica de Enfermedades Infecciosas y Microbiología Clínica del Hospital Virgen de las Nieves de Granada, España».

RESULTADOS

Se detectaron 289 episodios de candiduria durante el periodo de estudio. Del total de muestras de orina con presencia de levaduras, 240 (83,0%) pertenecían a pacientes con algún



	2011	2012	2013	2014	2015
C. albicans	8	4	0	44	99
LNCA	13	11	22	29	59
candidurias totales	21	15	22	73	158

Figura 1 Distribución de los aislamiento de *Candida* en los diferentes años del periodo de estudio.

LNCA: levaduras no *C. albicans*

Tabla 1 Sensibilidad total del periodo de estudio a fluconazol, anfotericina B y voriconazol.

			Porcentaje de sensibilidad[b]		
Especies	Aislados[a]	Porcentaje[a]	Fluconazol	Anfotericina B	Voriconazol
C. albicans	16	10,3	100%	100%	100%
C. glabrata	46	80,7	85,9%	98,4%	---
C. parapsilosis	21	84,0	86,9%	95,6%	100%
C. tropicalis	33	89,2	92,1%	94,7%	89,5%

[a]Número y porcentaje de aislados estudiados, sobre el total de los obtenidos durante el periodo de estudio para esta especie.
[b]Por obtener valores de CMI en mg/l de anfotericina B < 1; de fluconazol < 2 para *C. albicans*, *C. parapsilosis* y *C. tropicalis*, < 0,002 para *C. glabrata*; y de voriconazol < 0,125, sin interpretación para *C. glabrata*.

tipo de sonda urinaria y en 49 (17,0%) no constaba el uso de sonda. Un total de 152 (52,6%) aislados procedían de muestras de orina pertenecientes a hombres, mientras que 137 (47,4%) pertenecían a mujeres. Hasta 155 aislados (53,6%) pertenecían a la especie *C. albicans*, el resto, 134 (46,4%), eran LNCA: 57 (19,7%) *C. glabrata*, 37 (12,8%) *C. tropicalis*, 25 (8,6%) *C. parapsilosis*, 10 (3,5%) *Candida lusitaniae*, 2 (0,7%) *Candida krusei*, y 3 (1,0%) de otras especies de *Candida*. En la figura 1 se observa la distribución de los aislamientos en *C. albicans* y LNCA por años: 21 en el año 2011; 15 en el 2012; 22 en el 2013; 73 en el 2014 y 158 en el 2015. La edad media de los pacientes con candiduria fue de 75,8 años en 2011; 64,8 en 2012; 73,3 en el año 2013; 65,6 en el año 2014 y finalmente, 71,9 en 2015.

Del total de candidurias, 62 provenían de pacientes ingresados en la Unidad de Cuidados Intensivos (21,4%), 118 (40,8%) de ingresados en Medicina Interna, y 38 (13,1%) de los servicios de Nefrología y Urología. Cuando se estudió la evolución anual, entre 2011 y 2015, de las candidurias diagnosticadas en los servicios se observó un incremento en valores absolutos en el servicio de Medicina Interna (6, 5, 9, 33 y 65, respectivamente) y un descenso en la Unidad de Cuidados Intensivos (8, 7, 8, 16 y 23, respectivamente).

En la tabla 1 se observa la sensibilidad total del periodo de estudio a fluconazol, anfotericina B y voriconazol de 116 (40,1% del total) aislados de las 4 principales especies.

DISCUSIÓN

En este trabajo se revisa la presencia de candiduria en pacientes hospitalizados debido a la relativamente escasa existencia de trabajos sobre series recientes en pacientes hospitalizados. Un estudio a nivel europeo determinó que el 9,4% de las ITU nosocomiales están causadas por *Candida* [11], cifra que justifica el interés por la comunidad científica.

Se trata de un problema clínico posiblemente subestimado ya que el urocultivo estándar puede resultar poco sensible si no se actúa de manera correcta, y puede llevar a la infraestimación de la candiduria, sobre todo en aquellas especies LNCA.

Así *C. glabrata* puede tardar más de 48horas en crecer en el medio de cultivo de agar sangre de cordero al 5%. Además, los métodos automatizados de cribado mediante citometría suelen tener problemas de sensibilidad para la detección de candiduria por *C. glabrata* debido a su pequeño tamaño [12].

En la mayoría de los estudios *C. albicans* es el microorganismo más aislado, con porcentajes entre el 50% y el 70%, 53,6 % en nuestro área, seguida por *C. glabrata* (19,7% en este estudio) y en tercer lugar *C. tropicalis* (37 aislamientos, 12,8%) [13].

Actualmente, *E. coli* sigue siendo el microorganismo más aislado en las muestras de orina de pacientes sondados, pero las especies *Candida* le siguen, destacando dentro de ellas *C. albicans*. Cuando se ha obtenido crecimiento de *Candida* en muestras de orina se abre un debate clínico acerca de su posible significación, pues puede tratarse de una contaminación, por una recogida errónea de la muestra, una colonización o de una verdadera ITU [14]. Los procedimientos básicos del laboratorio, como los criterios estrictos de significación microbiológica y una buena recogida de muestra, sin duda ayudan a resolver la cuestión. La contaminación puede diferenciarse de otros procesos mediante la obtención adecuada de una nueva muestra de orina [5]. Esto ocurre con frecuencia en pacientes femeninos de edad avanzada, cuyo introito suele estar colonizado por *Candida* y que tienen dificultad para la obtención adecuada de una muestra de orina de micción media. En estos casos, si en la segunda muestra, obtenida en adecuadas condiciones, no hay crecimiento de levaduras no es necesario proseguir con el diagnóstico.

Existen diversos estudios que enumeran los diferentes factores de riesgo para la candiduria: inmunodepresión, diabetes mellitus, ingreso hospitalario prolongado, sondaje urinario, antibioterapia de amplio espectro previa, sexo femenino y edad superior a los 65 años [6]. En nuestro trabajo, en el año 2012, tenemos una media de edad de los pacientes con candiduria inferior a lo observado en estudios previos, de 64,8 años, aunque es muy cercana a los 65 años, de modo que en nuestro área sanitaria la edad también podría ser un factor de riesgo como indican estudios previos. En este trabajo no parece que el sexo influya en la presencia de candiduria, ya que la distribución por sexos es prácticamente la misma. También en otros estudios previos se ha visto igual distribución por sexos [15]. Sin embargo, sí observamos una gran presencia de candiduria entre pacientes con sonda urinaria (83,0%). También se sabe que el uso de antibióticos de amplio espectro facilita la colonización por *Candida* tras reducir la carga bacteriana propia, intestinal y genital, y posiblemente también en áreas cercanas a la uretra; pero en este estudio no hemos recogido la información sobre la ingesta previa de antibióticos.

Generalmente, la mayoría de pacientes con candiduria se encuentran escasamente sintomáticos [2]. Pero en aquellos pacientes con sintomatología, estos son indistinguibles de los que aparecen en la ITU bacteriana. Hay síntomas poco frecuentes pero muy sugestivos de ITU por levaduras, como son la oliguria, la eliminación de material floculoso y la pneumaturia, que se presentan cuando hay una bola fúngica vesical, típico de los pacientes diabéticos [16].

En un estudio español se encontró que la candiduria aparece tardíamente durante la estancia hospitalaria, alrededor de la segunda semana [6], y solo entre el 1% y el 8% de los pacientes con candiduria desarrollan candidemia, con mayor riesgo en pacientes ingresados en UCI [17]. De este modo, globalmente, la mayoría de los pacientes con candiduria no presentarán candidemia. Pero en pacientes críticos, la candiduria siempre ha de considerarse como un potencial marcador de riesgo de infección fúngica invasiva [18]. En tal caso, es muy importante la obtención de muestras de hemocultivo y la minuciosa exploración en busca de lesiones sugerentes de candidiasis invasiva [3]. Se ha comprobado que la candiduria aumenta la mortalidad en los pacientes ingresados en UCI [19]. Hasta un 22% de los pacientes ingresados en este servicio desarrollan candiduria tras 7 días de estancia [6]. En nuestro trabajo el porcentaje de candidurias totales procedentes de UCI al final del periodo de estudio fue bajo, con respecto a estudios previos, del 21,4%, sobre todo si comparamos la incidencia con la procedente del Servicio de Medicina Interna. Podemos observar que se trató de una disminución relativa de las candidurias en UCI con respecto a las producidas en otros servicios, principalmente en el Servicio de Medicina Interna, ya que realmente van aumentando en otros lugares del hospital con los años. Al inicio del periodo, en Medicina Interna la incidencia de candidurias fue inferior a UCI (año 2011, 8 en UCI frente a 6 en MI), pero ya en el año 2015 las candidurias en Medicina Interna fueron casi el triple que en la UCI (65 frente a 23). Esto podría deberse a un aumento de los pacientes sondados en Medicina Interna, muy frecuente en los pacientes pluripatológicos o paliativos. Los porcentajes de candiduria en los servicios de Urología y Nefrología son los más elevados tras los Servicios de UCI y Medicina Interna, pero a lo largo del periodo de estudio se han mantenido fluctuantes.

C. albicans es un microorganismo tanto comensal como patógeno, que puede colonizar e invadir diferentes espacios anatómicos. La morfología de levadura se asocia con la diseminación, mientras que la morfología de pseudohifa se relaciona con la adhesión, invasión y actividad proteolítica [20, 21]. Durante muchos años *C. albicans* ha sido la especie más aislada en el tracto urinario, y en nuestro estudio continuó siéndolo, pero pensamos que quizás debido al aumento del uso del antifúngico fluconazol, las LNCA han aparecido haciéndose cada vez más dominantes. Así, muchos estudios aportan ya aislamientos de LNCA superiores al 50% [2], no en nuestro caso con un 46,36%. Estas especies son más difíciles de erradicar y están mejor adaptadas al tracto urinario, como ocurre con *C. glabrata*, con una mejor adaptación al pH y osmolaridad de esa zona [22]. El único factor de riesgo que se ha asociado de forma significativa con la selección de especies de LNCA es la administración previa de fármacos antifúngicos; y el uso de fluconazol se ha asociado específicamente a la presencia de *C. glabrata* [1]. El aislamiento de *C. glabrata* ha aumentado de forma importante en las candidurias, y en este estudio es la segunda especie más identificada, con un total de 57 aislamientos (19,7%).

Para tratar la candiduria habitualmente se tiene en cuenta su carácter sintomático o no. Actualmente, en los pacientes asintomáticos se recomienda disminuir los factores de riesgo, si es posible, y hacer un recambio de la sonda urinaria si la poseen, ya que esto puede ser suficiente para eliminar la candiduria sin necesidad de tratamiento antifúngico [23]. Sin embargo, aquellos pacientes asintomáticos pero con elevado riesgo de diseminación, como los inmunodeprimidos y los recién nacidos prematuros deben ser tratados con altas dosis fluconazol intravenoso o anfotericina B en caso de tratarse de LNCA resistentes [24]. Si se trata de candiduria sintomática el tratamiento dependerá de la presentación clínica [16]. Fluconazol es el principal antifúngico para las candidurias, en adultos o recién nacidos, por su elevada biodisponibilidad, superior al 90%, concentrándose tanto en la piel como en la orina [7, 25]. En escasas ocasiones el tratamiento recomendado es la irrigación intravesical con anfotericina B. En nuestro estudio sólo *C. albicans* sigue teniendo una sensibilidad del 100% a estos fármacos antifúngicos.

En nuestro medio, fluconazol, tratamiento de elección en candidurias, fue el antifúngico al que los aislamientos de LNCA fueron menos sensibles, con valores del 85,9%, 86,9% y 92,1% para *C. glabrata*, *C. parapsilosis* y *C. tropicalis*, respectivamente. La anfotericina B tiene un amplio espectro, pero se trata de un antifúngico con gran toxicidad renal, limitando su uso. Para disminuir la nefrotoxicidad se crearon las formulaciones lipídicas. Aunque la resistencia a anfotericina B es rara en *Candida* ya se ha registrado en estudios previos [26]. En nuestro estudio se obtuvo una resistencia a este antifúngico en las especies *C. glabrata*, *C. parapsilosis* y en *C. tropicales*, que no superó el 10%. Todos los aislamientos de *C. albicans* fueron sensibles a anfotericina B. Pensamos que la sensibilidad podría, independientemente de la especie, estudiarse en todos los aislamientos de pacientes con candiduria y terapia antifúngica previa debido a que esta puede suponer un factor de riesgo para la presencia de especies farmacorresistentes. Las técnicas comerciales, como Vitek 2® (bioMérieux), se han implantado en muchos laboratorios clínicos, aunque se ha descrito la posibilidad de falsos positivos en la detección de resistencia de *C. tropicalis* frente a fluconazol y voriconazol [27]. Entonces, en base a los datos obtenidos, fluconazol puede seguir siendo el tratamiento de elección para las candidurias, siendo especialmente válido para *C. albicans*, aunque también para el resto de LNCA sería válido como tratamiento empírico en nuestro entorno ya que la resistencias son menores al 15%.

En conclusión, los estudios de urocultivos en pacientes hospitalizados deben incluir la posible presencia de candiduria, que es más prevalente en los procedentes de los Servicios de Medicina Interna, portadores de sonda urinaria y con una edad superior a los 65 años. Aunque la resistencia a fluconazol es baja en nuestro medio, dada la elevada prevalencia de LNCA, se recomienda incorporar, al menos, estudios de identificación de especie para predecir la posible resistencia a aquel.

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CONFLICTO DE INTERESES

Los autores declaran no tener ningún conflicto de intereses.

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Original

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Changes in tuberculosis in human immunodeficiency virus infected patients in a Spanish tertiary hospital (1995-2013)

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ABSTRACT

Objectives. Although the incidence of human immunodeficiency virus (HIV)-associated tuberculosis (TB) has decreased, changes in other characteristics of the disease are largely unknown. To describe the trends in TB in patients infected with HIV from 1995 to 2013.

Methods. We review all cases of TB in a tertiary hospital in Madrid, Spain.

Results. Among 1,284 patients diagnosed of TB, 298 (23%) were coinfected with HIV. The prevalence of HIV infection during the period of study has decreased from 40% to 14% (p for the trend < 0.001). Clinical presentation has also changed. Although pulmonary and extrapulmonary TB has remained unchanged, miliary presentation has significantly decreased (from 36% to 22%, p = 0.005). The 4-drug regimen was the preferable scheme, with higher implementation at the end of the study period (82% from 1995-1999 to 95% in 2010-2013, p = 0.43). Factors such as treatment failure (OR: 11.7; CI 95%: 3.12-44.1) and miliary form (OR: 2.8; CI 95%; 1.09-7.3) were independently associated with TB related mortality, while the longer duration of treatment was as a protective factor (OR 0.7; CI 95%: 0.6-0.8).

Conclusions. HIV has decreased very significantly as a risk factor for the development of TB. Despite improvement in the treatment of both TB and HIV, and in overall mortality, deaths attributable to the disease in this population remain high mostly in miliary and relapsing forms.

Key-words: Tuberculosis, VIH, Acquired Immune-deficiency Syndrome, Antiretroviral Therapy, Multidrug Resistant Tuberculosis.

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Cambios en los pacientes coinfectados por tuberculosis y por el virus de la inmunodeficiencia humana en un hospital terciario español (1995-2013)

RESUMEN

Objetivos. Aunque la incidencia de la coinfección por el virus de la inmunodeficiencia humana (VIH) y la tuberculosis (TB) ha disminuido, los cambios ocurridos en otras características de la enfermedad son desconocidos. El objetivo de nuestro trabajo fue describir las tendencias en los pacientes con tuberculosis infectados por el VIH en un periodo de casi dos décadas (1995-2013).

Métodos. Se revisaron todos los casos de TB en un hospital terciario en Madrid, España.

Resultados. De los 1.284 pacientes diagnosticados de TB, 298 (23%) estaban coinfectados por el VIH. La prevalencia de la infección por el VIH durante el periodo del estudio disminuyó del 40% al 14% (p para estudio de tendencias < 0,001). La presentación clínica también se modificó. Aunque las formas pulmonares y extrapulmonares permanecieron invariables a lo largo del estudio, la presentación miliar disminuyó de modo significativo (del 36% al 22%, p = 0,005). El esquema de 4 fármacos fue el mayormente elegido, con un incremento de la implementación de dicho tratamiento al final del periodo de estudio (82% desde 1995-1999 frente a 95% en el periodo 2010-2013, p = 0,43). El fracaso del tratamiento (OR 11,7; IC 95%: 3,12-44,1) y las formas miliares (OR 2,8; IC 95%; 1,09-7,3) se asociaron de forma independiente con la mortalidad atribuida a TB, mientras que la mayor duración del tratamiento se comportó como un factor protector (OR 0,7; IC 95%: 0,6-0,8).

Conclusión. La infección por el VIH ha disminuido de forma significativa como factor de riesgo principal del desarrollo de TB en nuestro medio. A pesar de la mejora en el

tratamiento de la TB y de la infección por el VIH, así como una menor mortalidad total, los fallecimientos atribuidos en esta población permanecen muy elevados, sobre todo en las formas recidivantes y en la TB miliar.

Palabras clave: Tuberculosis, HIV, Síndrome de inmunodeficiencia adquirida, Tratamiento Antiretroviral, Tuberculosis Multirresistente.

INTRODUCTION

The importance of tuberculosis (TB) in Western countries has been dropping during the last years [1]. Despite the decline in its incidence, more than 9 million people were diagnosed with TB in 2015 [2], and it is estimated that 1.5 million deaths could be related to TB worldwide. Traditionally, Spain ranks one of the regions in European Union in TB and acquired immunedeficiency syndrome (AIDS) incidence [3].

Since the introduction of highly active antiretroviral therapy (HAART) the number of patients infected with HIV and TB is declining. However, the burden of this association remains too high in resource-poor settings with more than 80% of notified TB patients being HIV infected [2].

In our environment, different changes in HIV epidemic have modified the TB pattern. The present study aimed to describe the changes in demographical and clinical characteristics as well as in treatment outcomes of HIV infected patients with TB over a long period in Spain.

METHODS

Study design and setting. We conducted an observational retrospective study at the Ramón y Cajal Hospital, a 1200-bed tertiary referral center in Madrid that provides medical care to a population of 600,000 inhabitants.

Patients. We included all adult patients with a TB diagnosis from January 1995 to December 2013. TB was considered only when *Mycobacterium tuberculosis* was isolated from culture of a clinical sample or, in the absence of identifying the organism, a compatible clinical picture together with the finding of granulomas in a tissue biopsy and/or an elevated adenosine deaminase level in an organic fluid plus a positive PCR for *M. tuberculosis* in a clinical sample. Patients who were less than 16 years old were excluded from the analysis. HIV-infected patients were selected from the TB cohort. All the patients included in the study were admitted to the Respiratory Isolation Unit, where HIV testing is routinely performed to all patients with TB. In order to make comparative analysis, the years of the study were divided into four periods: 1995-1999, 2000-2004, 2005-2009 and 2010-2013.

Patients were identified by cross-matching two hospital registries: the Microbiology Department database and the internal server of the center with discharge diagnoses. Data on all patients were obtained from the medical records. Ethics consent was obtained and approved by the local ethic committee of our institution.

Definitions. TB was considered to be pulmonary if *M. tuberculosis* was isolated in culture of respiratory samples or/and if chest X-ray was suggestive of pulmonary involvement. The extrapulmonary forms were considered if the isolation was from a non-pulmonary source or histologically confirmed in patients with TB proven by culture. In extrapulmonary patients with negative cultures, TB was established if: adenosine deaminase cut-off levels were higher than 35 Ud/L in pleural and peritoneal liquid and more than 6 Ud/L in cerebrospinal fluid; a positive polymerase chain reaction (PCR) in smear other than sputum and positive acid fast bacilli (AFB) was obtained. TB was considered to be miliary if chest X-ray showed a miliary pattern and TB was confirmed by culture of pulmonary or non-pulmonary samples.

Data collection. The following data of the diagnosis of TB was registered: date of diagnosis, type of TB and socio-demographic variables that included age, gender, and country of origin. Associated risk factors and comorbidities (smoking habit, drug use, alcohol abuse, diabetes, neoplasia, chronic renal disease, liver disease, chronic obstructive pulmonary disease, malnutrition and social status) were also recorded. Regarding tuberculosis, the following variables were included: date of diagnosis, type of TB, microbiological and other diagnostic procedures (tuberculin skin test (TST), AFB smear, PCR, culture, histological examination, ADA and drug susceptibility testing), antituberculosis drugs administered, duration of treatment, associated adverse events, treatment adherence and outcome. Finally, the following information related to HIV infection was registered: risk practice for and time of the HIV infection, last count of CD4+ T-lymphocytes, and plasma viral load before the diagnosis of TB, antiretroviral treatment administered before the diagnosis of TB. As a retrospective cohort, no information could be collected on the timing of initiation of antiretroviral therapy, nor on the development of immune reconstitution inflammatory syndrome (IRIS) in patients who initiated treatment after the diagnosis of TB. The main point of the study, trends in HIV, is hard enough to prevail over less relevant complications such as side effects not affecting prevalence.

Laboratory procedures. *M. tuberculosis* culture of samples was performed according to Tacquett & Tison method and inoculated on solid media, Lowenstein Jensen and Coletsos media (Bio Medics SL, Tres Cantos, Madrid, Spain) and, since 1996, in liquid medium (Veersa TREK system, formerly ESP culture System II). The strains isolates were identified using DNA probes (Gen Probe, San Diego, California, USA) and phenotypic tests. *In vitro* susceptibility tests were undertaken according to Canetti's method on Lowenstein Jensen medium and 7H10 agar medium until 1996, and later in liquid medium with antibiotic concentrations according to the manufacturer's protocol (Versa TREK system, formerly ESP culture System II). Multidrug resistant TB (MDR-TB) was defined when the organism was resistant to at least isoniazid and rifampin. If the organism had additional resistance to a fluoroquinolone and a second-line injectable drug was considered extensively drug-resistant TB (XDR-TB).

Transcription-mediated amplification was applied for molecular diagnosis. The Amplified *M. tuberculosis* Direct Test (AMTD; Gen-Probe Inc.) is a rapid isothermal (42º C) method based on the amplification of 16S-rRNA. Reverse transcriptase is used to copy rRNA to a cDNA-RNA hybrid, and the chemiluminescent method is then applied using specific DNA probes. This procedure was directly applied on clinical samples, including sputum, bronchoalveolar lavage, tissue biopsy specimens and urine.

Outcome. Treatment for TB was considered completed if correct therapy and follow-up were confirmed and clinical features showed a favorable outcome. In patients who had abandoned therapy and who had received more than one course of therapy, only the last episode was included for analysis. Patients whose smear or culture remained positive during the monitoring in an outpatient setting were considered failure.

Statistical analysis. The epidemiology of the disease and changes occurring during the study period as well as risk factors related to mortality were analyzed. The descriptive statistical analysis included medians and interquartile ranges (IQR) for continuous variables, and frequencies and proportions for categorical variables. The chi square test and Student t test were used to compare continuous and categorical data, respectively. Odds ratio (OR), 95% confidence interval (CI), and P values were estimated. A $P < 0.05$ was considered statistically significant.

Univariate and multivariate logistic regression analyses were performed to assess factors associated with poor outcomes. Multiple logistic regression analysis was used to determine the independent risk factors associated with mortality. All variables with a $p< 0.1$ in the univariate analysis, as well as those clinically significant that could have an impact on mortality, were entered in the multivariate model. Software SPSS Statistics 19® was used for the statistical analyses.

RESULTS

Patients characteristics. During the study period 1,284 patients were diagnosed with TB. HIV infection at the time of TB diagnosis was present in 298 patients (23.2%). The diagnoses of HIV-infection and TB were made concurrently in 67 cases (22.4%). The median CD4+ T cell count and plasma HIV RNA was 100 cells per cubic millimeter and 100000 HIV RNA copies per milliliter, respectively. HAART was being administered to only 42 cases (14%). Characteristics of the patients according to the HIV infection status are shown in table 1. HIV infected patients were significantly younger (mean age: 36 vs. 47, $P < 0.001$), predominantly men (79% vs. 59%, $P < 0.001$), and with more comorbidity, regarding coinfection with hepatitis C virus infection (57% vs. 7%, $P <0.001$), alcoholism (27% vs. 19%, $P <0.001$) and drug abuse (65% vs. 3%, $P < 0.001$).

There were significant changes in the different study periods. From 1995 to 2013, the prevalence of HIV infection decreased from 40% to 14% (X^2 trend: 94.5; $P < 0.001$, figure 1) while the median age at diagnosis of patients with HIV infection and TB increased (35 to 45 years, $P < 0.001$). Regarding the clinical forms, both pulmonary (85% in 1995-1999 to 89% in 2010-2013, $P = 0.7$) and extrapulmonary (61% in the two periods, $P = 0.68$) involvement remained stable. However, miliary TB showed a significant decrease in the period of study, from 36% in 1995-1999 to 22% in 2010-2013 ($P = 0.005$) (figure 2).

Table 1 Patients's characteristics at diagnosis of tuberculosis according to HIV status.

	HIV positive (n= 298)	HIV negative (n= 986)	P value
Gender, male	236 (79%)	585 (59%)	< 0.001
Age, years	36 (32-41)	47 (29-64)	< 0.001
Foreign-born	30 (10%)	274 (28%)	< 0.001
Alcohol abuse	80 (27%)	191(19%)	< 0.001
Injection drug users	195 (65%)	30 (3%)	< 0.001
Chronic liver disease	111 (37%)	60 (6%)	< 0.001
HCV	169 (57%)	53 (7%)	< 0.001
Chronic renal disease	2 (4%)	36 (1%)	< 0.001
Diabetes Mellitus	3 (1%)	89 (9%)	< 0.001
Pulmonary TB	249 (84%)	669 (68%)	< 0.001
Extrapulmonary TB	180 (60%)	437 (44%)	< 0.001
Miliary TB	86 (29%)	30 (3%)	< 0.001
Previous TB episode	35 (12%)	97 (10%)	0.34
Marginality	45 (15%)	35 (3%)	< 0.001

Data are reported as number (%) of patients or main value (interquartile range). HIV = human immunodeficiency virus, TB = tuberculosis, HCV = Hepatitis C virus.

Diagnostic procedures. A tuberculin skin test (TST) was performed in 161 (54%) patients. In 93 patients (58%) the TST was positive. The sputum smear was positive in 154 of 272 (57%), and the culture was positive in 205 out of 272 (75%). PCR in sputum was positive in 37 out of 45 patients (82%). The remaining specimens submitted for microbiological studies and the results are summarized in table 2. Of note, the yield of sputum smears and cultures, as well as that of other microbiological and biochemical studies did not change during the study period.

Anti-tuberculosis drug resistance. Susceptibility testing was undertaken in 268 isolates (90%). Resistance ≥ 1 drug was detected in 37 isolates (14%). Primary isoniazid resistance was documented in 8 cases (3%). MDR-TB and XDR were observed in 10 (4%) and 10 (4%) isolated, respectively. Most resistant isolates were detected in the first period, although changes in the rates of resistance during the study period were not significant.

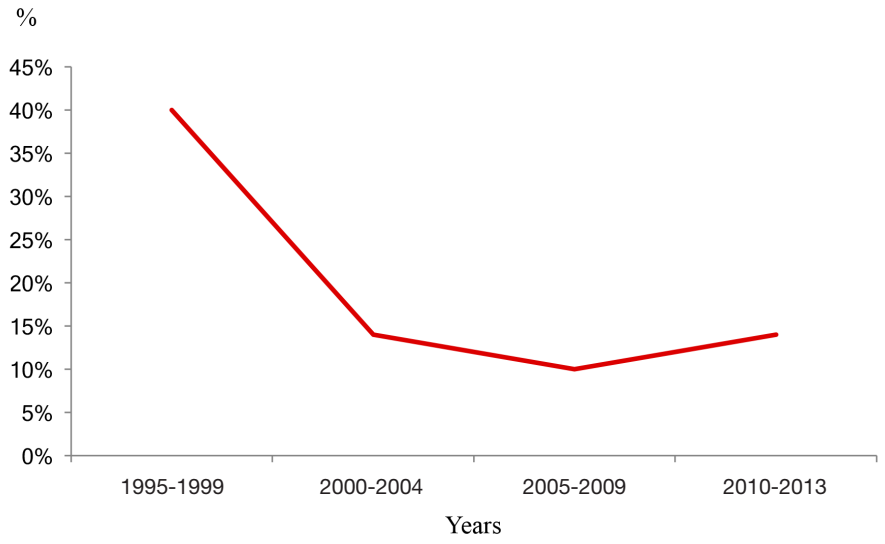

Figure 1 | **Changes in human immunodeficiency virus prevalence among patients with tuberculosis from 1995 to 2013.**

Data are expressed as number of HIV-infected patients with tuberculosis/total number of patients with tuberculosis.

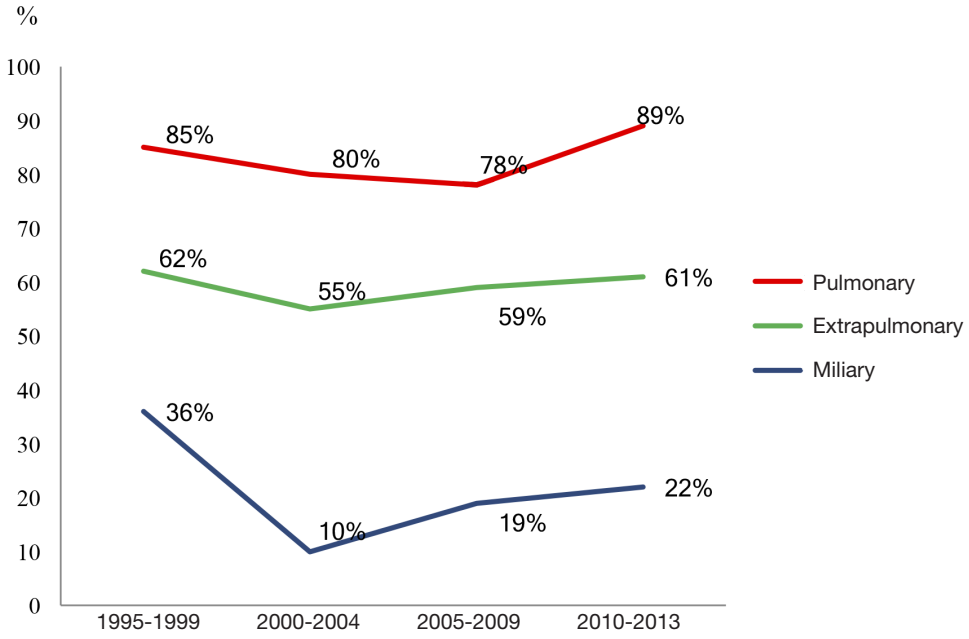

Figure 2 | **Changes in clinical involvement of tuberculosis in HIV infected patients from 1995 to 2013.**

Outcomes. TB-treatment was administered in 291 (98%) episodes. The remaining patients did not receive treatment due to early death during the episode or loss of follow-up. In patients with isolated pulmonary involvement, the median duration of treatment was 9 (IQR = 6-12) months which was not different to that in patients with extrapulmonary or disseminated disease [median 10 (IQR = 6-12) months]. A 4-drug regimen was the therapy most commonly used, with higher implementation at the end of the study period without significant differences (82% from 1995-1999 to 95% in 2010-2013, P = 0.43). A completed treatment was documented in 182 (71%) patients, 44 (17%) discontinued the therapy and 71 (24%) were lost during follow up.

Table 2 Yield of different diagnostic laboratory methods for the diagnosis of tuberculosis in human immunodeficiency virus infected patients

Specimen	Stain	Culture	PCR	ADA[a]
Sputum	154/272 (57)	205/272 (75)	37/45 (82)	-
Urine	23/129 (18)	47/128 (43)	19/49(39)	-
Pleural fluid	1/14 (7)	6/14 (43)	-	37 (33-67)
Ascitic fluid	1/3 (33)	1/2 (50)	-	37 (37-51)
Cerebrospinal fluid	1/18 (6)	4/19 (21)	-	11 (7-12.5)
Lymph node[b]	58/72 (81)	61/70 (87)	19/23 (83)	-
Bone marrow[c]	6/18 (25%)	9/24 (37)	-	
Blood	-	6/15 (40)	-	-

Data are expressed as number positive / number tested (%). PCR = polymerase chain reaction.
ADA = adenosine deaminase.
[a]Data are expressed in median (interquartile range). [b]Lymph tissue evaluated by fine needle aspiration.
[c]Bone marrow examination.

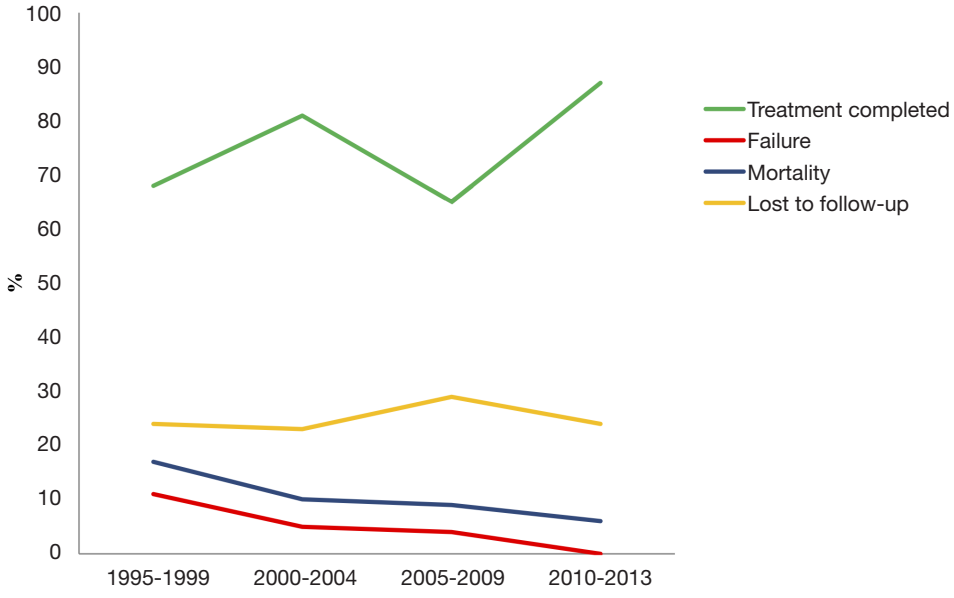

Figure 3 Trends in TB treatment outcomes from 1995 to 2013.

A total of 57 (19%) patients deceased, and death could be directly attributed to TB in 43 cases (14%). Throughout the study period a trend to a better success rate of completed treatment was observed (68% vs. 87%, p =0.1), while mortality ($p < 0.05$) and treatment failure ($p < 0.05$) decreased. The number of patients lost during follow-up remained stable (figure 3).

After adjusting for all variables, treatment failure (OR: 11.7 CI 95%; 3.12-44.1) and miliary TB (OR: 2.8; CI 95% 1.09-7.3) were independently associated with mortality related with TB. The longer duration of treatment was identified as a protective factor (OR: 0.7; CI 95% 0.6-0.8).

DISCUSSION

This study reports the changes in TB in HIV infected patients during two decades in a tertiary center. We have confirmed the importance of HIV infection as a risk factor

for TB. Although the number of patients with TB and HIV infection had decreased during the study period, HIV continues to be an important risk factor for TB even after the advent of combination antiretroviral therapy. At the beginning of the 90's, Spain was a high incidence country in TB with highest rate of AIDS [5]. Over the last few years, important changes have taken place in our country which explains the observed decline in TB-HIV coinfection [6-9].

Most studies have consistently showed a reciprocal interaction among the two diseases which leads to a significant impact [10]. The great spread of the HIV epidemic in the 90's contributed to the resurging global epidemic [11]. Although there has been a fall in Western countries which could be attributable to the introduction of HAART as well as the new development of other policy health control measures [6], it still remains high in other settings. In Europe, Eastern-countries such as ex-former Soviet Union countries or Portugal have the highest incidence rates for coinfection of TB and HIV [12].

Interestingly, our data shows a very low percentage of patients with HAART at TB diagnosis. This issue could be due to the increase of both diagnoses at once.

We have observed no changes in the frequency of overall pulmonary and extrapulmonary involvement in HIV infected patients with TB, but a marked decline in miliary forms. The latter is possibly related to the better control of AIDS with less severe immunodeficiency during shorter periods of time. In fact, the few cases of military TB were diagnosed in patients with very low CD4 counts, as commented in previous reports [9, 13].

Our microbiological results are similar to those of other large cohorts [14], with no relevant changes regarding the diagnostic yield regardless the specimen tested and the methods used. It is true, however, that PCR in sputum led to an increase in the accuracy of diagnosis of almost 10%. Similarly, PCR has been used successfully in patients with several extrapulmonary and pulmonary forms [15-17], and it was helpful in some cases to detect earlier resistant isolates [18].

It is worthy to highlight the low percentage of primary isoniazid resistance in our cohort (3%). This could explain a higher use of the three drugs regimen at the beginning of the study. The American Thoracic Society consensus guidelines [19], as well as the Spanish Health Authorities recommendation [20] have established the four-drug regimen as the recommended starting regimen which is the preferable scheme in our cohort at the end of the study.

Our data show better outcomes throughout the study in completed treatment and mortality. Although mortality is still high in HIV infected patients, it has changed dramatically with respect to the 90's, where AIDS related infections and delayed treatment for TB were the main causes of death [21]. Moreover, HIV is a well- known risk factor for TB mortality in low-income countries [22,23], and has also been a matter of interest in Western countries [24]. In our patients, the failure of treatment and the miliary forms were independently related with death attributable to TB. Several factors could help to mitigate the risk for death in this population. For instance, in a prospective study in Brazil, an earlier timing for HAART initiation was associated to a lower risk for death related to TB [25], as shown in clinical trials [26, 27].

We are aware of the limitations of our study. Those related with the retrospective nature of the analysis are most significant, specially the lack of some important information. In this sense, we could not provide any results on the impact of the timing of initiation of antiretroviral therapy on overall and TB related mortality, as well as on the development of IRIS. It is very likely that an association be found, as shown in clinical trials mentioned earlier [26,27]. We could only show an indirect association between the availability of combination ART and the decrease on the incidence and mortality of HIV-associated TB. The goal of our study was to describe the changes of TB in HIV infected patients during a long period, and hope that we have succeeded with the picture described, despite the admitted limitations.

In summary, our study shows that HIV and TB coinfection has steadily decreased during the last two decades. Miliary TB has been significantly reduced, although other extrapulmonary and pulmonary forms have remained stable. Although the burden of HIV infection in patients with TB is still relevant, a better prognosis has been observed over the years with lower rate of mortality and lost to follow-up.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

FUNDING

None to declare

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Original

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Role of *Pneumocystis jirovecii* in patients with different pulmonary underlying condition using a nested-PCR

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ABSTRACT

Introduction. The prevalence of *Pneumocystis jirovecii* colonization and its role in pulmonary disease remains unclear. PCR methods have shown an improved sensitivity in the detection of this fungus. It has been suggested that the PCR results be combined with another test such as IFA to create a diagnostic algorithm.

Material and methods. A multiplex nested-PCR procedure with a 16S rRNA gene as the internal amplification control was evaluated to determine the role of *P. jirovecii* in pulmonary disease.

Results. A 20% of the 199 bronchoalveolar lavage samples were PCR-positive, 13.5% samples were PCR-inhibited, and the rate of Pneumocystis-colonisation was 6.4%. The sensitivity, specificity, positive predictive value and negative predictive value of the nested-PCR were 100%, 93%, 70% and 100%, respectively. The sensitivity of the nested-PCR was higher than the current "gold standard" immunofluorescence assay (IFA) (p< 0.0001). PCR-negative and PCR-positive patients did not show any clinical or radiological differences in the medical variables studied.

Conclusion. PCR could help the diagnosis of *Pneumocystis* pulmonary disease given the high negative predictive value of the technique. *P. jirovecii* DNA can frequently be detected in healthy population, so the analysis of the patient medical history is critical to make the correct clinical decision.

Keywords: *Pneumocystis jirovecii*; nested-PCR; internal control; colonisation; pulmonary disease.

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Papel de *Pneumocystis jirovecii* en pacientes con diferente patología pulmonar de base usando una PCR anidada

RESUMEN

Introducción. La prevalencia de la colonización por *Pneumocystis jirovecii* y su papel en la enfermedad pulmonar sigue sin estar clara. Los métodos de PCR han demostrado una sensibilidad mejorada en la detección de este hongo. . Se ha sugerido que los resultados de PCR se combinen con otra prueba como IFA para crear un algoritmo de diagnóstico.

Material y métodos. Se evaluó una PCR múltiple anidado con el gen 16S rRNA como control interno de amplificación para determinar el papel de *P. jirovecii* en la enfermedad pulmonar. **Resultados.** Un 20% de las 199 muestras de lavado broncoalveolar fueron positivas para PCR, 13,5% muestras fueron inhibidas por PCR, y la tasa de colonización por *Pneumocystis* fue de 6,4%. La sensibilidad, especificidad, valor predictivo positivo y valor predictivo negativo de la PCR fueron del 100%, 93%, 70% y 100%, respectivamente. La sensibilidad de la PCR fue mayor que el ensayo de inmunofluorescencia "gold-standard" (IFA) actual (p <0,0001). Los pacientes PCR-negativos y PCR-positivos no mostraron diferencias clínicas o radiológicas en las variables médicas estudiadas.

Conclusión. La PCR podría ayudar al diagnóstico de la enfermedad pulmonar por *Pneumocystis* dado el alto valor predictivo negativo de la técnica. El ADN de *P. jirovecii* se puede detectar con frecuencia en poblaciones sanas, por lo que el análisis del historial médico del paciente es fundamental para tomar la decisión clínica correcta.

Palabras clave: *Pneumocystis jirovecii*; PCR anidada; control interno; colonización; enfermedad pulmonar.

INTRODUCTION

Pneumocystis jirovecii is an opportunistic fungal pathogen that causes pneumonia (PJP) in immunocompromised hosts. Over the past decade, despite the decreased number of PJP cases among HIV-infected patients, PJP has become a serious problem in immunodeficient patients with other immunosuppressive conditions [1, 2]. *P. jirovecii* has a global distribution; most people have serologic evidence of infection during early childhood [3, 4], and normal healthy individuals can carry this fungus. The colonisation may develop into PJP if there is a worsening of the underlying disease and the patients does not receive appropriate prophylaxis [2, 5, 6]. However, the prevalence of *P. jirovecii* infection without disease remains unclear and complicates the interpretation of positive results becoming the clinical diagnosis of PJP into a challenging question.

The detection of the fungus in the laboratory by direct microscopic immunofluorescent staining of respiratory smears and tissue specimens has long been considered the major diagnostic tool and the current "gold standard". The development of PCR technology with increased sensitivity allowed the detection of subclinical infections and colonisations [7-9]. These new approaches are especially important for non-HIV immunocompromised patients where the diagnosis is more difficult [10]. Currently, molecular detection is superior to microscopic evaluation [11, 12].

PCR assays vary significantly in their detection technology, turnaround time, type of clinical sample and capacity to yield a quantitative versus qualitative result [8, 13-20]. Simple DNA extraction and nested PCR in bronchoalveolar lavage specimens has been shown to be a sensitive test [6], which may be performed in clinical laboratories [21], with similar or even better sensitivity than real-time PCR [22]. Nevertheless, bronchoalveolar lavage (BAL) samples may contain inhibitors of the PCR reaction. The use of an internal control (IC) can identify inhibitory substances and monitor the PCR reaction [23].

The objectives of this work were to determinate the role of *Pneumocystis jirovecii* in the pulmonary disease and to evaluate a simple multiplex nested-PCR in bronchoalveolar samples to detect *P. jirovecii*.

MATERIAL AND METHODS

From April 2013 to April 2014, 199 BAL samples sent to the laboratory for the detection of any infectious agents from 197 patients at the 1250-tertiary bed University Hospital (CHUVI) were collected.

Sample preparation for staining. BAL samples were mixed V/V with Sputasol (Oxoid) and mixed vigorously for 5 min. Samples were centrifuged at 3000 g for 10 min, and the pellets were resuspended 1/10 in 0.9%NaCl. The resuspended pellet was used to prepare smears. An immunofluorescence assay (IFA) was performed with a flouresceinisothiocyanate-conjugated monoclonal antibody to *P. jirovecii* following the manufacturer's instructions (MONOFLUO™ *Pneumocystis jirovecii* IFA Test Kit, Bio-Rad, Laboratories, USA).

Sample preparation for DNA extraction. One ml of BAL samples were aliquoted in 200 µl each and were stored at -20°C until they were studied. DNA was extracted from stored BAL samples using an InstaGene Matrix kit (Bio-Rad Laboratories, USA) according to the manufacturer's recommendations.

The nested-PCR protocol for amplification of mtLSUrRNA in *P. jirovecii* was performed as previously described

Table 1 **Primers sequences and thermocycling nested-PCR conditions.**

Reaction	Target	Primer name	Primer sequences	Cycling conditions	
Primary amplification					
	mtLSUrRNA	pAZ102-E	5´-GATGGCTGTTTCCAAGCCCA-3´	94°C 10 min	
		pAZ102-H	5´-GTGTACGTTGCAAAGTACTC-3´	40 cycles	94°C/1 min
					50°C/1 min
		U1	5´-CCAGCAGCCGCGGTAATATCG-3´		72ºC/2 min
	16sRNA	U2	5´- ATCGG(C/T)TACCTTGTTACGACTTC-3´		
				72°C 10 min	
Secondary amplification					
	mtLSUrRNA	pAZ102-X	5´-GTGAAATACAAATCGGACTAGG-3´	94°C 5min	
		pAZ102-Y	5´-TCACTTAATATTAATTGGGGAGC-3´	45 cycles	94°C/20sec
					50°C/20sec
					72°C/20sec
				72°C 10 min	

by Wakefield et al.[13]. The external primers pAZ102-E and pAZ102-H to mtLSUrRNA *P. jirovecii* gene amplification were used in the first amplification round which produced a 346 bp amplicon, being included in the same reaction as an internal control 16s rRNA gene amplification primers (U1 and U2), which produced a 996 bp product [24]. The internal primers pAZ102-X and pAZ102-Y were used in a second round to amplify a 260 bp fragment.

Each PCR reaction contained 5 µL of the extraction product, 12.5 µL QIAGEN Multiplex PCR MasterMix and 0.2 µM concentration of each primer in a total volume of 25 µL (QIAGEN Multiplex PCR kit,Qiagen, Hilden, Germany);5 µL of the first PCR product was used as the DNA template for the second PCR reaction. The PCR products were analysed by electrophoresis on 1% agarose gel stained with RedSafe TM Nucleic Acid Staining Solution (iNtRON Biotechnology Inc., Sungnam, Kyungki-Do, Republic of Korea) (table 1).

External controls, contamination prevention and validation of the nested-PCR product. During each PCR, a positive control (a BAL fluid sample from a patient with PJP, *P. jirovecii* positive immunofluorescence assay) and ultra-pure water as the negative control were used. Amplification of the 16s RNA was performed to confirm successful DNA extraction and the absence of PCR inhibitions. To avoid contamination, all steps (master mix preparation, DNA extraction, amplification, and addition of the PCR product) were performed in separate areas. To validate the usefulness of the primers U1/U2 to identify oropharyngeal bacteria by PCR that usually contaminate the BAL samples, the following bacteria were studied: *Streptococcus mitis* ATCC 49456, *Streptococcus oralis* ATCC 35037, *Streptococcus dysgalactiae subps equsimilis* ATCC 10009, *Corynebacterium striatum* ATCC 7094, *Hemophilus parainfluenzae* ATCC 9796 and *Neisseria cinerea* ATCC 14685, getting a 996 bp product in all of them. The nested-PCR product was purified using a QIAquik PCR purification Kit (Qiagen, Hilden, Germany) and sequenced with the forward primer pAZ102-X. A BLAST search was performed to verify the PCR-amplicon result.

Clinical and microbiological data collection. Demographic and clinical data including: age, sex, haematological malignancies, solid tumours, transplant recipients (bone marrow or solid organ), immunosuppressive therapy, tobacco smoke exposure, inflammatory lung disease, HIV infection, anti-PJP prophylaxis or treatment, clinical symptoms, season of the year and other organisms identified in the specimen. All samples were studied according to the procedures, serological studies and molecular studies for difficult to culture bacteria and virus were performed on request by the clinician [25].

Colonisation or Pneumocystis infection. Patients with a negative staining result who were PCR-positive were classified as colonised or infected based on a medical chart review. Colonisation was defined in cases involving a patient without symptoms, no previous infection or treatment for PJP, no radiological abnormalities and favourable clinical outcome without specific treatment or symptoms attributed to another basic pathology. *Pneumocystis* infection (true-PJP) was defined in the setting of symptoms (fever or low grade fever, cough, dyspnea, weight loss) and compatible radiography or CT scan [26], provided that no other infectious agent or immune-allergic aetiology were identified.

Statistical analysis. Analysis was performed using SPSS version 20.0 (Chicago, IL, USA). All clinical variables were compared between the PCR-positive and PCR-negative groups. Continuous variables were compared using Student`s *t*-test, and categorical data were compared using the Chi-squared test. A two-tailed p value <0.05 was considered statistically significant.

Sensitivity, specificity and positive predictive value (PPV) and negative predictive value (NPV) of IFA and PCR were calculated. Positive-PCR results in patients with pneumonia were considered true-positive. False positive was considered the positive-PCR results in colonized patients. To determine the predictive values, we assumed a PJP prevalence of 20%, which is considered the median rate of colonisation in immunocompetent adults [27].

RESULTS

Nested-PCR results. For the 199 BAL specimens tested by nested-PCR, 35 (20.3%) were positive, 137 (68.8%) were negative and 27 (13.5%) were PCR-inhibited.

There was an initial inhibition of the PCR reaction in 47 samples, but the inhibition problems were resolved in 20 of them, with a second DNA extraction of the original specimens. This produced a total of 27 samples that were definitely considered as PCR-inhibited.

Comparison of classic staining/nested-PCR. Only 6 of the PCR-positive samples were positive based on IFA, and no negative PCR results with positive microscopy results were found.

Based on the criteria established in the materials and methods section, we estimated the sensitivity and specificity of each technique: IFA sensitivity and specificity was 25% (IC 95%: 0.08-0.42) and 100% (IC 95%: 0.97-1), respectively, and the nested-PCR sensitivity and specificity was 100% (IC95%: 0.85-1) and 93% (IC 95%: 0.88-0.97), respectively. There was a significant difference in sensitivity between PCR and IFA (P<0.0001). The PPV of the technique was 70% (IC95%: 0.5-0.9), and the NPV was 100%.

A good correlation between the IFA and PCR results was found in HIV-positive patients (2/2), but PCR detected more possible PJP patients among the non-HIV immunocompromised groups.

Characteristics of the patients. The mean age of the 170 patients was 61 +- 14 years, 105 (62%) were male. The most common underlying diseases were haematologic malignancies or solid tumours (35%); only 11 patients were HIV positive (6.5%), 88 (51.8%) had chronic lung disease (CPOD, asthma, pneumoconiosis, or interstitial lung disease). Active or previous exposure to tobacco was identified in 50% of the patients and

Table 2 **Clinical data of positive-PCR patients**

Age	Sex	Underlying disease	Clinical presentation	Immunosuppressive therapy	Tobacco[a] exposure	Pneumocystis prophylaxis	Radiological signs	Sampling season	Colonisation/PJP
62	Male	Crohn´s disease	Asymptomatic	Yes	Ex	No	Infiltrate	Spring	Colonisation
71	Female	Chronic lymphocytic leukaemia B	Dyspnoea, cough, fever	Yes	No	No	Infiltrate	Spring	Pneumonia
44	Female	Bronchial asthma	Asthma exacerbation	Yes	No	No	Infiltrate	Spring	Pneumonia
64	Female	Microscopic polyangiitis	Cough, fever	No	Ex	Yes	Normal	Spring	Pneumonia
39	Female	Breast cancer	Cough, dyspnea	Yes	No	No	Infiltrate	Spring	Pneumonia
68	Male	Hypersensitivity pneumonitis	Asymptomatic	No	Yes	No	Normal	Spring	Colonisation
46	Male	Silicosis	Asymptomatic	No	No	No	Infiltrate	Spring	Colonisation
70	Male	Colon cancer	Dyspnoea, cough, fever	Yes	Ex	No	Consolidation	Summer	Pneumonia
69	Female	COPD	Cough, fever	Yes	Yes	No	Infiltrate	Summer	Pneumonia
61	Male	Silicosis	Dyspnoea, cough	No	Ex	No	Infiltrate	Summer	Pneumonia
33	Male	HIV	Dyspnoea, cough	No	No	No	Infiltrate	Summer	Pneumonia
53	Male	Chest chondrosarcoma	Dyspnoea, cough, fever	Yes	No	No	Infiltrate	Summer	Pneumonia
64	Female	Peritoneal pseudomyxoma	Dyspnoea, fever	No	No	No	Infiltrate	Autumn	Pneumonia
55	Male	Bronchial asthma	Asymptomatic	Yes	No	No	Normal	Autumn	Pneumonia
73	Male	Pulmonary fibrosis	Asymptomatic	No	Ex	No	Infiltrate	Autumn	Colonisation
64	Male	ILD	Asymptomatic	Yes	No	No	Infiltrate	Autumn	Colonisation
56	Female	ILD	Dyspnoea	Yes	Ex	No	Infiltrate	Autumn	Pneumonia
60	Male	Silicosis	Asymptomatic	Yes	No	No	Nodule	Autumn	Colonisation
63	Female	Chronic bronchitis	Asymptomatic	No	Yes	No	Infiltrate	Autumn	Colonisation
52	Female	Hypersensitivity pneumonitis	Asymptomatic	No	Yes	No	Infiltrate	Autumn	Colonisation
75	Female	Common variable immunodeficiency	Dyspnoea, weight loss	Yes	No	No	Infiltrate	Autumn	Pneumonia
53	Male	Lung cancer	Cough, fever, chest pain	Yes	Yes	No	Infiltrate	Autumn	Pneumonia
63	Male	Myelofibrosis	Fever	Yes	Yes	Yes	Infiltrate	Winter	Pneumonia
79	Female	Bronchiectasis	Dyspnoea, cough, fever	No	No	No	Infiltrate	Winter	Pneumonia
66	Female	Breast cancer	Fever, cough	No	No	No	Infiltrate	Winter	Pneumonia
53	Male	Silicosis	Asymptomatic	No	Ex	No	Consolidation	Winter	Colonisation
64	Female	Bronchiectasis	Cough, low grade fever	No	Yes	No	Nodule	Winter	Pneumonia
73	Male	Acute pneumonia	Dyspnoea, cough, fever	No	Ex	No	Nodule	Winter	Pneumonia
81	Male	Lung abscess	Cough, weight loss	No	No	No	Infiltrate	Winter	Colonisation
84	Male	No disease	Dyspnoea	No	Yes	No	Infiltrate	Winter	Pneumocystis pneumonia
74	Male	Ureter cancer and leukaemia	Cough, fever	Yes	No	No	Infiltrate	Winter	Pneumonia
73	Female	Pulmonary fibrosis	Cough, fever	Yes	Yes	No	Infiltrate	Winter	Colonisation
37	Female	No disease	Cough, fever	No	Yes	No	Infiltrate	Winter	Pneumonia
57	Female	Common variable immunodeficiency	Cough, fever	No	No	No	Nodule	Winter	Pneumonia
49	Male	HIV	Dypsnoea, chest pain	No	No	No	Infiltrate	Spring	Pneumonia

[a]Ex: previous exposure to tobacco. IDL: Interstitial lung disease. COPD: chronic obstructive pulmonary disease. PJP: *Pneumocystis jirovecii* pneumonia

43.5% of the patients received immunosuppressive therapy including corticosteroids or chemotherapeutic agents. Seventeen percent of patients were treated or received prophylactic therapy for PJP.

PJP was diagnosed in 24 patients, and *P. jirovecii*-colonisation was diagnosed in 11 patients on basis of the criteria described in the material and methods. Table 2 summarises the clinical data from *P. jirovecii* PCR-positive patients. Direct flu-

Table 3 Comparisons of the clinical features PCR+ and PCR- patients.

	PCR-positive (n=35)	PCR-negative (n=137)	p-value
Age, years	62 ± 13	61 ± 14	0.954
Sex, Female	15 (42.8%)	49 (35.7%)	0.279
Underlying disease			
Solid tumor	6 (17.1%)	21 (15.3%)	0.42
Haematological malignancy	4 (11.4%)	29 (21.1%)	
Immunosuppressive disease	5 (14.3%)	19 (13.9%)	0.949
Inflammatory lung disease (COPD/Asthma)	11 (31.4%)	77 (56.2%)	0.0015
HIV infection	2 (5.7%)	9 (6.5%)	0.726
Organ transplantation	3 (8.5%)	16 (11.7%)	0.601
Corticosteroids	14 (40%)	60 (4.4%)	0.68
Tobacco exposure			
Active	10 (28.6%)	34 (24.8%)	0.89
Previous exposure to tobacco	10 (28.6%)	32 (23.4%)	
Anti- PcP prophylaxis	3 (8.5%)	27 (19.7%)	0.12
Positive microbiology culture results	8 (22.8%)	35(25.5%)	0.74
X-ray findings			
Nodules	4 (11.4%)	25 (18.2%)	0.142
Infiltrates	25 (71.4%)	70 (51.1%)	
Consolidations	3 (8.6%)	30 (21.9%)	
Normal	3 (8.6%)	12 (8.8%)	

orescence microscopy examination was ordered by clinicians in 15 (42.8%) of the PCR-positive samples. Furthermore, all these patients had some immunosuppressive condition that increased the risk of PJP. The seasonality of the infection was related with the cold months of the year, 12 patients in winter and 10 patients in autumn.

Comparisons of clinical and radiological results between PCR-positive and PCR-negative patients. The clinical features of the PCR-positive and PCR-negative patients are shown in table 3. The PCR-positive results were more likely in females and in patients with prophylaxis, although there was no statistical significance. Nodules, consolidations and normal X-ray findings were also more common than infiltrates in PCR-negative patients. A diagnosis of an underlying lung disease was significantly associated with a negative PCR result. Other clinical conditions were not associated with a positive result for *P. jirovecii*.

Microbiological results. Eight (22.8%) of the 35 PCR-positive samples and 35 (25.5%) of the PCR-negative samples were positive for other pathogens. In the PCR-positive samples, the most commonly isolated microorganisms were *Staphylococcus aureus* (n=2), *Aspergillus fumigatus* (n=2) and *Haemophilus* spp. (n=2). In the PCR-negative samples, non-fermenting gram-negative bacilli (n=7) were the most common isolated microorganisms, followed by *Enterobacteriaceae* spp. (n=5), *Haemophilus* spp. (n=5) and *A. fumigatus* (n=5). Microbiological results of the culture positive samples are summarised in Table 4.

Clinical outcome. IFA and PCR positive patients were treated for PJP. Three patients of the 35 PCR-positive patients died, the cause of death could not be attributable to PJP.

DISCUSSION

PJP is an opportunistic infection of increasing importance in non-HIV patients. In this study, only 2/35 (5.3%) of the PCR-positive patients were HIV-infected. It is well acknowledged that the diagnosis of PJP is particularly difficult in non-HIV patients, who can develop rapidly progressive PJP even with low fungus loads. In addition, in non-HIV patients, false negative results from tinctorial methods are more common [8, 9].

In most studies that compared microscopy with PCR methods for PJP diagnosis, PCR exhibited superior sensitivity for organism detection in patients with chronic lung disease and/or those on steroid treatment [3, 28, 29]. A recent me-

Table 4 **Microbiological isolates obtained from *P. jirovecii* PCR positive and negative samples.**

P. jirovecii PCR-positive	*P. jirovecii* PCR-negative
8/37 (23%)[a]	31/137 (23%)[a]
Staphylococcus aureus (n=2)	*Aspergillus fumigatus* (n=5)
Aspergillus fumigatus (n=2)	*Haemophilus* spp.[b] (n=5)
Haemophilus spp. (n=2)	*Pseudomonas* spp.[c] (n=4)
Pseudomonas aeruginosa (n=1)	*Escherichia coli* (n=3)
Mycobacterium lentiflavum (n=1)	*Candida* spp.[d] (n=3)
	Mycobacterium spp.[e] (n=3)
	Streptococcus pneumoniae (n=3)
	Other non-fermenting gram-negative[f] (n=3)
	Staphylococcus aureus (n=2)
	Serratia marcescens (n=2)
	Metapneumovirus (n=1)

[a]Number of isolated microorganisms/total samples studied
[b]*H. influenzae* (n=6); *H. parainfluenzae* (n=1)
[c]*P. aeruginosa* (n=3); *P. fluorescens* (n=1)
[d]*C. albicans* (n=3); *C. glabrata* (n=1)
[e]*M. avium* (n=1); *M. lentiflavum* (n=1); *M. tuberculosis* (n=1)
[f]*Burkholderia cepacia* (n=1); *Brevundimonas diminuta* (n=1); *Stenotrophomonas maltophilia* (n=1).

ta-analysis showed a very high accuracy of PCR in BAL samples for the diagnosis of PJP in patients who are at risk and a pooled sensitivity of 98.3% and a specificity of 91.0% [9]. To select the targets and primers, Roberts et al. compared 9 PCR assays with different primers/targets and found that the most sensitive PCR technique should consider a mtLSUrRNA nested reaction with the potential of producing false positive results [30]. Consistent with published data [11, 29, 31] the mtLSUrRNA PCR method used in the present study detected 18 more *P. jirovecii*-infected and 11 more *P. jirovecii*-colonised patients than IFA, improving the sensitivity from 25% to 100% ($p<0.01$). IFA exhibited excellent specificity but lacked sensitivity, whereas PCR was much more sensitive and also detected colonised patients. In particular, all confirmed-PJP cases in the HIV patient group were detected by both methods, perhaps in relation to the higher fungus loads in the HIV-infected patients [32, 33]. However, nested PCR exhibited a higher sensitivity in the non HIV group.

The high NPV allowed excluding PJP. Nevertheless, clinical and radiological criteria are essential to interpret a PCR-positive result because the low PPV of this technique. Clinical diagnosis in conjunction with IFA and PCR are considered the cornerstones for PJP patient management [10]. Although *P. jirovecii* was not recognised as the main cause of disease, it might play an important role as a comorbidity cofactor in patients with a severe underlying disease [33].Recent studies showed that 31.8% of the patients with positive PCR results have a history of PJP or will develop PJP [29]. Surprisingly, in this study, the laboratory test to detect *P. jirovecii* was requested in less than 50% of patients, and half of the unrequested PCR-positive samples were from patients who were at risk of developing PJP.

The actual prevalence of *P. jirovecii* colonisation or subclinical infection in immunocompetent patients remains unclear. The results of the current study show a low rate of colonisation of 6.4%. Nevertheless, similar studies reported differences in the rate of colonisation. Recent studies have shown a *P. jirovecii* colonisation prevalence from 2.6% to 55% [27, 28]. The differences in the prevalence rate could be due to the respiratory sample used (BAL vs. sputum), differences in the studied patients, such as underlying disease and comorbidities or differences in the recognition of a positive case given the absence of universally accepted criteria to establish the diagnosis for PJP. On the other hand, recent studies have shown that colonised-patients with low loads could be candidates for *P. jirovecii* prophylaxis [34]. Obviously, the detection of a *P. jirovecii* infection has a direct therapeutic impact on the choice of appropriate antimicrobial therapy. Understanding the role of *P. jirovecii* colonisation in patients with underlying pulmonary or systemic disease may help identify patients at risk of developing PJP [35].

Calderón et al. showed that *P. jirovecii* carriage could be involved in the progression of COPD by means of the capacity of *P. jirovecii* during very early stages of the infection to induce, in animal models, alveolar macrophage activation, pro-inflammatory interleukin elevation, and changes in pulmonary surfactant [36]. In the present study, 10% of the CPOD patients were PCR positive for *P. jirovecii*. Recent studies in Europe found rates of carriage in patients with chronic diseases between 6 and 40% [37, 38]. However, the association between high rates of *P. jirovecii* colonisation and chronic lung diseases is debatable. In the present study, there were more PCR-positive *P. jirovecii* patients in the group without any lung disease. This result could be related to the proportion of patients with malignancies that were higher in the group of patients without any pulmonary disease than in the group of patients with lung disease (48% versus 21%).

In the present study, it was not clear whether the presence of *P. jirovecii* with other pathogens contributed to the exacerbation of pneumonia, because of the low number of PJP in which another potential respiratory pathogen was isolated.

As far as we know, this is the first conventional-PCR method to detect *P. jirovecii* that uses a non-competitive bacterial internal amplification control (IAC) to reveal reaction failure due to the presence of inhibitory substances in the sample [39]. A current issue that limits the reliability and sensitivity of PCR is the degree of inhibition caused by inhibitory substances, especially in respiratory samples [40]. Many other PCR designed to detect *P. jirovecii* do not use IAC [13, 22], use nonbacterial IAC as exogenous internal commercial control or complex recombinant plasmids [7, 8, 15, 19]. Another strategy used was

a second round of amplification with the addition of a DNA template to exclude the presence of inhibitors [18, 41]. In this work, 13.5% of samples were inhibited, demonstrating the clinical utility of IAC. Therefore, reporting false-negative results is avoided. Previous studies found inhibition rates of 23.7% [40]. As Döskaya et al. observed in retesting diluted samples [40] in 20 (11.6%) samples, inhibition problems were resolved with a second extraction round. Maybe the use of more efficient extraction systems would improve the results.

In conclusion, PJP could be a serious problem for non-HIV patients, where the diagnosis by PCR has produced better results than traditional staining methods. The use of an internal control is necessary to ensure the reliability of the results, especially in samples with a high presence of PCR inhibitors such as respiratory samples. The PCR strategy used in this work has proven to be useful for routine clinical laboratories without access to more specialized diagnostic procedures, which are more expensive for the detection of *P. jirovecii* in respiratory samples. Our results could help in the understanding of the clinical features that are associated with colonisation or infection with this microorganism. However, more studies are needed to clarify these findings.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

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None to declare

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Original

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Vaccination of pregnant women in the Valencian Community during the 2014-15 influenza season: a multicentre study

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ABSTRACT

Background. To study influenza vaccination uptake in pregnant women from three Health Departments in the Valencian Community (Spain) during the 2014-15 flu season, to identify degree of knowledge, sources of information and attitudes toward immunization against influenza.

Methods. Multicentre cross-sectional descriptive study during the 2014-15 vaccination campaign. Vaccine coverage was determined using the Nominal Vaccination Registry (NVR). Subsequently, a telephone survey was carried out on a sample of vaccinated and unvaccinated postpartum women.

Results. The NVR had information on 934 (59.5%) out of 1,569 postpartum women; distribution per Health Departments was: 420 (44.9%), 161 (17.2%) and 353 (37.8%) in La Ribera, Torrevieja and Elx-Crevillent respectively. Vaccine uptake was 27.9% (n = 261). According to the "Country of Origin" variable, 77.5% (n = 724) of women were Spanish, with a vaccination rate of 26.7% (n = 193), compared to 22.5% (n = 210) who were non-Spanish, with a rate of 32.4% (n = 68). The main source of information was midwives for 83.7% (n = 159) of vaccinated pregnant women and for 44.6% (n = 127) of non-vaccinated women. The main reasons for vaccine refusal were lack of awareness (29.5%, n = 84) and not considering it necessary (25.6%, n = 73).

Conclusion. Despite their high willingness to be vaccinated after receiving information about the flu vaccine, the vaccination coverage in pregnant women studied is still low and can be improved. Health professionals need new information strategies to extend vaccine uptake to a larger number of pregnant women in Spain. Midwife advice plays an essential role in transmitting information on influenza vaccination in pregnant women and has a significant impact on uptake.

Keywords: Influenza, Pregnancy, Immunization, Vaccine coverage, Vaccination uptake, Midwife, Health professionals.

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Vacunación de mujeres embarazadas en la Comunidad Valenciana durante la temporada de gripe 2014-15: un estudio multicéntrico

RESUMEN

Objetivos. Investigar la cobertura de la vacunación antigripal en gestantes en tres Departamentos de Salud de la Comunidad Valenciana (España) durante la temporada 2014-15, y evaluar su aceptabilidad, fuentes de información y motivos de rechazo hacia la inmunización contra la gripe.

Métodos. Estudio descriptivo transversal multicéntrico en la campaña vacunal 2014-15. La cobertura vacunal se identificó a través del Registro Nominal de Vacunas (RVN). Posteriormente, se realizaron 2 encuestas telefónicas a un mínimo muestral de puérperas vacunadas y no vacunadas.

Resultados. De 1.569 puérperas, 934 (59,5%) disponen de información en el RVN; la distribución por Departamentos: 420 (44,9%), 161 (17,2%) y 353 (37,8%) en La Ribera, Torrevieja y Elx-Crevillent respectivamente. Se obtuvo una cobertura vacunal del 27,9% (n=261). Según la variable "País de Origen", el 77,5% (n=724) es española, con una tasa vacunal del 26,7% (n=193), frente al 22,5% (n=210) extranjera, con el 32,4% (n=68). La principal fuente de información fue la matrona en el 83,7% (n=159) de gestantes vacunadas y el 44,6% (n=127) en no vacunadas. Los principales motivos de rechazo fueron el desconocimiento (29,5%; n=84) y el no considerarse imprescindible (25,6%; n=73).

Conclusiones. A pesar de su alta predisposición a

vacunarse después de recibir información sobre la vacuna contra la gripe, la cobertura de vacunación en mujeres embarazadas estudiadas es aún baja y puede mejorarse. Son necesarias nuevas estrategias de formación e información por parte de los profesionales sanitarios para obtener un mayor número de gestantes vacunadas. El consejo de la matrona es un factor esencial en la emisión de la información sobre la vacunación antigripal recibida por las gestantes estudiadas.

Palabras claves: Gripe, Embarazo, Inmunización, Cobertura Vacunal, Vacunación, Matrona, Profesionales Sanitarios.

BACKGROUND

Pregnant women present a higher number and increased severity of diverse infections [1, 2], making them especially vulnerable to influenza [3-5]. This disease is one of the main causes of hospitalization during any pregnancy trimester, as well as respiratory infection in children aged under one year [6, 7].

The need of pregnant women and newborns for immunological protection has led the World Health Organization (WHO) to recommend influenza vaccination in any trimester of pregnancy [8, 9]. This recommendation is supported by results showing a reduction in influenza in 70% of immunized women [10] demonstrating its efficacy and safety [11, 4] as well as extending its protection to infants of up to six months of age [8, 9].

Following the recommendations of the WHO, the Valencian Community (Spain) included pregnant women in the influenza risk group as from 2013 [12]. Spain does not have official influenza vaccination records in pregnant women and the few published studies that do exist reveal vaccine coverage rates below 40% [6, 13, 14]. This figure contrasts with data reported in the United States, where uptake in pregnant women rose to up to 70% following the 2009 influenza pandemic [15].

Fear of possible adverse effects was the most cited reason by pregnant women for avoiding influenza vaccination [16].

Health professionals have a key role in promoting immunization in pregnant women. However, it has been found that a significant percentage of this group is unaware that pregnant women are included in the risk group subject to vaccination [17].

The purpose of this study is to investigate influenza vaccination coverage among pregnant women in three Health Departments of the Valencian Community (Spain): La Ribera (LR), Torrevieja (TV) and Elx-Crevillent (EC) during the 2014-15 season, and identify their degree of knowledge, sources of information and attitudes towards immunization against influenza.

METHODS

An observational, multicentre, descriptive and cross-sectional study was performed among pregnant women who gave birth in the three referral hospitals of the health departments under study (LR, TV and EC) between October 20, 2014 and January 31, 2015. These hospitals provide health care to a total of 570,000 inhabitants (250,000 in LR; 170,000 in TV and 150,000 in EC).

Once the total sample of pregnant women having given birth during the study period was obtained, their vaccination uptake was determined based on the Nominal Vaccination Registry (NVR).

The NVR portal was set up by the autonomous government and it electronically stores information on people vaccinated in hospitals, health centres and clinics in the Valencian Community since 1994 [12].

To analyse vaccination coverage and factors influencing vaccination, the following variables were used: health department (LR / TV / EC), age (<25 years, between 25 and 35 years,> 35 years), country of origin (Spain, Others), parity (1 child, 2 children, >3 children) and influenza vaccination status (yes/no/no data in NVR) during the 2014-15 season.

To identify the degree of knowledge, sources of information and attitudes towards the immunization of pregnant women, a subgroup of 100 pregnant vaccinated women and a subgroup of 100 non-vaccinated women were selected within each department. Inclusion in these two subgroups was determined randomly from among the studied population, in order to identify possible differences between them, with a level of significance of <0.05. The subgroup of vaccinated pregnant women did not reach the minimum sample of 100 women in two health departments (TV, 49 and 55, EC) due to a lack of records in the NVR or because of its low vaccination coverage.

The study was conducted according to the Declaration of Helsinki and current legislation and was approved by the Research Commission of the participating centres after obtaining authorization from the Spanish Agency of Medicinal Products and Medical Devices ("Agencia Española del Medicamento y Productos Sanitarios", AEMPS) (number CI2015-20).

Both subgroups were interviewed by telephone using a closed ad hoc survey, a survey conducted by authors of the current manuscript published in similar studies [14]. Telephone calls were made between March and May 2015, respondents were informed about confidentiality, protected anonymity, as well as the right not to answer questions.

We asked vaccinated women about the source of the information of influenza vaccine, the health provider involved in recommending the vaccination, and whether they would opt to be vaccinated again in their next pregnancy the health provider involved in recommending the vaccination, and whether they would be vaccinated again in their next pregnancy. For the group of women who did not receive vaccines, asked if they had heard about the vaccine and, if so, which healthcare provider recommended it, the reasons

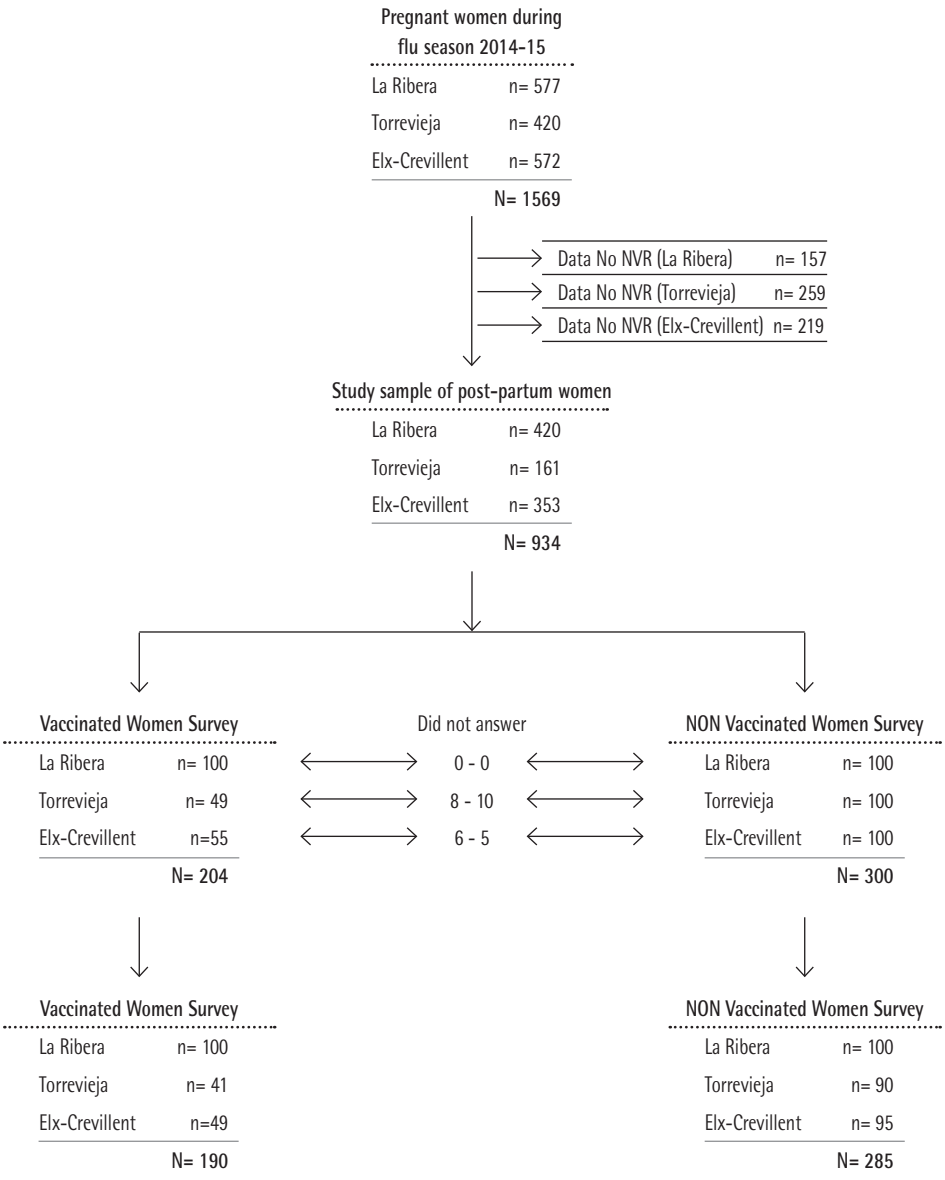

Figure 1 | **Flow chart of study**

for vaccine rejection, their knowledge about the vaccine and whether they would have the vaccine given in their next pregnancy.

The inclusion criteria in the study consisted in being pregnant and cared for by the Primary Health Care centres, as well as being assisted with labour diagnosis in the health departments (LR, TV and EC). Women having given birth in other hospitals were excluded, as well as those with contraindications to influenza vaccination, occurrence of antenatal death or who refused the telephone survey.

Statistical analysis was performed using the statistical software SPSS version 20.0. Quantitative and qualitative variables were expressed as absolute frequencies, mean and ratios. Vaccination coverage was calculated as the percentage of women vaccinated with respect to the total number of pregnant women, and their 95% confidence interval (CI) was calculated. The Chi-square test was used to analyse the statistical significance of differences in vaccine coverage percentages between the categories of variables. To evaluate the adjusted effect of age, parity, country of origin and health department variables on non-vaccination, multivariate logistic regression models were constructed, taking non-vaccination as a response variable and the rest as explanatory variables.

Table 1 Pregnant women according to study variables and data in the Nominal Vaccination Registry (NVR).

		No NVR information		NVR information		
		n	%	n	%	Total
Total		635	40.5	934	59.5	1,569
Health department	La Ribera	157	27.2	420	72.8	577
	Torrevieja	259	61.7	161	38.3	420
	Elx-Crevillent	219	38.3	353	61.7	572
Country of Origin	Spain	374	34.2	724	65.8	1098
	Not Spain	261	55.4	210	44.5	471
Parity[a]	1	158	30.7	356	69.3	515
	2	224	40.5	329	59.5	553
	3 or more	253	49.2	248	50.2	286
Age	<25 years	73	33.2	147	66.8	220
	25-35 years	409	40.8	593	59.2	1002
	>35 years	153	44.1	194	55.9	347

[a]Total number of pregnancies, including the current pregnancy.

Table 2 Influenza vaccination coverage among pregnant women during the 2014-15 season

		n	Vaccinated women	%	IC95%
Total		934	261	27.9	[25.0-30.7]
Health department	La Ribera	420	157	37.4	[33.1-42.3]
	Torrevieja	161	49	30.4	[23.3-37.5]
	Elx-Crevillent	353	55	15.6	[11.8-19.4]
Country of origin	Spain	724	193	26.7	[23.5-29.9]
	Not Spain	210	68	32.4	[26.1-38.7]
Parity[a]	1	357	96	27.8	[22.4-31.6]
	2	329	93	28.3	[23.4-33.2]
	3 or more	248	72	29.0	[23.3-34.6]
Age	<25 years	147	38	25.9	[18.8-32.9]
	25-35 years	593	162	27.3	[23.7-30.8]
	>35 years	194	61	31.4	[24.8-37.9]

[a]Total number of pregnancies, including the current pregnancy.

RESULTS

After applying the exclusion criteria (figure 1), a total of 934 patients were finally selected out of a total of 1.569 postpartum patients. A total of 635 (40.4%) postpartum women were discarded from the study because they did not have any data recorded in the NVR. In this respect, the TV department stood out because it disposed of no recorded data on 61.7% of postpartum women (table 1). The final sample under study turned out not to be homogeneous across the different health departments (LR, 420 (44.9%); TV, 161 (17.2%); and EC, 353 (37.8%)). Vaccination coverage was 27.9% (n=261 [CI 95%: 25.0-30.7]), with a higher coverage in LR, with 37.7% (n = 157) and TV, with 30.4% (n = 49) (table 2).

Table 3 **Attitudes and information sources on influenza vaccination in pregnant women vaccinated and not vaccinated during the 2014-15 season**

Pregnant women vaccinated	Total (n=190) %	CI (95%)	Pregnant women not vaccinated	Total (n=285) %	CI (95%)
Have you heard of influenza vaccination during pregnancy?					
			SI	67.7	[62.3-73.1]
			No	32.3	[26.9-37.7]
Where did you receive information?					
Primary Care Centre	94.7	[91.5-97.8]	Did not receive	31.9	[26.4-37.3]
Media	2.1	[0.1-4.1]	Media	8.8	[5.5-12.1]
Private consultation	2.6	[0.3-4.8]	Family/Friends	7.7	[4.6-10.8]
The pregnant woman is a health care professional	0.5	[0.0-1.5]	At work	0.7	[0.0-1.6]
Who recommended the influenza vaccine?					
Family doctor	7.9	[4.1-11.7]	Midwife	44.6	[38.8-50.4]
Midwife	83.7	[78.4-88.9]	Gynaecologist	0.4	[0.0-1.1]
Health care professional	1.6	[0.0-3.4]	Primary Care doctor	2.1	[0.4-3.8]
Gynaecologist	4.2	[1.3-7.0]	The pregnant woman is a health care professional	3.9	[1.6-6.1]
Other	4.2	[1.3-7.0]			
If you became pregnant again at the same time of year, would you get vaccinated again?			**If you became pregnant again at the same time of year, would you get vaccinated then?**		
Yes	96.8	[94.3-99.3]	Yes	61.8	[56.1-67.4]
No	3.2	[0.7-5.7]	No	31.6	[26.2-36.9]
			Not sure	6.7	[3.8-9.6]

Spain was the country of origin accounting for 77.5% (n=724) of the sample, presenting vaccination coverage of 26.7% (n = 193 [95% CI: 23.5-29.9]). The remaining 22.5% (n=210) were non-Spanish, with a coverage of 32.4% (n=68 [CI 26.1-38.7]) (table 2).

Concerning the parity variable, higher coverage was observed in multiparous pregnant women (> 3 children), 29.0% (n = 72 [95% CI: 23.3-32.9]). This result is inversely related to the distribution of the study's population, since the number of first parity (38.1% (n = 356)) or Second parous (35.2% (n = 329)) women predominated.

Women between 25 and 35 years represented 63.5% (n = 593) of the total, followed by 20.7% (n = 194) of pregnant women> 35 years. Vaccination coverage in the > 35 years group was highest, with 31.4% (n = 61 [95% CI: 24.8-37.9]); this data had a descending orientation, 27.3% (n=162) and 25.9% (n=38), according to the established age segments (table 2).

Out of a total number of 190 surveys carried out on vaccinated pregnant women aiming at identifying the degree of knowledge, attitudes and sources of information, 52.6% (n=100), 21.6% (n=41) and 25.8% (n=49) corresponded to LR, TV and EC respectively (table 3).

A total of 94.7% (n = 180 (95% CI: 91.5-97.8) received information on the influenza vaccine at their Primary Health Care Centre, and the LR and EC departments stood out with 98% (n = 98) and 96 % (N = 47) respectively. Midwives accounted for 83.7% of the total number of health professionals involved (n = 159 [95% CI: 78.4-88.9]) and played a major role in recommending the vaccine to pregnant women. The 96.8% of vaccinated pregnant women (n = 184 [95% CI: 94.3-99.3]) would get vaccinated again if they got pregnant again during the seasonal period of the vaccination campaign (table 3).

A total of 285 non-vaccinated pregnant women were contacted for the telephone survey, with a distribution of 35.1% (N = 100), 31.6% (n = 90), and 33.3% (n = 95), in LR, TV and EC respectively. Of these, 67.7% (n = 193 [95% CI: 26.4-73.1]) had heard of influenza vaccination during pregnancy, EC showing the lowest level of information with 45.3% (n = 43) (table 3). Midwives, with 44.6% (n = 127), were again the main source of information in all three departments studied (table 3).

The main arguments put forward for rejecting

Table 4 Reason for influenza vaccine refusal in pregnant women not vaccinated during the 2014-15 season

Pregnant women not vaccinated	Total (n=285) %	CI (95%)
Could you indicate your reason for refusal?		
Unawareness	29.5	[24.2-34.8]
Not necessary	25.6	[15.5-35.6]
Not receiving information from a health worker	12.3	[8.5-16.1]
Giving birth soon	8.1	[4.9-11.3]
Fear of adverse effects	8.1	[4.9-11.3]
Not effective	6.7	[3.8-9.6]
Never get vaccinated	3.9	[1.6-6.1]
Advised against by doctor	2.8	[0.9-4.7]
Have a cold	2.1	[0.4-3.7]
Long waiting list	1.1	[0.0-2.3]

administration of the vaccine were: unawareness of the recommendation of influenza vaccination during the gestational period with 29.5% (n = 84 [95% CI: 24.2-34.8, EC data standing out with 44.2% (n = 42); and 25,6% (n=73 [IC95%: 15,5-35,6], not considering it necessary as a preventive measure, especially in the LR department with a total of 39% (n=39) (table 4).

A total of 61.8% (n = 176 [95% CI: 56.1-67.4]) of unvaccinated pregnant women interviewed showed a predisposition to get vaccinated against influenza in the case of a new pregnancy.

An analysis of the logistic regression model of the department, country of origin, age and parity (modelling the probability of non-vaccination) variables, showed that there was no statistically significant relationship between influenza vaccination and the rest of the variables, except for the department variable. The EC department showed worse vaccination coverage than the LR department, these differences being statistically significant and adjusted by the multivariate model.

Differences with the TV department were not significant, although this may be due to lack of data on pregnant women profiles in the NRV; the latter were significantly elder than those in the RN department, with a greater number of children and a much higher percentage of non-Spanish women.

DISCUSSION

Vaccination coverage registered in the Valencian Community during the 2014-15 season in the so-called risk groups was less than 80% of the immunization rates recommended by the WHO. Vaccination coverage of 50% was obtained in the population with cardiac pathology, 43% in health professionals and 17.4% in pregnant women [18].

Although vaccination is an essential recommendation to prevent and combat maternal and neonatal morbidity [1], vaccination coverage of pregnant women was low, showing unequal behaviour across the three health departments under study. The EC department, with 15.6%, presented the lowest rates, a figure similar to the 17.4% obtained in the southern area of Madrid during the 2004-05 season [6].

In Europe, data on vaccine coverage of pregnant women are disparate. A study conducted in Germany during the 2012-13 season turned out a coverage of 15.9% [19], another in the UK showed a coverage between 14.9% and 21.6% during the 2009-10 season [20] and in Belgium a rate of 42.8% for the 2013-14 season [21] was found. These latter figures are similar to those in different areas of the United States (41%) during the same season [22].

Although they are far from acceptable, our results reveal higher coverage rates than those in other similar studies, such as those described in the Barcelona health department (5%) during the 2007-08 season [13].

The influenza surveillance program developed in the Valencian Community shows a progressive increase in the number of influenza vaccine doses administered to pregnant women compared to previous seasons (7.6%; 9.2% and 14.8% for season 2011-12; 2012-13 and 2013-14 respectively) [12, 18, 23].

These reports do not give real figures on the total number of pregnant women, but they do provide an estimate, so this study allows updating the situation of influenza immunization in the group of pregnant women in the Valencian Community.

Significantly, non-Spanish pregnant women had better vaccination coverage than Spanish pregnant women.

Data relating to age and parity variables were similar across studied departments, presenting a pattern of higher vaccination rates in pregnant women who were elder and had previously been mothers. This result coincides with that revealed in a recent systematic review of studies predominantly in Europe and America. This latter review concludes that older ages of pregnant women, previous experiences and recommendations of medical staff were the most influential factors in the acceptance of the vaccine against seasonal influenza [24].

Vaccinated pregnant women reported receiving information on the vaccine at their health centre and their midwife recommending it. The bond of trust established between midwives and pregnant women during the months of pregnancy could explain these results [14]. In addition, these data coincide with a similar study conducted in London [25], where recommendation by midwives accounted for 76% of cases. However, at the international level, the family doctor is found to be the health professional having the greatest impact in recommending influenza vaccination [20, 26]. Almost all

vaccinated pregnant women surveyed said that in the case of a new pregnancy, they would get vaccinated again. Nonetheless, the behaviour of unvaccinated pregnant women was uneven according to the department, where a higher percentage of pregnant women were located in EC and TV, with the intention of not being vaccinated again. One study identified a greater predisposition to immunization in women who had already been vaccinated prior to their pregnancy during other flu seasons [27].

Unawareness that influenza vaccination is recommended during pregnancy is the main reason vaccine administration is rejected, thus corroborating data from previous studies. [28]. Other arguments against vaccination were that of considering it as non-effective and non-essential, which is part of the perception of lack of scientific evidence about the efficacy and safety of the vaccine [18, 29, 30].

Nevertheless, the safety of the influenza virus vaccine in pregnant women has been evaluated in observational studies [31] and to date, there is no evidence of an increase in adverse effects such as late antenatal death, miscarriage or congenital malformations [32]. Nor is there any evidence that pregnant women immunized against influenza develop a greater number of adverse effects compared to those who are not pregnant and are vaccinated [33].

The importance of information strategies during the vaccination season highlights the key role that health professionals have in transmitting such information [34] in socio-sanitary contexts as disparate as the United Kingdom [35] or Pakistan [36]. However, in other studies, the effectiveness of these interventions to increase vaccination coverage is put into question, and health professionals in contact with pregnant women are recommended to verbalize the benefits of the vaccine for newborns [37]. In this line of research, the majority of studies was situated in North America and was published in 2011, as a consequence of the 2009 influenza pandemic. From that year onwards, a decrease of interest in scientific literature can be observed on influenza vaccination among pregnant women [30].

The recent inclusion of the dTpa vaccine as a recommendation for pregnant women may improve adherence to influenza vaccination. Both can be administered simultaneously and are safe for the mother and the child [38].

The study has some limitations since pregnant women without data in the RVN have been excluded from the study. A greater participation of the health professionals in charge of the registration of vaccines in the RVN would imply a considerable improvement of the vaccination program.

Vaccination coverage among the studied population of pregnant women was low and could be improved. In order to reach higher coverage rates, new strategies are needed to encourage health professionals to inform pregnant women, minimize excessive self-confidence and perceptions of the vaccine as dispensable. Disseminating scientific evidence available on the risks of influenza and the safety of the influenza vaccine as a Public Health preventive measure is essential. In Spain, midwife advice is a crucial factor in transmitting information to pregnant women on the influenza vaccine.

Both vaccinated and non-vaccinated pregnant women are highly predisposed to accepting immunization against influenza in the case of a new pregnancy, and this fact reinforces the importance of communication strategies directed at this group.

Finally, in the light of the lost records observed, the NVR should be used with caution when determining the vaccination status of pregnant women. The duty of professionals to record each vaccination should be reinforced.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

FUNDING

None to declare

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Original

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Staphylococcus aureus bacteremia in a secondary level Spanish hospital: clinical implications of high vancomycin MIC

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ABSTRACT

Background. One of the most controversial issues in recent years has been the clinical significance of high vancomycin MIC in *Staphylococcus aureus* bacteremia. The aim of this study was to elucidate the clinical implication that this parameter has in the staphylococcal bacteremia of a second level hospital.

Material and methods. Retrospective descriptive study between January 2014 and September 2016 with 138 records from the blood culture Severo Ochoa University Hospital registry. A total of 98 cases were finally analized. Microbiological analysis of vancomycin MIC was performed using micro dilution technique.

Results. The mean age was 71.4 ± 12.45 and 63.26% of the patients had a Charlson index ≥6. A 30.61% were carriers of a venous central catheter. The most frequent source was venous central catheter (26.53%). There were 14.24% metastatic events. Global mortality rate at 30 days was 25.51%. The 43.87% of strains had a vancomycin MIC ≥ 2 mg/L. High vancomycin MIC was significantly associated with persistent bacteremia (OR 3.12 [1.13-8.93]), maintaining this statistical significance in methicillin-resistant *S. aureus* (MRSA) group (p =0.001) but no in methicillin-susceptible *S. aureus* (MSSA) group (p = 0.13). Persistent bacteremia was also significantly related with permanent catheter carriers (OR 4.18 [1.38-12.61]), peripheric catheter source (OR 5.18 [1.13-8.93]) and metastatic complications (OR 3.82 [1.03-12.81]). There was no significant association between high vancomycin MIC and mortality.

Conclusions. High vancomycin MIC may be useful in daily clinical practice as a marker of poor clearance of *S. aureus* bacteremia, specially when is due to MRSA strains.

Keywords: *Staphylococcus aureus* bacteremia, high vancomycin MIC, persistent bacteremia.

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Bacteriemia por *Staphylococcus aureus* en un hospital de segundo nivel en España: implicaciones de la CMI elevada a vancomicina

RESUMEN

Introducción. En los últimos años, el significado clínico de la CMI elevada a vancomicina en la bacteriemia por *Staphylococcus aureus* ha sido un tema de una enorme controversia científica. El objetivo de este estudio fue dilucidar la implicación clínica que este parámetro tiene en la bacteriemia estafilocócica de un hospital de segundo nivel.

Material y métodos. Estudio descriptivo retrospectivo entre enero 2014 y septiembre 2016 con 138 entradas del registro de hemocultivos del Hospital Universitario Severo Ochoa de Leganés. Se analizaron un total de 98 casos. El análisis microbiológico de la CMI a vancomicina se realizó mediante técnica de microdilución. El análisis estadísitico se realizó mediante SPSS 20.0: Shapiro Wilk, $\chi 2$, Mann Whitney, regresión logística y Kaplan Meier.

Resultados. La media de edad fue 71,4 ± 12,45. Un 63.26% de los pacientes tenían un índice de Charlson ≥6. El 30,61% eran portadores de vía venosa central (VVC). El foco más frecuente fue la VVC (26,53%). Hubo un 14,24% de embolismos a distancia. La mortalidad global a los 30 días fue de 25,51%. El 43,87% de las muestras tenían una CMI≥ 2mg/L a vancomicina. La CMI elevada a vancomicina se asoció de forma significativa con la bacteriemia persistente (OR 3,12 [1,13-8,93]), manteniendo esta significación estadística en el grupo de *S. aureus* resistente a meticilina (SARM) (p=0,001), pero no en el grupo

de *S. aureus* sensible a meticilina (SASM) (p=0,13). La bacteriemia persistente se relacionó también con portadores de VVC permanente (OR 4,18 [1,38-12,61]), con el foco asociado a vía venosa periférica (OR 5,18 [1,13-8,93]) y con las embolismos a distancia (OR 3,82 [1,03-12,81]). No se encontraron diferencias significativas entre la CMI a vancomicina y la mortalidad.

Conclusiones. La CMI elevada a vancomicina podría ser un parámetro útil como marcador de retraso en el aclaramiento de la bacteriemia por *S. aureus*, especialmente en el contexto de bacteriemia por SARM.

Palabras clave: bacteriemia por *Staphylococcus aureus*, CMI alta a vancomicina, bacteriemia persistente.

INTRODUCTION

Staphylococcus aureus is the most frequent pathogen in many situations such as nosocomial bacteremia, endocarditis and arthritis [1] and is also characterized by its high morbidity and mortality [2]. Since the first series of methicillin-resistant *S. aureus* (MRSA) were described in the 1960s, over the past 40 years the resistance to beta-lactams have been dispersed from nosocomial environment to the community. Furthermore, the already classic Cosgrove meta-analysis observed that MRSA bacteremia mortality rate is higher than associated with methicillin-susceptible *S. aureus* (MSSA) [3,4]. All of this has led to the fact that in recent years, vancomycin has become the drug of choice for the empirical treatment of *S. aureus* bacteremia (SAB) and practically, until 10 years ago, the only option for MRSA targeted treatment. In the last few years, several authors have described a progressive increase in *S. aureus* vancomycin minimal inhibitory concentration (MIC) [5-10]. This relies on the conformation of a thicker bacterial wall that makes vancomycin binding to peptidogly can D-Alanine residues in bacterial wall more difficult. Moreover, it has been observed that in apparently susceptible to vancomycin isolates it is possible to identify *S. aureus* sub populations with vancomycin-intermediate susceptibility (hVISA) [11]. The clinically distinct behavior of the strains with a vancomycin MIC in the upper limit of the sensitivity has also been reported [12-14]. However, the concrete role of this finding remains controversial [15]

The aim of this study was to identify possible factors associated with complicated SAB, its associated mortality and to establish a relationship between this outcomes and high vancomycin MIC.

MATERIAL AND METHODS

A retrospective descriptive study was performed between January 2014 and September 2016 from blood culture registry of Severo Ochoa University Hospital in Leganés, Madrid, Spain. It is a 382-bed hospital that serves to an estimated population of 250,000 habitants. It is also a reference hospital for 5 different socio-sanitary residence for elderly patients. A total of 138 records were retrospectively collected. Exclusion criteria were: recurrent bacteremia within 8 weeks, cases with definitive follow-up in another hospital, and cases in which no information was available about antibiotic therapy used. Clinical, microbiological, therapeutic and prognostic variables were analyzed for the 98 cases selected through paper and electronic medical records.

SAB was defined as at least one positive blood culture for *S. aureus*. Co-morbidity was measured by Charlson score index and it was later stratified in two groups (≥6 score vs. <6 score). Particular comorbid conditions were also collected, so as being central venous catheter (CVC) carrier and vascular device carrier (including pacemakers and endovascular grafts). Three acquisition categories were considered according to Friedman criteria [16]: 1) nosocomial SAB ifthe episode was diagnosed at least 48 hours after hospital admission; 2) healthcare related-SAB if the patient had had contact with healthcare system in the previous 3 months; 3) community acquired SAB source was defined as any event detected within 48 hours of hospital admission. Unknown source bacteremia was set out when its origin was uncertain after careful examination of clinical an microbiological data. Empirical antibiotic was defined as any treatment administered in the first 48 hours after bacteremia, despite of the lack ofmicrobiological information. Definitive antibiotic was considered as the treatment administered after appropriate microbiological isolation and susceptibility tests. Antibiotic treatment was considered appropriate if the strain was susceptible to at least one of the administered antibiotics, with the exception of aminoglycosides, which were considered inappropriate regardless of the sensitivity test. Guidelines concordant treatment was determined as any treatment that is contemplated in Spanish Society of Clinical Microbiology and Infectious Diseases (SEIMC) guidelines (only vancomycin, daptomycin, linezolid, cloxacillin, fosfomycin) [17]. Persistent bacteremia was defined as positive blood cultures after more than 48 hours of appropriate antibiotic therapy. General complications were defined as every kind of complication during the hospitalization directly related or not with SAB. Metastatic complications were considered as the present of at least one secondary focus to bloodstream seeding. Focus control was considered if the catheter or foreign body was removed or if a surgical intervention was performed. Global mortality rate at 30 days was defined as death by any cause 30 days after the initial bacteremia. In-hospital mortality rate was defined as *exitus letalis* by any cause during the admission.

Blood cultures were processed by BACTEC 9240© (Becton Dickinson Diagnostic Instrument Systems, USA). Isolates were identified according to standard microbiological techniques. Minimum inhibitory concentration (MIC) was determined by microdilution method (ESTEN© 2009) in accordance with CLSI criteria in 2014 (S < 2 mg/L, I 4-8 mg/L, R ≥ 16 mg/L). All determinations were made under blind conditions without knowledge of any clinical outcome.

Shapiro-Wilk test for was performed to check normal distribution of the sample. Continuous variables were compared using Mann Whitney U-test. Qualitative and stratified continuous variables were compared using Pearson's chi-squared test. Multivariate analysis with logistic regression was performed for

Table 1 **Global series characteristics and outcomes. Univariate analysis between MRSA and MSSA subgroups.**

	SAB (98; 100%)	MRSA bacteremia (40; 40.81%)	MSSA bacteremia (58; 59.18%)	p (IC 95%)
Age	71.41 ± 12.45	75.65 ± 11.35	68.48 ±12.37	0.003
Sex				
Women	40 (40.80)	16 (40)	24 (41.37)	1.00
Men	58 (59.18)	24 (60)	34 (58.62)	
Charlson				
≥6	62 (63.26)	31 (77.5)	31 (53.44)	0.01
<6	36 (36.73)	9 (22.5)	27 (46.55)	
Morbidity				
Cardiovascular	51 (52.04)	25 (62.50)	26 (44.82)	0.10
Hepatic	15 (15.30)	5 (12.50)	10 (17.24)	0.58
Nephropaty	22 (22.44)	10 (25)	12 (20.68)	0.63
COPD	30 (30.61)	17 (42.5)	13 (22.41)	0.04
Arteriopathy	26 (26.55)	14 (35)	12 (20.68)	0.16
Immunosupression	14 (14.28)	8 (20)	6 (10.34)	0.24
Neoplasia	22 (22.44)	8 (36.36)	18 (24.13)	0.80
Diabetes	28 (28.57)	14 (35)	14 (24.13)	0.26
VCC	30 (30.61)	13 (32.5)	17 (29.3%)	0.82
Permanent	22 (22.44)	11 (27.5)	12 (20.7%)	0.47
Transient	8 (8.16)	3 (7.5)	5 (8.6)	1.00
Vascular device	9 (9.18)	6 (15)	3 (5.17)	0.15
Nosocomial	47 (47.95%)	21 (52.5)	26 (44.82)	0.53
Health-care related	24 (24.48%)	8 (20)	16 (27.58)	0.47
Community	27 (27.55%)	11 (27.5)	16 (27.58)	1.00
ICU admission	23 (23.46)	10 (25)	13 (22.80)	0.81
Source				
Endovascular	39 (39.79)	9 (22.5)	30 (51.72)	0.006
VCC	26 (26.53)	9 (22.5)	17 (29.31)	0.49
VPC	11 (11.22)	–	11 (18.96)	0.002
Vascular graft	2 (2.04)	–	2 (3.44)	0.51
Primary	23 (23.46)	11 (27.50)	12 (20.66)	0.47
Respiratory	9 (9.18)	5 (12.50)	4 (6.89)	0.48
Soft tissue	11 (11.22)	7 (17.5)	4 (6.89)	0.11
Osteoarthricular	2 (2.04)	–	2 (3.44)	0.51
Urinary	3 (3.06)	2 (5)	1 (1.70)	0.56
TTE done	54 (55.10)	22 (40)	32 (55.17)	1.00
TEE done	17 (17.34)	8 (20)	9 (15.51)	0.59
Empirical treatment				
Correct	71 (72.44)	23 (57.5)	48 (82.75)	0.11
Guidelines concordant	36 (36.73)	13 (32.5)	17 (29.31)	0.80
Vancomycin	27 (27.55)	2 (5)	14 (24.13)	0.37
Daptomycin	3 (3.06)	–	1 (1.72)	0.56
Linezolid	–	–	–	-
Cloxacillin	–		–	-
Definitive treatment				
Vancomycin	31 (31.62)	18 (45)	13 (22.41)	0.02
Daptomycin	10 (10.20)	7 (17.5)	3 (5.17)	0.08
Linezolid	15 (15.30)	11 (27.5)	4 (6.89)	0.009
Cloxacillin	23 (23.46)	–	23 (39.65)	0.000
Not concordant	19 (19.38)	4 (10)	15 (25.86)	0.06
Control blood cultures	51 (52.04)	21 (52.5)	30 (51.72)	1.00
Persistent bacteremia	24 (24.48)	8 (20)	16 (27.58)	0.47
Source control	38 (38.77)	11 (27.50)	27 (46.55)	0.26
General complications	41 (41.83)	17 (42.5)	24 (41.37)	1.00
Metastasic infection	14 (14.28)	5 (12.5)	9 (15.51)	0.74
Endocarditis	4 (4.08)	1 (2.5)	3 (5.17)	0.64
Thrombophlebitis	3 (3.06)	1 (2.5)	2 (3.44)	1.00
Osteomielitis	7 (7.14)	3 (7.5)	4 (6.89)	1.00
30 days mortality	25 (25.51)	10 (25)	15 (25.86)	1.00
In-hospital mortality	27 (27.55)	12 (30)	15 (25.86)	0.65

Data are no. (%) of patients. SAB (*S. aureus* bacteremia), COPD (Chronic Obstructive Pulmonary Disease), ICU (Intensive Care Unit), Venous Central Catheter (VCC), Venous Peripheral Catheter (VPP), TTE (Transthoracic Echocardiography), TEE (Transesophageal Echocardiography)

Table 2 **Comparative between MIC ≥ 2 mg/L and MIC <2 mg/L strains: characteristics and outcomes with univariate and multivariate analysis.**

	MIC ≥ 2 mg/L (n; %) (43/98; 43.87)	MIC < 2 mg/L (n; %) (55/98; 56.12)	p (IC 95%)	Multivariate analysis (p <0.20)
Age	71.49 ± 13.73	71.35 ±11.42	0.53	
Sex				
Women	20 (46.51)	20 (36.36)	0.40	
Men	23 (53.48)	35 (63.63)		
Charlson				
≥6	29 (67.44)	33 (60)	0.52	
<6	14 (32.55)	22 (40)		
Morbidity				
Cardiovascular	26 (60.46)	25 (45.45)	0.15	
Hepatic	4 (9.30)	11 (20)	0.16	
Nephropathy	12 (27.90)	10 (18.18)	0.30	
COPD	14 (32.55)	16 (29.09)	0.82	
Arteriopathy	15 (34.88)	11 (27.27)	0.11	
Immunodeficiency	4 (9.30)	7 (12.72)	0.75	
Neoplasia	9 (20.93)	13 (23.63)	0.81	
Diabetes	14 (32.55)	14 (25.45)	0.50	
Vascular device	6 (13.95)	3 (5.45)	0.17	
VCC	14 (32.55)	16 (29.09)	0.82	
Permanent	11 (25.6)	12 (21.8)	1.00	
Transient	4 (9.30)	4 (7.27)	0.72	
Nosocomial	19 (44.18)	28 (50.90)	0.54	
Health related	12 (27.90)	12 (21.81)	0.63	
Community	12 (27.90)	15 (27.27)	1.00	
ICU admission	10 (23,25)	13 (23.63)	1.00	
Source				
Endovascular	17 (39.53)	22 (40)	1.00	
VCC	11 (25.58)	15 (27,27)	1.00	
VPC	4 (9.30)	7 (12.72)	0.75	
Vascular graft	2 (4.65)	0 (0)	0.19	
Desconocido	11 (25.58)	12 (21.18)	0.81	
Respiratory	3 (6.97)	6 (10.90)	0.72	
Soft tissue	5 (11.62)	6 (10.90)	1.00	
Osteoarthricular	7 (16.27)	6 (10.90)	0.55	
Urinary	-	3 (10.90)	0.25	
TTE	26 (60.46)	28 (50.90)	0.41	
TEE	8 (18.60)	9 (16.36)	0.79	
Empirical treatment				
Correct	32 (74.41)	39 (70.09)	0.81	
Guidelines concordant	16 (37.20)	20 (36.36)	1.00	
Vancomycin	14 (32.55)	13 (23.63)	0.36	
Daptomycin	2 (4.65)	1 (1.81)	0.58	
Linezolid	–	–	-	
Cloxacillin	–	–	-	
Definitive treatment				
Vancomycin	10 (23.25)	21 (38.18)	0.13	
Daptomycin	9 (20.93)	1 (1.81)	0.04	P 0.01 OR 14.76 (1.75 – 124.09)
Linezolid	7 (16.27)	8 (13.54)	1.00	
Cloxacillin	10 (23.25)	13 (23.63)	1.00	
Not concordant	7 (16.27)	12 (21.81)	0.60	
MRSA	19 (47.5)	21 (52.2)	0.67	
MSSA	24 (41.4)	34 (58.6)	0.67	
Control blood cultures	22 (51.62)	30 (54.54)	0.68	
Persistent bacteremia	15 (34.88)	9 (16.36)	0.05	P 0.04 OR 2.83 (1.05 – 7.62)
Source control	16 (37.20)	22 (40)	1.00	
General complications	17 (39.53)	24 (43.63)	0.85	
Metastasic infection	6 (13.95)	8 (14.54)	1.00	
Endocarditis	3 (6.97)	1 (2.32)	0.31	
Thrombophlebitis	-	3 (5.45)	0.25	
Osteomyelitis	3 (6.97)	4 (7.27)	1.00	
30 days mortality	10 (23.25)	15 (27.27)	0.81	
In-hospital mortality	12 (27.90)	15 (27.27)	1.00	

Data are no. (%) of patients. COPD (Chronic Obstructive Pulmonary Disease), ICU (Intensive Care Unit), Venous Central Catheter (VCC), Venous Peripheral Catheter (VPP), TTE (Transthoracic Echocardiography), TEE (Transesophageal Echocardiography)

Table 3 **Univariate and multivariate analysis for factors associated with persistent *S. aureus* bacteremia.**

	Persistent bacteremia (24; 24.48%)	NO persistent bacteremia 74 (75.51%)	p (IC 95%)	Multivariate analysis (p<0.20)
Age	70.54 ± 14.33	71.69 ± 11.83	0.92	
Sex				
Women	11 (45.83)	29 (39.18)	0.63	
Men	13 (54.16)	45 (60.81)		
Charlson				
≥6	13 (54.16)	49 (66.21)	0.32	
<6	11 (45.83)	25 (33.78)		
Morbidity				
Cardiovascular	14 (58.33)	37 (50)	0.49	
Hepatic	2 (8.33)	13 (17.56)	0.34	
Nephropathy	5 (20.83)	17 (22.97)	1.00	
COPD	5 (20.83)	25 (33.78)	0.31	
Arteriopathy	6 (25)	20 (27.02)	1.00	
Immunodeficiency	2 (8.33)	9 (12.16)	1.00	
Neoplasia	4 (16.66)	18 (24.34)	0.57	
Diabetes	6 (25)	22 (29.79)	0.79	
Catheter	12 (50)	18 (24.3)	0.02	P 0.008, OR 4.08 (1.43 – 11.61)
Permanent	9 (37.5)	13 (17.6)	0.05	
Transient	3 (12.5)	5 (6.8)	0.40	
Vascular device	1 (4.16)	8 (10.81)	0.44	
Nosocomial	10 (41.66)	37 (50)	0.49	
Health-care related	7 (29.16)	17 (22.97)	0.58	
Community	7 (29.16)	20 (27.02)	1.00	
ICU admission	18 (75)	56 (75.67)	1.00	
Source				
Endovascular	12 (50)	27 (36.48)	0.30	
CVC	7 (29.16)	19 (25.67)	0.79	P 0.88, OR 3.50 (0.831 – 14.74)
PVC	5 (20.83)	6 (8.10)	0.13	
Vascular graft	–	2 (2.70)	1.00	
Unknown	7 (29.16)	16 (21.62)	0.58	
Respiratory	2 (8.33)	7 (9.45)	1.00	
Soft tissue	2 (8.33)	9 (12.16)	1.00	
Osteoarthricular	1 (4.16)	12 (16.21)	0.17	
Urinary	–	3 (4.05)	1.00	
TTE done	11 (14.78)	43 (58.10)	0.34	
TEE done	3 (12.5)	15 (20.27)	0.54	
Empirical treatment				
Correct	18 (75)	53 (71.62)	1.00	
Guidelines concordant	8 (33.33)	28 (37.83)	0.80	
Vancomycin	6 (25)	21 (28.37)	1.00	
Daptomycin	1 (4.16)	2 (2.7)	1.00	
Linezolid	–	–	-	
Cloxacillin	–	–	-	
Definitive treatment				
Vancomycin	5 (20.83)	26 (35.13)	0.21	
Daptomycin	2 (8.33)	8 (10.81)	1.00	
Linezolid	6 (25)	9 (12.16)	0.18	
Cloxacillin	5 (20.83)	18 (24.32)	1.00	
Not ideal	6 (25)	13 (17.56)	0.55	
Source control	11 (45.83)	27 (36.48)	0.47	
General complications	7 (29.16)	34 (45.94)	0.16	
Metastasic infection	6 (25)	8 (10.81)	0.10	P 0.04; OR 3.82 (1.03 – 12.81)
Endocarditis	3 (12.5)	1 (4.16)	0.44	
Thrombophlebitis	1 (4.16)	2 (2.70)	1.00	
Osteomyelitis	2 (8.33)	5 (6.75)	1.00	
MIC ≥ 2 mg/L	15 (62.5)	28 (37.83)	0.05	P 0.02; OR 3.12 (1.13– 8.93)
MRSA	8 (33.33)	32 (43.24)	0.47	
MSSA	16 (66.66)	42 (56.75)	0.47	
30 days mortality	6 (25)	20 (27.02)	1.00	

Data are no. (%) of patients. COPD (Chronic Obstructive Pulmonary Disease), ICU (Intensive Care Unit), Venous Central Catheter (VCC), Venous Peripheral Catheter (VPP), TTE (Transthoracic Echocardiography), TEE (Transesophageal Echocardiography)

Table 4 **Univariate and multivariate analysis for factors associated with persistent MRSA bacteremia and persistent MSSA bacteremia.**

	Persistent MRSA bacteremia YES (8/40; 20%)	Persistent MRSA bacteremia NO (32/40; 80%)	P	Persistent MSSA bacteremia YES (16/58; 27.58%)	Persistent MSSA bacteremia NO (42/58; 72.41%)	p
General complications	4 (50)	13 (40.62)	0.70	5 (31.25)	19 (45.23)	0.38
Metastasic infection	3 (37.5)	2 (6.25)	0.04	3 (18.75)	6 (14.28)	0.69
Endocarditis	1 (12.5)	–	0.20	2 (12.5)	1 (2.38)	0.18
Thrombophlebitis	1 (12.5)	–	0.20	–	2 (4.76)	1.00
Osteomyelitis	1 (12.5)	2 (6.25)	0.49	1 (6.25)	2 (4.76)	1.00
MIC ≥ 2 mg/L	6 (75)	2 (6.25)	0.001	9 (56.25)	14 (33.33)	0.13
30 days mortality	2 (25)	8 (25)	1.00	3 (18.75)	12 (28.57)	0.52

Data are no. (%) of patients. COPD (Chronic Obstructive Pulmonary Disease), ICU (Intensive Care Unit), Venous Central Catheter (VCC), Venous Peripheral Catheter (VPP), TTE (Transthoracic Echocardiography), TEE (Transesophageal Echocardiography)

all variables which achieved p<0.20 in univariate analysis. Odds ratio were calculated with 95% confidence interval. Kaplan Meier survival was determined up to week 135 from the start of the study date (January 1st, 2014). Associations that reached p value <0.05 (CI of 95%) were considered statistically significant. Analysis were performed using SPSS 20.0.0 (Microsoft, USA).

The regional ethical comitte aproved this work (EC 27/18), and because no direct patient contact was planned, the requirement for informed consent was waived.

RESULTS

Patients and episode characteristics. The mean age was 71.41±12.45 years with 59.2% of men and 63.3% of the patients had a Charlson comorbidity index ≥ 6. A 47.95% of patients had a SAB nosocomial episode and a 27.98% a health care related episode. There were 40 cases of MRSA bacteremia (40.81%) and 58 cases of MSSA bacteremia (59.18%). Other baseline characteristics are shown in table 1.

The most frequent bacteremia source was catheter (34.88%) followed by primary bacteremia (23.46%). Endovascular source was more common in MSSA than in MRSA group (51.72% vs. 22.50%, p=0.006) and this was at expense of peripheral venous catheter bacteremia (18.96% vs. 0, p=0.002). A 41.8% of patients had some type of complication during the episode and 14.24% had an episode attributable to blood spread: 7 cases of osteomyelitis, 4 cases of endocarditis and 3 cases of thrombophlebitis. A 63.26% of patients received an empirical antibiotic different than recommended by the SEIMC guidelines and vancomycin was also the drug of choice for definitive treatment (31.62%). Other clinical and episode characteristics are shown on table 1.

There were 43 cases (43.87%) with a vancomycin MIC³2 mg/L: 42 cases with MIC=2 mg/L and 1 case with MIC=4 mg/L. Distribution between MRSA and MSSA group was 47.5% and 41.1% respectively. Differences in patients characteristics according to vancomycin MIC were not found. There were no significant differences in age, comorbidity, source or complications according to vancomycin MIC. Daptomycin was the definitive treatment in vancomycin MIC ≥ 2 mg/L SAB (20.93% vs. 1.81%, p=0.01; OR 14.76 [1.75–124.09]). Other items are described in table 2.

Outcomes. Persistent bacteremia was present in a total of 24 patients (24.48%). Differences between MRSA and MSSA group were not found. However, persistent bacteremia was significantly more common in high vancomycin MIC SAB (34.88% vs. 16.36%, p 0.05). A multivariate analysis of risk factors for persistent SAB was performed (table 3). Having a CVC was the main risk factor (OR 4.18; 1.38-12.61; p=0.008) to have a persistent SAB, followed by metastatic infection in general (OR 3.82; 1.03–12.81; p=0.04) and vancomycin MIC=2 mg/L (OR 3.12; 1.13–8.93; p= 0.02). In the MRSA subgroup analysis this was more evident showing that vancomycin MIC ≥ 2 mg/L it is more frequently associated with persistent MRSA bacteremia (75% vs. 6.25%; p=0.001). Persistent MRSA bacteremia is also more associated with metastatic complications (37.5% vs. 6.25%, p=0.004). Other items are shown in table 4.

Overall mortality at 30 days was 25.5%. There were no differences between MRSA and MSSA group, although there were more general in-hospital mortality in the first group. Survival analysis confirms these findings, with a survival rate of 70% at 135 weeks of follow-up that occurs mainly in the first week after the episode. Differences according to vancomycin MIC were not found. Multivariate analysis (table 5) showed that mortality was independently associated with general complications (OR 4.03; 1.42–1.44; p=0.009), other definitive treatment different from the contemplated in national guidelines (OR 3.72; 1.12–12.63; p=0.03), respiratory source (OR 3.72; 1.12–12.63; p=0.09) and age (OR 1.04; 0.99–1.09; p=0.05).

Table 5 **Univariate and multivariate analysis for factors associated with global mortality at 30 days for *S. aureus* bacteremia between January 2014 and September 2016 in Severo Ochoa University Hospital.**

	Mortality 30 days YES (26; 26.53%)	Mortality 30 days NO (72; 73.46%)	P <0.05 (IC 95%)	Multivariate analysis (p <0.20)
Age	75.73 ± 10.47	69.85 ± 12.76	0.05	P 0.05 OR 1.04 (0.99 – 1.09)
Sex				
Women	10 (38.46)	30 (41.66)	0.82	
Men	16 (61.53)	42 (58.33)		
Charlson				
≥6	20 (76.92)	42 (58.33)	0.10	
<6	42 (58.33)	30 (41.66)		
Morbidity				
Cardiovascular	10 (38.46)	35 (48.61)	0.36	
Hepatic	6 (23.07)	9 (12.5)	0.21	
Nephropathy	8 (30.76)	14 (19.44)	0.27	
COPD	11 (42.30)	19 (26.38)	0.14	
Arteriopathy	9 (34.61)	17 (23.61)	0.30	
Immunodeficiency	3 (11.53)	8 (11.11)	1.00	
Neoplasia	5 (19.23)	17 (23.61)	0.78	
Diabetes	7 (26.92)	21 (29.16)	1.00	
Catheter	6 (23.10)	24 (33.33)	0.45	
Permanent	2 (7.69)	20 (27.8)	0.05	
Transient	4 (15.4)	4 (5.60)	0.20	
Vascular device	2 (7.69)	7 (9.72)	1.00	
Nosocomial	11 (42.30)	36 (50)	0.64	
Health-care related	6 (23.07)	18 (25)	1.00	
Community	9 (34.61)	18 (25)	0.44	
ICU admission	6 (23.07)	17 (23.61)	1.00	
Source				
Endovascular	6 (23.07)	33 (45.83)	0.61	
CVC	3 (11.58)	23 (31.94)	0.06	
PVC	2 (7.69)	9 (12.5)	0.72	
Vascular graft	1 (3.84)	1 (1.38)	0.46	
Unknown	8 (30.76)	15 (20.83)	0.41	
Respiratory	5 (19.23)	4 (5.55)	0.05	P 0.09 OR 3.72 (0.80 – 17.25)
Soft tissue	3 (11.53)	8 (11.11)	1.00	
Osteoarthricular	3 (11.53)	10 (13.88)	1.00	
Urinary	1 (3.84)	2 (2.77)	1.00	
TTE done	11 (42.30)	43 (59.72)	0.16	
TEE done	2 (7.69)	15 (20.83)	0.22	

Table 5 **Univariate and multivariate analysis for factors associated with global mortality at 30 days for *S. aureus* bacteremia between January 2014 and September 2016 in Severo Ochoa University Hospital (cont.).**

	Mortality 30 days YES (26; 26.53%)	Mortality 30 days NO (72; 73.46%)	P <0.05 (IC 95%)	Multivariate analysis (p <0.20)
Empirical treatment				
Correct	18 (69.23)	53 (73.61)	0.79	
Guidelines concordant	9 (34.61)	27 (37.5)	1.00	
Vancomycin	6 (23.07)	21 (29.16)	0.61	
Daptomycin	1 (3.84)	2 (2.77)	1.00	
Linezolid	–	–	-	
Cloxacillin	–	–	-	
Definitive treatment				
Vancomycin	7 (26.92)	24 (33.33)	0.62	
Daptomycin	4 (15.38)	6 (83.33)	0.44	
Linezolid	2 (7.69)	13 (18.05)	0.34	
Cloxacillin	5 (19.23)	18 (25)	0.78	
Not concordant	8 (30.76)	11 (15.27)	0.14	P 0.03 OR 3.72 (1.12 – 12.63)
Control blood cultures	13 (50)	34 (47.22)	0.82	
Persistent bacteremia	6 (23.07)	18 (25)	1.00	
Source control	5 (71.42)	33 (80.48)	0.62	
General complications	17 (65.38)	24 (33.33)	0.006	P 0.009 OR 4.03 (1.42 – 11.44)
Metastasic infection	2 (7.69)	12 (16.66)	0.34	
Endocarditis	-	4 (5.55)	0.57	
Thrombophlebitis	1 (3.84)	2 (2.77)	1.00	
Osteomyelitis	1 (3.84)	6 (8.33)	0.67	
MRSA	10 (38.46)	30 (41.66)	P 0.82	
MSSA	16 (61.53)	42 (58.33)	P 0.82	
MIC ≥ 2 mg/L	32 (41.66)	11 (42.30)	P 1.00	
MRSA MIC ≥ 2 mg/L	5/10 (50)	15/30(50)	P 1.00	
MSSA MIC ≥ 2 mg/L	6/16 (37.5)	17/42 (40.47)	P 1.00	

Data are no. (%) of patients. COPD (Chronic Obstructive Pulmonary Disease), ICU (Intensive Care Unit), Venous Central Catheter (VCC), Venous Peripheral Catheter (VPP), TTE (Transthoracic Echocardiography), TEE (Transesophageal Echocardiography)

DISCUSSION

A retrospective descriptive study of *S. aureus* bacteremia series with 98 episodes in a Spanish secondary hospital between January 2014 and September 2016 is presented. Firstly, SAB continues to be an entity related mainly to elderly and comorbid patients. This fact is particularly emphasized in MRSA subgroup in consonance with previous literature [18]. On the contrary on the literature reviewed [18-20], bacteremia of endovascular source is predominantly associated with MSSA group (p=0.006). This has to do with the association between MSSA bacteremia and peripheral venous catheter. Secondly, although empirical treatment is in most cases correct (72.44%), empirical treatment remains no concordant with guidelines (63.26%). What's more, follow-up cultures are only taken in half of the patients. Since there were no differences in follow-up time between patients who had follow-up cultures and those who did not, this may be related to the type of patient in our setting (elderly and with great comorbidity) and a tendency towards a more conservative attitude that limitates venipuncture. Furthermore, definitive treatment is not always adjusted to the clinical practice national guidelines (30.76%). As several jobs have shown in recent years [21-22], quality of care in SAB has an impact on prognosis. This is expressed in the study with the fact that no concordant definitive treatment is associated with mortality(OR 3.72; 1.12–12.63; p=0.003). The fact that our center does not have a multidisciplinary nosocomial infection management team in which the infectious diseases physician

advises other professionals on the adequate management of complex processes such as SAB, could explain this poor adherence to the guidelines.

Thirdly, the more important factors associated with persistent SAB are being a CVC carrier, having a metastatic complication and vancomycin MIC ≥ 2 mg/L. In the past few years, several authors have reported this vancomycin progressive high creep, with different results in clinical practice. There are several opinions on this point [23-25], but most of the research is in favor of high vancomycin MIC turns into a thickness of bacterial cell wall peptidoglycan and a delayed transition to the postexponential growth phase because of alterations in bacterial metabolism and possibly a blockage in the adhesive phase of *S. aureus* [26-28]. The *agr* system is a genetic regulon that encodes most of the proteins that have to do with these functions and it also has been observed that there is a correlation between reduced susceptibility to vancomycin and reduce *agr* regulon expression, particularly with *agr* genotype II [29]. All this could lead to a greater tendency to persistent bacteremia. As REIPI group has recently reported [30], in MRSA subgroup this is more evident and it seems that it could be related also with metastatic complications. In our series, any predisposing factor to having a high vancomycin MIC SAB was found. Although, high vancomycin MIC was related with persistent bacteremia, there were not any relationship between high vancomycin MIC and mortality or general complications. Contrary to many authors, no statistically significant association was found between persistent bacteremia and vancomycin MIC in the subgroup of MSSA, probably due to sample size. However, in MRSA subgroup analysis, this association was more evident showing that high vancomycin MIC it is more frequently associated with persistent MRSA bacteremia(75% vs. 6.25%, p=0.001) and also, that persistent MRSA bacteremia is more associated with metastatic complications (37.5% vs. 6.25%, p=0.004).

The strength of our study is the great proportion of high vancomycin MIC. *S. aureus* strains. We think that this may have to do with the fact that vancomycin is the most used antibiotic for gram positive bacteremia in our area, but we are aware of a genetic study of is maybe needed. Because this is a retrospective and descriptive study, it has many limitations. The reduced sample size make difficult to establish a statistically significant relationship between persistent MSSA bacteremia and high vancomycin. It also make difficult to establish risk factors for metastatic complications. Moreover, a multivariate analysis in persistent MRSA bacteremia subgroup could not be performed. There is also a lack of information related to days of persistent bacteremia and the lack of control blood cultures in 47.96% of patients. Furthermore, the fact that vancomycin MIC is measured with microdilution test, may compromise the external validity with other works that are mainly done with E-test.

In conclusion, high vancomycin MIC could be a marker of virulence that guides treatment optimization in SAB. The prevalence of this population in certain settings could also motivate greater compliance with national guidelines for the management of this entity. Although, the impact of this factor on mortality or metastatic complications in this study is low, it has a greater importance in MRSA bacteremia. Prospective and center individualized studies with bigger samples, could help to improve the care and prognosis of this entity in each center.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

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None to declare

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Brief report

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Seroprevalence of *Toxoplasma gondii* infection in pregnant women attending antenatal care in southern Ethiopia

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ABSTRACT

Objetives. The aim of the study was to assess the prevalence and possible risk factors of *Toxoplasma gondii* (toxoplasmosis) infection in pregnant women attending antenatal care at Gambo General Rural Hospital, southern Ethiopia.

Methods. Hospital-based, prospective cross-sectional study. We collected 401 serum samples from September 1 to October 30, 2015, along with sociodemographic data and data on potential risk factors, using a simple random sampling technique.

Results. The overall seroprevalence of *T. gondii* in pregnant women (mean age 23.1 years) was 23.9% (95% confidence interval [CI] 20.0, 28.3). We did not find any significant risk factors associated with seropositivity in relation with participants' level of education; occupation; contact with cats; consumption of raw or uncooked meat, vegetables, or milk; or type of flooring (soil versus cement) at home. The women who were aware of the risk of toxoplasma infection on the fetus had fewer *T. gondii* antibodies. Drinking unsafe water was associated with a higher risk of toxoplasmosis (p = 0.08).

Conclusion. The seroprevalence of toxoplasmosis among pregnant women was relatively lower.

Key words: Seroprevalence, *Toxoplasma gondii*, Maternal health, Ethiopia

Seroprevalencia de *Toxoplasma gondii* en mujeres embarazadas que acuden a la atención prenatal en el sureste de Etiopía

RESUMEN

Objetivos. Evaluar la prevalencia y los posibles factores de riesgo de la infección por *Toxoplasma gondii* (toxoplasmosis) en mujeres embarazadas que reciben atención prenatal en el Hospital Rural General de Gambo, en el sureste de Etiopía.

Métodos. Estudio prospectivo transversal de base hospitalaria. Se tomaron 401 muestras de suero desde el 1 de septiembre hasta el 30 de octubre de 2015, junto con a datos sociodemográficos y factores de riesgo potenciales, utilizando una técnica de muestreo aleatorio simple.

Resultados. La seroprevalencia general de *T. gondii* en mujeres embarazadas (edad media de 23,1 años) fue del 23,9% (intervalo de confianza [IC] del 95%: 20,0; 28,3). No encontramos ningún factor de riesgo significativo asociado con la seropositividad en relación con el nivel de educación de los participantes; ocupación; contacto con gatos; consumo de carne, verduras o leche crudas o sin cocinar; o tipo de piso de la casa(suelo versus cemento) en casa. Las mujeres que conocían el riesgo de tener infección por toxoplasma tenían menos anticuerpos contra *T. gondii*. Beber agua no segura se asoció con un mayor riesgo de toxoplasmosis (p = 0,08).

Conclusión. La seroprevalencia de toxoplasmosis entre las mujeres embarazadas fue relativamente baja.

Palabras clave: Seroprevalencia, *Toxoplasma gondii*, Salud Materno-infantil, Etiopía

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INTRODUCTION

Toxoplasmosis is one of the primary foodborne parasitic diseases [1]. It affects one-third of the world's population, with prevalence rates in low- and middle-income countries ranging from 30% to 60% [2]. Toxoplasmosis had a burden of approximately 1.68 million (95% uncertainty interval [UI] 1.24, 2.45) disability-adjusted life years (DALYs) [1]. Primary infections with *Toxoplasma gondii* acquired during pregnancy are usually asymptomatic for the mother but can lead to serious neonatal complications for the newborn [3], including miscarriage, hydrocephalus, cerebral calcification, and chorioretinitis [4].

Serological screening of pregnant women or *T. gondii*-specific antibodies is not practiced in antenatal care in Ethiopia, but there are several studies providing data about its prevalence [5-11]. This study aimed to assess the seroprevalence and associated risk factors of *T. gondii* infection in a rural area of southern Ethiopia.

MATERIAL AND METHODS

The study population was pregnant women attending Gambo Rural Hospital (GRH), a 150-bed rural general hospital located in the West-Arsi region, 250 km south of Addis Ababa. The GRH is a private mission hospital. Due to an inadequate transportation network, the coverage area of the GRH is restricted to approximately 75,000 inhabitants, most of whom live in a rural setting and work in agriculture and farming.

We performed a cross-sectional study in consecutive pregnant women attending a mother and child healthcare clinic in GRH from September 1 to October 30, 2015. Blood samples were collected on Whatman filter paper, stored at 2º-8º C, and subsequently transported to the Microbiology Laboratory of Hospital General Universitario de Alicante, Spain. We performed an elution for serological determinations in a volume of 300 µL, as described elsewhere [6]. Investigators collected demographic information from participants at the same time of blood drawing. A commercial enzyme immunoassay (EIA) was used to detect immunoglobin G (IgG) type antibodies for *T. gondii* (Demeditec Diagnostics GmbH, Germany) according to the manufacturer's instructions.

Participant data were anonymized, and the study received ethical approval from the local Research and Publication Committee of the GRH and the Health Unit and Ethical Review Committee of the Ethiopian Catholic Secretariat (GH/MSMHF/723).

Data were collected and entered into Excel software. The data were then cleaned and imported into SPSS statistical software, version 22 (IBM, Chicago, Ill, USA) for analysis. We used the Student's t test to evaluate continuous data and the Chi-square test to analyze categorical variables. We estimated prevalence with 95% confidence intervals (CI) using the Wilson procedure. We expressed the measure of association as an odds ratio (OR) with 95% CI. P values less than 0.05 were considered statistically significant.

RESULTS

We initially included 408 participants in the study, but seven were excluded due an error in numbering or blood droplets on the filter paper. The mean age of the 401 analyzed participants was 23.1 years (standard deviation [SD] 4.9). Three patients had HIV infection (0.8%). About one in every 10 pregnant women had knowledge of toxoplasmosis infection and its risk to the fetus.

Participant characteristics, including sociodemographic data and history of exposure to known risk factors for *T. gondii* infection, are presented in table 1. The overall seroprevalence of *T. gondii* in our sample was 23.9% (95% CI 20.0, 28.3). We did not find any significant factors associated with seropositivity related to participants' level of education; occupation; contact with cats; consumption of raw or uncooked meat, vegetables or milk; or type of flooring (soil versus cement) in the home. The pregnant women who know the risk of toxoplasma infection on fetus had fewer *T. gondii* antibodies. Drinking unsafe water was associated with a higher risk of toxoplasmosis than drinking pipe water, with borderline statistical significance (p = 0.08) (table 1).

DISCUSSION

A wide variability in the prevalence of toxoplasmosis among pregnant women has been reported worldwide. The present study observed a 23.9% prevalence of anti-toxoplasma antibodies in pregnant women in a rural area of southern Ethiopia, which is higher than that observed in another study in Felege Hiwot Referral Hospital, in Bahi Dar, northwest Ethiopia [5], but similar to that reported in other low-income countries [11]. However, our results are lower than the seroprevalence reported in pregnant women in Addis Ababa (85.4%) [6]; in Jimma town (83.6%) [7]; in four central Ethiopian municipalities (Addis Ababa, Ambo, Debre-Zeit, and Metehara) (81.4%) [8]; in Debre Tabor, northwest Ethiopia (68.4%) [9]; and in the general population of Nazareth, central Ethiopia (60%) [10]. The differences observed may be partly attributable to the different procedures used to measure antibodies, with either EIA [7,8,9] or latex agglutination slide test [5,6,10].

Studies have reported several risk factors for toxoplasma in pregnant women, including age [9], illiteracy [9], contact with cats at home [5,7,9], or consumption of raw/undercooked meat [5] or vegetables [8]. We assessed these known risk factors as well as other potential ones such as gestational age, occupation and various sociodemographic characteristics. However, like the report by Gelaye et al in Addis Ababa [6], our study did not observe any statistically significant association between *T. gondii* infection and any risk factor in pregnant women attending routine antenatal care.

One limitation of our study is the possibility that the method of specimen storage (on Whatman paper) decreased the sensitivity of antibody detection. Moreover, we only measured type IgG antibodies to *T. gondii*, whereas type IgM antibodies are more suggestive of recent infections.

Table 1 **Participant characteristics and seroprevalence of *T. gondii* infection**

Variables (N)	N (%)	Positive N (%)	Negative N (%)	ORc (95% CI)	P value
Age in years (N = 396)					0.7
15-19	55 (13.9)	11(20)	44 (80)	1.0	
20-24	146 (36.9)	37 (25.3)	109 (74.7)	2.1 (0.7, 6.9)	
25-29	115 (29.0)	28 (24.3)	87 (75.7)	1.9 (0.6, 5.6)	
30-34	64 (16.2)	14 (21.9)	50 (78.1)	1.8 (0.6, 5.1)	
35-39	16 (4.0)	6 (37.5)	10 (62.5)	2.4 (0.7, 8.0)	
Residence (N = 401)					0.7
Urban	74 (18.5)	19 (25.7)	55 (74.3)	1	
Rural	327 (81.5)	77 (23.5)	250 (76.5)	0.8 (0.5, 1.6)	
Type of flooring in home (N = 385)					0.1
Cement	41 (10.6)	6 (14.6)	35 (85.4)	1.0	
Soiled	345 (89.4)	85 (24.6)	260 (75.4)	1.9 (0.8, 4.7)	
Knowledge of toxoplasmosis (N = 395)					0.6
Yes	11 (2.8)	0	11 (100)	1.0	
No	384 (97.2)	95 (24.7)	289 (75.3)	N.A	
Occupation (N = 396)					0.5
Housewife	363 (94.0)	90 (24.8)	273 (75.2)	1.0	
Other	23 (6.0)	5 (21.7)	18 (78.6)	0.7 (0.4, 1.2)	
Education (N = 391)					0.07
Illiterate	82 (21)	14 (17.1)	68 (82.9)	3.1 (0.4, 25)	
Read and write	201 (51.7)	52 (25.7)	150 (74.4)	5.2 (0.7, 40)	
High school	91 (23.3)	26 (28.6)	65 (71.4)	6.0 (0.7, 48)	
College and above	16 (4.1)	1 (6.3)	15 (93.8)	1.0	
Gestational age (N = 401)					0.5
1st trimester	75 (18.7)	19 (25.3)	56 (74.7)	1.0	
2nd trimester	158 (39.4)	42 (26.6)	116 73.4)	1.1 (0.6, 1.7)	
3rd trimester	168 (41.9)	35 (20.8)	133 (43.6)	0.9 (0.5, 1.7)	
Contact with cats (N = 401)					0.5
No	227 (56.6)	57 (25.1)	170 (74.9)	1.0	
Yes	174 (43)	39 (22.4)	135 (77.6)	0.8 (0.5, 1.4)	
Consumption of raw/uncooked meat (N = 400)					0.4
No	144 (36)	38 (26.4)	106 (73.6)	1.0	
Yes	256 (64)	58 (22.7)	198 (65.1)	0.8 (0.5, 1.3)	
Consumption of raw/uncooked vegetable (N = 401)					0.5
No	28 (7.0)	8 (28.6)	20 (71.4)	1.0	
Yes	373 (93.0)	88 (23.6)	285 (93.4)	0.8 (0.3, 1.8)	
Consumption of raw/uncooked milk (N = 401)					0.4
No	30 (7.5)	9 (30.0)	21 (70)	1.0	
Yes	371 (92.5)	37 (23.5)	284 (76.5)	0.7 (0.4, 1.4)	
Source of drinking water (N = 400)					0.1
Pipe	51 (12.8)	7 (13.7)	44 (86.3)	1.0	
Well	61 (15.3)	13 (21.1)	48.78.7)	1.7 (0.6, 4.6)	
Unsafe[a]	288 (72.0)	75 (26.0)	213 (74.0)	2.2 (0.9, 5.1)[b]	

[a]Unsafe water: river or spring water sources.

[b]p=0.08, comparing drink unsafe water with drink safe water (pipe and well)

ORc: crude odds ratio, CI: confidence interval

In conclusion, prevalence of *T. gondii* in pregnant women in our area is lower than that reported elsewhere in Ethiopia and similar to that reported in other low-income countries. In agreement with Gebremedhin et al [8], we believe that an educational program, antenatal screening of pregnant women and further epidemiological studies to uncover the economic and health impact of toxoplasmosis are called for.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

FUNDING

None to declare

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Clinical-Pathologic Conference

A patient with a rapidly lethal pneumonia after a visit to a touristic area in rural Leon (Spain)

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PRESENTATION OF CASE (DR. MARICELA VALERIO)

A 60 year old lady, presented to the Emergency Department of Hospital General Universitario Gregorio Marañón (HGUGM) in Madrid in November 2017 with a 4 day history of malaise, fever, chest pain and dyspnea.

She had a left side breast cancer in 2001 that received treatment with surgical resection of the nodule, axillary lymphadenectomy, chemotherapy, local radiotherapy and finally, hormonal therapy with tamoxifen for 5 years and letrozol for 2 years. She had no recent evidence of recurrence.

She was a smoker until several years ago, when she quit smoking and did not report any drug allergy or to other products. She had no underlying heart disease and was not receiving, regularly, drugs of any kind. She had never travel out of Spain. She had not been vaccinated recently.

Six days before her admission she had a family touristic trip to an area in the North West of Spain called Las Médulas, an historical roman gold mining area in the province of León. She entered several caves and tunnels but denied any contact with animals or birds, including bats and no particular exposure to dust. She denied eating uncooked products or unpasteurized milk or cheese.

During the trip, she started with fever, malaise and headache, and decided to prematurely return to Madrid and stayed at her home with symptomatic treatment, 4 days later she began with dry cough and dyspnea. Due to the rapid deterioration of her clinical condition she went to the Emergency Department (Day 0). On admission she had dyspnea, tachypnea, tachycardia and hypotension, blood cultures were obtained and IV fluids and antibiotic treatment with ceftriaxone (2 g IV q.d.) and levofloxacin (750 mg q.d.) was administered before her transfer to the Intensive Care Unit.

A chest X-ray taken on admission is shown in figure 1. Radiologic report states that "there is an Upper Right Lobe (URL) consolidation with potential amputation of the superior URL bronchus. Increased density in the right lung hilum. Possible pneumonia. A central hilum tumor should be ruled out".

Other complementary data obtained on admission were the following: hemoglobin 12.7 g/dL, hematocrit value 35.9 %, mean corpuscular volume 93.2 fL, platelet count 170,000 uL, white blood count 6,800/uL (neutrophils 6,200 uL, lymphocytes 400 uL, monocytes 10 uL, eosinophils 300 uL). Prothrombin time 25.5 sec, I.N.R. 2.11, fibrinogen >1000 mg/dL, A.P.T.T. 35.8 sec.

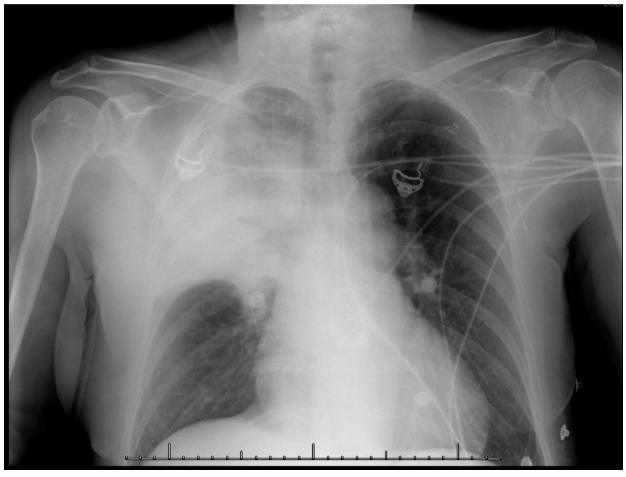

Figure 1 | **Day of admission**

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Venous blood data: pH 7.43, pCO_2 34 mmHg, pO_2 28 mmHg, O_2 saturation of 55 %, HCO_3 23 mmol/L, BEb -1.2 mmol/L, lactate of 5.0 mmol/L, glucose 107 mg/dL, ALT 46 U/L, total billirubin 1.2 mg/dL, GGT 19 U/L, alkaline phosphatase 49 U/L, CK 47 U/L, amilase 18 U/L, lipase 15 U/L, creatinine 1.03 mg/dL, glomerular filtrate 55 mL/min/1.73 m^2, Na 139 mmol/L, K 3.6 mmol/L, Cl 99 mmol/L, Ca 8.5 mg/dL. troponin T 6 ng/L , Nt-proBNP 5,204 ng/L, PCR 34.0 mg/dL, PCT 8.39 µg/L. Normal ECG.

ICU admission. Due to shock and progressive hypoxia (O_2 sat 91%) despite 100% ventimask and noradrenalin, she was transferred to the ICU. At the time of ICU admission she was conscious and mentally oriented with a Glasgow coma score of 15. The patient had sinus tachycardia. No heart murmurs were present. Abdomen was soft with no liver nor spleen enlargement. There was a discrete abdominal pain on palpation of the right hipocondrium. No signs of peritoneal irritation were present. No peripheral edema nor signs of deep venous thrombosis were present. Peripheral arteries pulsed symmetrically.

Legionella antigenuria was informed as negative and *Streptococcus pneumoniae* antigenuria as positive.

Day +2 after admission. A progressive deterioration of the respiratory function occurs and oro-tracheal intubation was required (Figure 2 Chest X ray). Persistent desaturation down to 75% occurred despite FiO_2 of 100% and PEEP of +18mmHg. Vasoactive drugs needs increased (adrenaline and noradrenaline) and a new right bundle block became evident in the urgently performed EKG. A trans-thoracic echocardiogram (TTE) revealed an important dilatation of the right ventricle with severely depressed function.

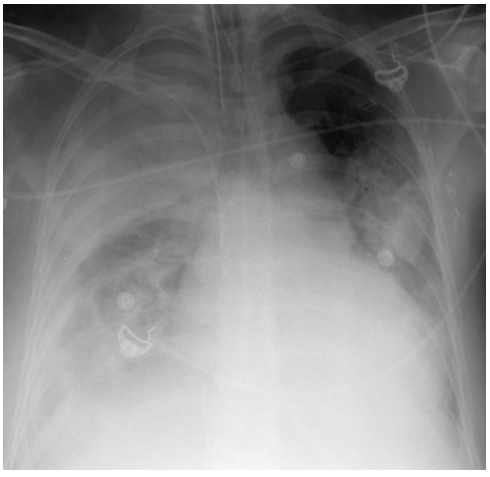

Figure 2 | **Day +2 after admission**

Day +3 to +6 after admission. An evolution to multi-organic failure occurred and blood cultures remained negative. Massive pulmonary thromboembolism was suspected and thrombolysis with alteplasa was performed. The patient required ECMO and continuous veno-venous hemofiltration (CVVHF) due to oliguric renal failure. On day +5 a fiberoptic bronchoscopy showed a permeable bronchial tree, with normal mucosa and no active bleeding. Samples for culture were obtained and antimicrobial treatment was modified, including now meropenem, vancomycin, and clindamycin.

A new TTE showed a severely dilated and severely dysfunctional right ventricle, with moderate-severe tricuspid valve dysfunction and a minimal pericardial fluid.

Consumption coagulopathy persisted despite the administration of vitamin K and progressive anemia and thrombocytopenia develops. Cultures taken during fiber optic bronchoscopy were all negative.

Complementary data requested include: Negative AVH, BVH, CVH, VIH, Rose of Bengal, *Rickettsia*, *Borrelia*, *Legionella*, *Mycoplasma*, *Coxiella*, *Chlamydia*, *Leptospira, Cryptococcal antigen* and *Aspergillus antigen.*

Blood PCR for *Bartonella and Coxiella burnetii* were both negative. The *Plasmodium antigen* was also negative and no microorganisms were seen in Giemsa stains of peripheral blood samples. CMV and EBV viremia were negative.

Other negative respiratory samples included: RSV, Influenza A and B.

Day +7 after admission. An abdominal echocardiogram showed: Enlarged liver (Up to 19 cm) of homogenous parenchyma. Normal biliary tree. Spleen-portal axis and pancreas were unremarkable as were both kidneys and the excretory system. Minimal pleural fluid and ascitic fluid were detected. CT scan could not be performed due to the critical and unstable situation of the patient.

Final evolution. The patient died on day +10th of admission and a limited, echography guided autopsy, was authorized by the family.

DIFFERENTIAL DIAGNOSIS (DR. FRANCISCO LÓPEZ-MEDRANO)

Thank you very much for inviting me to discuss this clinical case. I am totally unaware of the final diagnosis of the case. Three important factors have to be considered for the final approach to the diagnosis of a potential infectious disease: the clinical manifestations and syndromic diagnosis, the time of evolution and the risk factors of the patient. Those three aspects for this patient are, in my opinion: pneumonia, of acute evolution after a visit to an historical, not active, mining roman area in Leon, Spain.

Regarding the first aspect, the patient is a 60 year old

lady, apparently immunocompetent, that develops a rapidly evolving febrile disease with pulmonary infiltrates and a positive urine test for pneumococcal antigen. Here we may have, in my opinion, three potential scenarios. First, we are assisting to an episode of fulminant pneumococcal pneumonia affecting an immunosuppressed patient, second, we have a false positive pneumococcal antigenuria and there is an alernative etiology for the pneumonia or, finally, this is an episode of pneumococcal pneumonia with co-infection with another microorganism. In a recent article by Sanges et al [1], patients 18 to 40 year-old who had experienced an invasive infection with encapsulated bacteria were examined searching for primary immunodeficiencies (PIDs). Out of 36 such cases, 7 (19%) had a PID which included idiopathic primary immunodeficiency and hypogammaglobulinemia and also complement (C6 and C7) deficiencies. Authors concluded that PID screening should be considered after a first unexplained invasive encapsulated-bacteria infection in young adults.

Regarding the issue of pneumococcal antigen present in this patient's urine, Couturier et al [2] review the literature to that time and showed specificities of the test, generally from 90-100% with only occasional exceptions. My consideration is that this test is credible in this patient and very significant for my final diagnosis.

However, due to its fulminant course, we should consider other potentially treatable alternatives. We have to consider, either virus, bacteria, mycobacteria, fungi or parasites. Chapter 69 of the 2014 edition of Mandell's textbook of Infectious Diseases, written by Ellison and Donowitz [3], lists in several tables, common and uncommon causes of acute pneumonia, but we have to try to reduce the size of this long list to maintain only those etiologies that best fit with the case from a clinical, radiological and epidemiological point of view. Influenza and Respiratory Syncytial Virus heads the list of common viral agents, *S. pneumoniae* is the main cause of bacterial infections and Histoplasma the most common cause of fungal pneumonia, particularly after visiting caves. When considering specifically the main causes of non-resolving pneumonia, *Yersinia pestis*, *Burkholderia pseudomallei, Hantavirus* sp., *Coccidiodes immitis, Blastomyces* sp., *Histoplasma* sp. *and Cryptococcus gattii* are among the most commonly listed agents. Many of these agents are easily eliminated on the basis of epidemiological conditions and patient's history. *Cryptococcus gattii* has been reported occasionally in Spain [4] and *Strongyloides stercoralis* has been also diagnosed in autochthonous Spanish populations (Valencia and Alicante, but not in León) [5]. Hantaviruses are the etiological agents of hemorrhagic fever with renal syndrome in Europe and Asia, and hantavirus pulmonary syndrome is mainly an American entity. There is seropositiviy to Puumala, Hantaan and Seoul virus only in a low proportion of persons in some regions of Spain [6, 7] but cases with pulmonary involvement have been never described in Spain and the diagnosis of Hantavirus is highly improbable in this lady. Regarding the area of the patients visit we were not able to find any suggestive entity compatible with this case even when *Francisella tularensis* was reported in vole populations in that part of Spain [8] and has been described in outbreaks in the past [9-12].

My final consideration is the possibility of having a pneumococcal pneumonia and "something else".

In a review of the etiology of community-acquired pneumonia in the USA, reported by Jain et al [13], a bacterial and viral coinfection was demonstrated in 3% of the episodes. One virus of interest is adenovirus [14] that may occur in patients with no prior underlying condition. The same may occur with HSV in previously normal hosts that are able to cause a severe, non-resolving pneumonia in immunocompetent patients [15]. Bouza et al, reported Herpes simplex as a cause of worse prognosis when present in patients with ventilator-associated pneumonia [16].

Dr. López Medrano Diagnosis

My presumptive diagnosis is then: fulminant pneumococcal pneumonia due to co-infection with Herpes simplex virus or Adenovirus.

EVOLUTION OF THIS PATIENT (DR. MARICELA VALERIO)

In the final days of the life of the patient, or immediately after her death, the results of other requested tests were reported. Blood PCR test for Hantavirus and *C. burnetii* were reported negative and a blood test for *Histoplasma* spp. was also negative. Bacterial cultures of the bronchoalveolar lavage (BAL) samples were negative but a PCR test amplified *S. pneumoniae*. In the lung samples obtained by transthoracic biopsy after death, PCR was also positive for *S. pneumoniae*.

The liver and kidney echography guided biopsies obtained postmortem were negative by culture and PCR negative also.

Pathology reported changes compatible with disseminated intravascular coagulation. Bone marrow biopsy was

Table 1 Lymphocyte populations

	Percentage	Normal range (%)	Absolute value (cells/µL)	Normal range (cells/µL)
T cells (CD3+)	68%	55-82	191	700-2100
T cells (CD3+ CD4+)	37%	28-57	107	300-1400
T cells (CD3+ CD8+)	30%	10-39	87	200-1200
Coeficient CD4/CD8	1.2	1-3.6		
B cells (CD19+)	24%	6-19	65	100-500
LGL/NK cells (CD3-/ CD56+)	6%	7-31	16	90-600

hypo-cellular with marked decrease of megakaryocytic and granulocytic series and a relative increase in the red blood cell series, with dyserythropoiesis. Findings were interpreted as compatible with sepsis, disseminated intravascular coagulation (DIC), and haemophagocytosis.

The Immunology Laboratory reported: anti-cardiolipin IgG and IgM, Anti-Beta 2 GPI IgG and IgM, native Anti-DNA and Antinuclear antibodies, all negative. Immuneprotein levels in serum were reported as follows: IgG 893.0 mg/dL (normal range: 650-1610), IgA 221.0 mg/dL (normal range 90-497), IgM 123.0 mg/dL (normal range 42-255). Other serum values included complement C3 57.7mg/dL (normal range 91-190), C4 14.4mg/dL (normal range 18-56) and C-reactive protein 18.4mg/dL (normal range 0-0.8). Figures of different lymphocytic populations were clearly decreased and are summarized in table 1.

FINAL DISCUSSION

Several points of the presentation and evolution of this case deserve discussion, in our opinion.

First of all, the patient had, among the early laboratory tests, a positive pneumococcal antigen in urine. Determination of pneumococcal antigen in urine, is recommended by IDSA in patients with pneumonia that require ICU admission, those who fail response to the initial antibiotic treatment, patients with low white blood count, alcoholics and patients with pleural effusion and asplenia [17]. Sensitivity and specifcity of this test vary depending on different circumstances. Blaschke et al reported [18], sensitivities with the Binax NOW test from 70 to 90 % with specificities from 80 to 100% in adults with pneumonia. Results may be worse in patients who are nasopharyngeal carriers of *S. pneumoniae* and better in patients with severe infection and bacteremic pneumococcal pneumonia [19-21].

Another question in this case, is the interpretation of a specific PCR for *S. pneumoniae*, both in lower respiratory tract samples obtained by BAL and in lung biopsies postmortem. Sensitivity is considered variable but specificity could be superior to 95% according to different authors [22-25].

The reasons for the very aggressive behavior of pneumococcal infection in this patient, remain obscure for us. Fulminant pneumococcal infection is an uncommon but well known situation, particularly in immunocompromised and asplenic patients, either traumatic or functional [26-37]. However, it can also occur in non-immunocompromised subjects by mechanisms that are not totally clarified [38]. We could not demonstrate a situation of functional asplenia in our patient and only speculated with the potential radiation of the spleen while she received radiotherapy for her left breast cancer, several years before.

This patient, had very low figures of serum complement, that have also been associated with a risk of poor evolution in patients with pneumococcal infection [39-43]. Hypocomplementemia may be in the origin of the evolution of this patient, but in our opinion, it is the consequence of sepsis and DIC [44]. A previous report suggested that pneumococcal capsular polysaccharides (PCPs) were responsible for initiating DIC through inflammation induced by PCPs, per se, or an antigen–antibody reaction [45]. Also, certain serotypes of *S. pneumoniae* may be particularly invasive [46] but not having an isolate, we were unable to serotype this case.

Another probable diagnosis that needs to be mentioned, is the hemophagocytic syndrome secondary to the infection. The most common infectious trigger of this syndrome are viral infections. Bacterial causes are less common but cases of *S. pneumoniae* infection related hemophagocytic syndrome have been described [47, 48]. Hemophagocytic syndrome could be diagnosed if at least 5 out of 8 criteria are present, our patient only met 4 of them (fever, hypofibrinogenemia, hemophagocytosis and bicitopenia) but ferritin concentrations, CD25 levels and cytotoxic activity of NK cells were not determined [49].

Finally, we were surprised by the severe cardiovascular events of this patient. It is known that patients hospitalized for pneumococcal pneumonia have a higher risk of cardiovascular events than similar populations. Complications include, acute myocardial infarction, auricular fibrillation, ventricular tachycardia and acute heart failure [50-54]. Most commonly, those events occur very early in the natural history of pneumococcal infection with 55% of them reported in the very first day of admission, with a progressive decrease in the following month. Musher et al [55] found 33 cardiovascular severe events in a population of 170 cases admitted with pneumococcal pneumonia. A national retrospective cohort of patients with pneumococcal pneumonia in Taiwan was compared with a similar population without pneumonia, and the authors showed a higher risk of thromboembolic episodes (deep venous thrombosis and puylmonary emnbolisms) in the population with pneumonia, particularly within the first four weeks of evolution [56].

This patient had never received a pneumococcal vaccine, and our speculation in the discussion of the case was if vaccination could have avoided this episode or at least decreased its severity and preserve patient's life.

FINAL DIAGNOSIS

Invasive pneumococcal infection with pneumonia and fulminant sepsis.

Disseminated intravascular coagulation.

Right heart failure probably due to pulmonary embolism.

Hypocomplementemia and lypmphopenia

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None to declare.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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Letter to the Editor

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Postoperative soft-tissue infection due to multidrug-resistant *Pseudomonas aeruginosa*: usefulness of ceftolozane-tazobactam

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Sir,

Ceftolozane-tazobactam (CT) is a combination that contains a novel cephalosporin, ceftolozane, with activity against Gram-negative drug-resistant pathogens including *Pseudomonas aeruginosa* and Enterobacteriaceae, and tazobactam, an inhibitor of different beta-lactamases produced by some Gram-negative bacilli. This drug has been approved for the treatment of complicated urinary tract infections and complicated intra-abdominal infections [1], however its use is increasingly described in medical literature in other indications [2-4].

We report on a case of successful use of CT in a patient with a postoperative soft-tissue infection with repeated isolation of multi-resistant *P. aeruginosa*. Due to a lengthy course of therapy with colistin and tobramycin the patient developed acute kidney failure that required continuous renal replacement therapy and intensive care unit (ICU) admission.

The patient was a 52-year-old man with an history of heavy smoking, alcoholism, hypertension, dyslipemia and dilated cardiomyopathy. He was admitted to our hospital after suffering a traffic accident and was diagnosed of a comminute tibial and peroneal open fracture involving its middle third. Antibiotic prophylactic therapy with intravenous cefazolin and gentamycin was initiated and maintained during five days. Nail osteosynthesis surgery was then performed with good postoperative evolution so he was discharged six days later. Three weeks later, he was transferred from outpatient traumatology consultations to the emergency department for hospital admission due to skin necrosis with an eschar on the anterior side of the right leg with serohematic fluid discharge from which two samples for culture were positive for *P. aeruginosa* with susceptibility to all antimicrobial categories. Lab tests showed an increase in C-reactive protein (9.55 mg/dL) and globular sedimentation rate (98 mm). Treatment with intravenous ceftazidime (1 g t.i.d.) and tobramycin (100 mg t.i.d.) was started and maintained for two weeks. Daily cures were performed in the ward and in the surgery room, where the devitalized anterior tibial muscle was cleaned and the eschar and one bone fragment were removed. Subsequently, ceftazidime was replaced by imipenem (1 g t.i.d.) due to repeated isolation on wound cultures of a new strain of *P. aeruginosa* with resistance to aztreonam, cefepime, ceftazidime and piperacillin-tazobactam and susceptibility to quinolones, colistin, imipenem and tobramycin. Tobramycin was also withdrawn and ciprofloxacin was added but treatment with the latter was stopped due to the suspicion of an allergic reaction. In the following three weeks, a new isolate of *P. aeruginosa* appeared with resistance to imipenem so antimicrobial therapy was changed to intravenous tobramycin (300 mg in a once-daily dose) and colistin (2 million IU t.i.d.). At this time, susceptibility to CT of this *P. aeruginosa* strain was tested by the Etest method, on agar plates incubated at 35ºC for 18 hours. MIC_{90} was determined to be 0.50 mg/L, which is considered susceptible based on the EUCAST guidelines. After a prolonged therapy with intravenous tobramycin and colistin, the patient experienced sudden dysnea followed by a respiratory arrest requiring advanced cardiopulmonary reanimation, orotracheal intubation and Intensive Care Unit (ICU) admission. Laboratory workup revealed acute kidney failure with high blood levels of creatinine (10.4 mg/dL) and urea (102 mg/dL), severe metabolic acidosis and hyperlactatemia (6.6 mmol/L). Supportive treatment was started with mechanical ventilation, vasoactive drugs for vasodilation and cardiogenic shock, and renal replacement therapy with continuous venovenous hemodiafiltration (CVVHDF). Given the situation of acute kidney failure, repeated isolation on wound cultures of multidrug-resistant *P. aeruginosa* and susceptibility to CT, treatment was started with this antibiotic at doses of 0.5/0.25 g t.i.d. with an infusion time of 1 h. The patient´s clinical condition improved, allowing extubation and withdrawal of

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renal support and vasoactive drugs. Microbiological response was also favourable without any side effects of CT and negative results of wound and surveillance samples during ICU stay, so the antimicrobial treatment was withdrawn after seven days. He was discharged of ICU after 12 days and transferred to a conventional ward and then to a Plastic Surgery Service of our referral hospital with good evolution of the wound of his leg.

CT is considered the beta-lactam antibiotic most active against *P. aeruginosa* [5-6] due to its stability in the presence of AmpC beta-lactamases, higher affinity for penicillin-binding proteins, resistance to active efflux pumps and is not affected by the loss of outer membrane porins. The experience on its use, both in off-label indications and in critically ill patients is scarce. We conducted a search in MEDLINE (PubMed) using the Mesh terms "ceftolozane-tazobactam" and "soft-tissue infections" with no results. In addition to pneumonia [3,4], there are only some successful reports of its off-label use in bone and joint infections due to multidrug-resistant *P. aeruginosa*: two patients with osteomyelitis, one secondary to a vesical fistula [7] and other with multiple isolation in blood, lung and sternum [8]; and in a prosthetic hip joint infection [9]. Given the low inoculum after drainage and prescribing drug information in patients with impaired renal function we used a reduced dosage of CT. There are limited data evidence about CT dosing in critically ill patients under continuous renal replacement therapies, but data obtained in previous investigations suggest a decreased CT clearance with increased area under the plasma concentration-time curve and low extraction ratio by the CVVHDF filter, so a lower total daily dose might be utilized and continuous or extended-time infusions may not be necessary [8,9].

In conclusion, CT is a safe and highly effective therapy in multidrug-resistant *P. aeruginosa*. It could also be an alternative to other antibiotics in cases of infections by these bacteria or other Gram-negative bacilli with kidney failure or at high risk of nephrotoxicity. It remains to establish the optimal dosing in critically ill patients under continuous extrarrenal clearance techniques, although reduced dosage could be used without extended-time infusions, other factors such as the type of infection, bacterial load, MIC of the causative agent or the ultrafiltration dose might cause us to modify these approximate guidelines in the future.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest

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Carta al Director

Exantema postvacunal en paciente con enfermedad relacionada con IgG4

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Sr. Editor: La vacunación en los pacientes inmunodeprimidos y/o en situaciones especiales representa, actualmente, una prioridad en salud. El incremento del uso de las terapias biológicas y la mejora del diagnóstico de las enfermedades autoinmunes hace que aumente la necesidad de inmunización en estos grupos [1].

La seguridad de las vacunas es un hecho contrastado y con una amplia evidencia en la literatura científica [2]. Los ensayos clínicos y los controles de calidad durante la fabricación hacen que se hayan convertido en fármacos muy seguros [3]. No obstante, al igual que con el resto de medicamentos la notificación a los Sistemas de Farmacovigilancia de cualquier sospecha de reacción adversa es clave [4].

Se presenta un caso de exantema tras la vacunación frente a triple vírica (sarampión, rubeola y parotiditis) en un paciente candidato a inmunosupresión:

Se trata de un varón de 24 años diagnosticado de enfermedad relacionada con IgG4. Desde el punto de vista histopatológico esta enfermedad se caracteriza por la aparición de infiltrado linfoplasmocitario denso, fibrosis estoriforme y flebitis obliterante [5]. Habitualmente esta patología precisa tratamiento inmunosupresor [6] por lo que resulta de interés actualizar el calendario de vacunación.

En el caso que se expone, el paciente fue derivado a la Unidad de Vacunas del propio centro para valoración.

Una vez allí, se realizó la historia clínica vacunal y serología basal. El paciente refería no haber sido inmunizado en la infancia por rechazo activo de sus padres y solamente recordaba vacunación antitetánica con motivo de un accidente doméstico. Recordaba haber cursado infección por el virus de la varicela.

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En la tabla 1 se presentan los resultados de la serología basal.

Se inició la actualización del calendario con las vacunas frente a gripe estacional, neumococo conjugada de 13 serotipos, *Haemophilus influenzae* tipo b y meningococo C, descartando la aplicación de vacunas atenuadas dado que en ese momento el paciente recibía dosis altas de corticoides (40 mg/día de prednisona).

La segunda consulta de vacunación tuvo lugar a los 4 meses de la primera. En ese momento, el paciente realizaba pauta descendente de corticoides y llevaba más de 30 días con dosis inferiores a 20 mg/día. Por ello, se decidió administrar la primera dosis de triple vírica [7], además de una dosis de vacuna inactivada de polio. Como en la primera visita, se informó sobre las posibles reacciones adversas relacionadas con las vacunas y los signos y síntomas de alarma por los que debía consultar.

A los 6 días postvacunación el paciente comenzó con fiebre >38ºC y malestar general, que aumentó progresivamente hasta la aparición de un exantema máculo-papular en cabeza

Tabla 1 **Serología basal (prevacunal).**

Antígeno	Determinación	Resultado
Varicela	IgG	Positivo
Sarampión	IgG	Negativo
Hepatitis B	antiHBs	Negativo
	antiHBc tot	Negativo
	HBsAg	Negativo
Hepatitis A	IgG	Negativo

IgG: inmunoglobulina G; antiHBs: anticuerpo de superficie frente al HBsAg; antiHBc tot: anticuerpo total frente a las proteínas del core; HBsAg: antígeno de superficie.

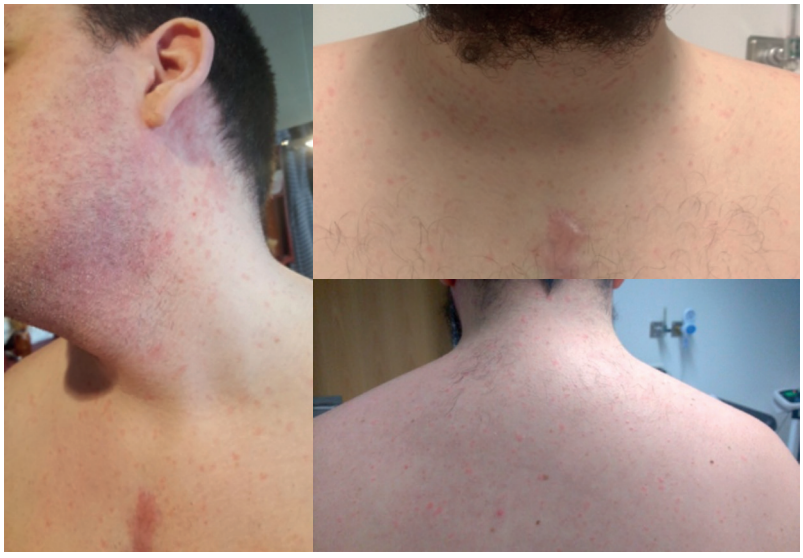

Figura 1 | **Exantema máculo-papular de predominio cefálico y tórax.**

y tórax el día 13 postvacunación (figura 1).

Ante cualquier episodio de exantema posterior a la administración de la vacuna triple vírica, es importante realizar un diagnóstico diferencial entre infección por virus salvaje o por virus vacunal. Según los protocolos de la Red Nacional de Vigilancia Epidemiológica, se considera que un caso de sarampión o rubeola es vacunal si hay *antecedente de vacunación en las 6 semanas previas al inicio del exantema, con IgM positiva y detección del genotipo vacunal* [8].

Con el fin de confirmar la implicación de los virus vacunales en el exantema se enviaron al Laboratorio de Virología un exudado faríngeo y una muestra de orina el mismo día de la aparición del exantema, (día 13) y una muestra de sangre a los 4 días del mismo (día 17). Posteriormente, se envió exudado faríngeo y orina de control a los 23 días. La figura 2 muestra la secuencia clínica y analítica.

Se confirmó la presencia de los virus sarampión y rubeola en el exudado faríngeo por amplificación genómica cuantitativa, con una carga viral de 5,2 y 4,3 log de copias del genoma del virus/10^3 células, respectivamente. La sangre, la orina y las muestras de control fueron negativas.

Para confirmar que el virus del sarampión implicado era una cepa vacunal, se amplificó un fragmento de 581 pb del gen de la nucleocápside utilizando cebadores diseñados en el propio laboratorio. Posteriormente, este fragmento se secuenció utilizando el kit "Big Dye Terminador v1.1" (Applied Biosystems, USA) y se analizó en el ABI PRISM 3700 DNA (Applied Biosystem, USA). La secuencia obtenida se comparó con las cepas del Gen Bank confirmando que se trataba de la cepa vacunal. No se investigó la presencia de un genotipo vacunal del virus de la rubeola por falta de disponibilidad de la técnica.

La evolución del paciente fue favorable y sin complicaciones. Se completó el calendario de vacunación con las vacunas inactivadas pendientes sin registrar incidencias y no se consideró la administración de la segunda dosis de triple vírica.

A pesar de ser numerosas las publicaciones relacionadas con el exantema posvacunal en niños y adultos sanos [9,10],

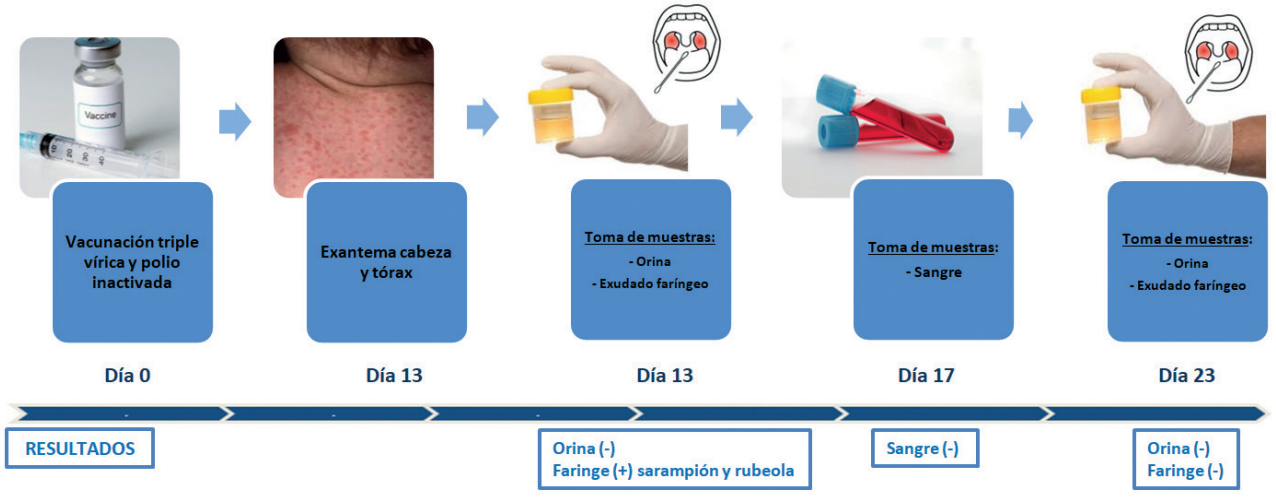

Figura 2 | **Secuencia clínica y analítica del caso.**

son escasas las de pacientes en situaciones especiales. Es importante mencionar que dado que el paciente fue informado sobre las reacciones adversas relacionadas con la vacunación, la identificación de los signos y síntomas por parte del propio paciente fue inmediata facilitando el diagnóstico y descartando, desde la aparición del exantema, una posible complicación de su enfermedad.

El caso fue notificado al Sistema de Farmacovigilancia (número 03-600307).

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CONFLICTO DE INTERESES

Los autores declaran no tener ningún conflicto de intereses.

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Carta al Director

Infección de marcapasos por *Mycobacterium neoaurum*

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Sr. Editor: El porcentaje de casos de infección por marcapasos y otros dispositivos cardíacos implantables se encuentra entre el 1 y 7% [1,2]. Los agentes etiológicos más comunes son las bacterias grampositivas, fundamentalmente *Staphylococcus* sp. Las bacterias gramnegativas representan el 30% de los casos y, excepcionalmente, están causadas por otros microorganismos [1]. Las micobacterias de crecimiento rápido (MCR) raramente causan infecciones de dispositivos cardíacos, siendo las micobacterias del grupo *fortuitum* las más frecuentes [1,3]. Estas infecciones pueden clasificarse en dos categorías: a) infección de la zona del generador y/o del trayecto subcutáneo de los electrodos (presentación más frecuente, producida generalmente durante la implantación del sistema); y b) infección de la porción transvenosa de los electrodos con o sin afectación del bolsillo del generador. La infección precoz depende de la contaminación intraoperatoria del dispositivo o del tejido donde se implanta [2].

Presentamos un caso de infección precoz de marcapasos por *Mycobacterium neoaurum*.

Mujer de 63 años, hipertensa e hipotiroidea en tratamiento con irbesartán/hidroclorotiazida 150 mg/12,5 mg (1 comprimido/día) y levotiroxina 50 µg (1 comprimido al día, 5 días/semana) y 75 µg (1 comprimido al día, 2 días/semana). Destaca en sus antecedentes personales, un cáncer de mama en remisión, tratado con quimioterapia y radioterapia hace 10 años.

Acude al Servicio de Urgencias presentando un cuadro de un mes de evolución de disnea y dolor en los miembros inferiores, con sensación de astenia y debilidad generalizada, acompañado de elevación de tensión arterial, sin dolor torácico, palpitaciones ni síncope. En la analítica destacó Hb 11,8 g/dL, leucocitos 13,70x10^3/µL (neutrófilos 67.4%) y NT Pro-BNP 4729,7 pg/mL (valores normales de 0 – 125 pg/mL). Tras la realización de un electrocardiograma, se objetiva bloqueo auriculoventricular completo, con escape de 34 latidos por minuto (lpm). Se decide el ingreso para colocación de marcapasos definitivo bicameral tipo Boston.

Cinco días después de la primera intervención, la paciente presenta un nuevo episodio de bradicardia de 32 lpm, con umbrales de estimulación muy altos y fallos de captura, acompañado de disnea y debilidad general. En la radiografía de tórax se objetiva un desplazamiento del electrodo ventricular derecho hacia el anillo. Ante esto, se decide realizar una nueva intervención para la recolocación del electrodo (en el ápex del ventrículo derecho) e implantación de marcapasos definitivo, abriéndose el bolsillo subcutáneo para la extracción del generador. Al día siguiente de esta segunda intervención, la paciente cursó con un episodio de fiebre (T=38,7ºC), por lo que, tras la extracción previa de hemocultivos, se pautó durante 10 días linezolid (600 mg cada 12 horas vía oral) y cefepima (2 g cada 12 horas vía IV). Presentó un episodio de dolor costal izquierdo que se relacionó con una contracción muscular, se acentuó por la noche como dolor centro-torácico tipo opresivo con sensación de ahogo. A raíz de ello, se realizaron nuevos estudios electrocardiográficos en tres ocasiones, objetivándose tensión arterial de 146/68 con frecuencia cardíaca de 48 lpm, 126/54 con 48 lpm en el segundo control; y 96/53 con 37 lpm en el último. La paciente continuó con dolor torácico a punta de dedo, con dolor a la palpación superficial y limitación respiratoria. Durante este período de 48 horas tras la segunda intervención quirúrgica, continuó con fiebre entre 38,4ºC y 39,8ºC, además de la sintomatología descrita, recogiéndose nuevos hemocultivos. Se decide reintervenir por tercera vez, tres días después de la segunda intervención, abriéndose de nuevo el bolsillo de marcapasos y recolocándose el electrodo ventricular dirigido a una nueva localización (septo ventricular). Tras el último procedimiento, la paciente permaneció estable y evolucionando favorablemente. La herida presentó buen aspecto, sin sangrado ni hematoma.

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En el 19º día de ingreso, se retiran las grapas de la herida quirúrgica en la zona precordial del bolsillo después de 11 días tras su colocación. En este momento se presenta un exudado seroso amarillento que se recoge para estudio microbiológico. Finalmente, se retiran las tres últimas grapas y la paciente es dada de alta al 20º día de ingreso con levofloxacino 500 mg cada 24 horas vía oral durante 10 días.

La muestra del exudado de herida quirúrgica se sembró en los medios sólidos habituales y en caldo de enriquecimiento (tioglicolato). Tras 72 horas de incubación, el cultivo en medio sólido resultó negativo. No obstante, al 10º día de incubación del caldo de enriquecimiento, se observó turbidez del medio líquido, por lo que se realizó una siembra en los medios sólidos. A las 48 horas se observó en agar Sangre y en agar Chocolate, el crecimiento de pequeñas colonias de aspecto dorado y apariencia suave y brillante (figura 1).

La tinción de Gram mostró bacilos grampositivos finos y cortos, y en la tinción de Ziehl-Neelsen se observaron bacilos ácido-alcohol resistentes. Mediante espectrometría de masas MALDI-TOF (Bruker®), se identificó como *M. neoaurum* con un score de 1.969.

La cepa se envió al Centro Nacional de Microbiología (ISCIII, Majadahonda, Madrid), donde se llevaron a cabo estudios moleculares para confirmar la identificación de la micobacteria, mediante el método de análisis de polimorfismo de fragmentos de restricción del gen *hsp*65 (PRA-*hsp*65) [4,5] y mediante secuenciación del gen 16S ARNr.

Para el estudio de sensibilidad antibiótica se testaron los siguientes antimicrobianos mediante Etest® (bioMérieux): amoxicilina/ácido clavulánico (CMI = 0,38 mg/L, sensible), cefoxitina (CMI = 2,0 mg/L, sensible), ciprofloxacino (CMI = 0,06 mg/L, sensible), levofloxacino (CMI = 0,016 mg/L, sensible), imipenem (CMI = 0,19 mg/L, sensible), tobramicina (CMI = 1,0 mg/L, sensible), claritromicina (CMI = 0,125 mg/L, sensible), tetraciclina (CMI = 1,0 mg/L, sensible), linezolid (CMI = 1,5 mg/L, sensible), moxifloxacino (CMI = 0,02 mg/L, sensible) y trimetoprim/sulfametoxazol (CMI = 32,0 mg/L, resistente).

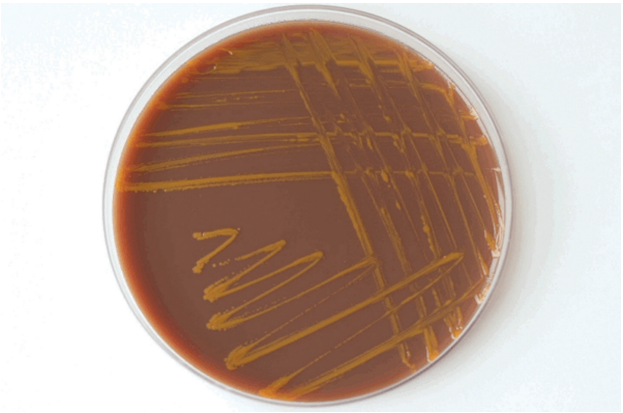

Figura 1 | **Cultivo en agar Chocolate de *M. neoaurum* a las 48 horas de incubación.**

Dos semanas tras el alta, y habiendo completado el tratamiento con levofloxacino, se le realiza un control para valorar el aspecto de la herida, que está cerrada y sin dolor a la palpación. Teniendo en cuenta el riesgo de adherencia al cable del dispositivo por parte del microorganismo, y para evitar otras complicaciones, se decide ampliar la cobertura antibiótica durante un mes con doxiciclina (100 mg cada 12 horas vía oral) y levofloxacino (500 mg cada 24 horas vía oral) como tratamiento definitivo. Una vez finalizado el tratamiento, se revalora el aspecto de la herida, cerrada y sin persistencia de recidiva.

Actualmente hay seis complejos de MCR. *M. neoaurum* es una especie incluida en un complejo junto con *M. canariasense*, *M. cosmeticum*, *M. monacense* y *M. bacteremicum* [6]. Se aisló del suelo y fue descrita por primera vez en 1972 (Japón) [7]. El primer caso de infección humana tuvo lugar en 1987 (Australia), presentándose como una bacteriemia relacionada con catéter en una paciente con cistadenocarcinoma de ovario metastásico. Esta micobacteria se encuentra de forma ubicua en suelo, agua y en otras superficies [3,8]. Es capaz de sobrevivir ante duras condiciones ambientales como temperaturas extremas y pH muy bajos [8]. Su alta hidrofobicidad le confiere capacidad para formar biofilms, lo que explica la adherencia a dispositivos como catéteres o implantes cardíacos [8-11]. Raramente se asocia a infecciones humanas [8], y solamente se ha registrado un caso de infección de marcapasos por *M. neoaurum*, el cual fue publicado en el año 2015 por Hayton et al [11] (tabla 1).

Las MCR están cobrando importancia como potenciales patógenos en las infecciones asociadas a dispositivos intravasculares y cardíacos [11]. *M. abscessus*, *M. fortuitum* y *M. mucogenicum* representan las especies más frecuentemente aisladas en estos casos [9,11].

La infección relacionada con la implantación de un dispositivo cardíaco eléctrico se presenta como infección en el lugar de inserción del mismo [10]. La implantación del generador o de los electrodos pueden producir lesiones en la piel adyacente y, debido a la posibilidad de contaminación del sistema a través de las mismas, implica que se tenga que actuar como si se tratara de una infección a dicho nivel [2]. A pesar de su baja virulencia, la tasa de mortalidad para infecciones asociadas a implantes de dispositivos eléctricos cardíacos o infecciones valvulares puede ascender al 25% [10]. Entre los factores de riesgo inherentes al paciente se encuentran procesos como malnutrición, neoplasias, diabetes mellitus, enfermedades cutáneas crónicas, y tratamientos prolongados de corticoides, inmunosupresores y anticoagulantes. La duración prolongada de la cirugía y reintervenciones para el recambio del generador, así como hemorragias del bolsillo, son factores relacionados con la aparición de infección [2].

Las manifestaciones clínicas de estas infecciones son muy variadas y dependen de diferentes variables como el momento de su aparición, localización y el agente etiológico responsable [2]. Sin embargo, en las diferentes series publicadas en las que una MCR estaba implicada, los pacientes presentaron una

Tabla 1 | **Características epidemiológicas de la infección de marcapasos por *Mycobacterium neoaurum*.**

Nº de pacientes	Edad / Sexo	Diagnóstico	Factores de Riesgo	Tratamiento	Evolución	Referencia
1	80 / F	Infección de marcapasos	Marcapasos permanente desde >9 años (reemplazado 7 años después), asma	Imipenem y amikacina durante 23 días, doxiciclina y ciprofloxacino durante 3 meses con linezolid el primer mes Retirada del marcapasos y colocación de uno nuevo 6 meses después de completar el tratamiento antibiótico	Favorable	Hayton et al. 2015 [11]
1	63 / M	Infección de marcapasos	Hipertensión, hipotiroidismo, cáncer de mama en remisión tratado con quimioterapia y radioterapia hace 10 años	Doxiciclina durante 30 días y Levofloxacino durante 40 días	Favorable	Caso presentado

sintomatología inespecífica, con fiebre de origen desconocido, y ausencia de signos locales de infección en algunos casos [11,12]. Para establecer el diagnóstico, es necesaria la extracción de hemocultivos y la obtención de muestras de exudado local. Se debe descartar la presencia de endocarditis mediante ecocardiografías (ETT y ETE), así como realizar radiografías de tórax para descartar una afectación pulmonar [2].

La identificación bioquímica de *M. neoaurum* resulta insuficiente para caracterizar de forma definitiva a la micobacteria. La espectrometría de masas constituye una alternativa en los laboratorios de rutina que proporciona una identificación fiable más rápida y sencilla, si bien no resulta totalmente adecuada para la distinción definitiva de las diferentes especies incluidas en este grupo, y siendo preciso recurrir a métodos moleculares confirmatorios [6].

A pesar de que los antibiogramas y las recomendaciones terapéuticas no han sido estandarizados de forma global, diferentes estudios consideran como terapia óptima el uso combinado de al menos dos antimicrobianos, tanto en el tratamiento empírico como definitivo, así como la retirada del dispositivo implantado [1,2,10,11,13].

Las infecciones por MCR suponen un reto a la hora de establecer un diagnóstico etiológico debido a la escasa sospecha clínica y que requiere prolongar el tiempo de incubación de los cultivos procedentes de muestras de piel y tejidos blandos.

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CONFLICTO DE INTERESES

Los autores declaran no tener ningún conflicto de intereses.

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Letters to the editor

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Osteoarticular tuberculosis of the hip and soft tissues: images of a diagnostic delay

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Sir,

A 54 year-old male patient, born in Ecuador, without any previous disease had been suffering from left hip pain and lameness that did not allow him to do his job as a farmer. Three cutaneous fistulas were persistently suppurating a caseous material, whose samples had been initially sent for bacterial culture and had yielded a coagulase-negative *Staphylococcus* that was treated with several antibiotics including fluoroquinolones. He had no fevers, sweats or gain loss. Three-years after his initial consult, the patient was re-evaluated with a hip X-ray, hip CT-scan and Tc99m-scintigraphy. They showed a destructive infectious process affecting the left femur and acetabular cavity with loss of bone substance and collections in the thigh root (figure 1A, B, and C). A surgical biopsy was performed and sent for bacterial culture (negative) and-mycobacterial culture. This last study allowed highlighting the presence of acid alcohol fast bacilli (AAFB) in the Ziehl-Neelsen (ZN) method and detection of the presence of DNA of *Mycobacterium tuberculosis* through nucleic acid amplification test (NAAT) (Fluorotype MTB, Hain Lifescience). Mutations of resistance to isoniazid and rifampicin were not detected by the technique Genotype MTBDRplus (Hain Lifescience). The antibiogram performed in the system BD Bactec MGIT 960 (Becton Dickinson) showed sensitivity for the five antibiotics tested: streptomycin (STR), isoniazid (INH), rifampin (RIF), ethambutol (EMB), and pyrazinamide (PZA).

The patient started to be treated with 4 first-line antituberculosis drugs (INH, RIF, PZA and EMB). A chest-CT scan was performed and a 15 mm upper-left lobe nodule with central calcification was found, consistent with a tuberculous granuloma (figure 1D). The patient had no respiratory symptoms so no sputum for culture could be collected. An aggressive surgery was performed: resection arthroplasty and surgical debridement of abscesses. The patient is still under antituberculosis treatment with rifampicin and isoniazid (27 months to day). Antituberculosis treatment has not yet been stopped because there was a delay in surgical treatment, all caseous material could not be removed and osteomyelitis focuses could have remained after surgery. Despite a positive clinical, analytical and radiological evolution after surgery, it has not been so fast as to ensure the microbiological cure with a standard treatment course.

Osteoarticular tuberculosis accounts for 10-11% of all extrapulmonary tuberculosis and 1-3% of all tuberculosis diagnoses [1]. Tuberculous arthritis is the second cause of osteoarticular tuberculosis after vertebral tuberculosis. Tuberculous arthritis usually affects joints that support large weight such as the hip or knee, and is usually a monoarticular condition. The delay in the diagnosis can be related to the assumption that the skin fistula colonizer is a colonizing fungus or bacteria, as in our case. In these cases a biopsy is mandatory in order to send samples for mycobacterial culture and PCR, in addition to routine bacterial cultures [2]. Diagnostic of osteoarticular tuberculosis can be challenging so a high index of suspicion is necessary and using deep biopsies not only for bacterial cultures, but also for mycobacterial cultures and PCR could help reducing delays in diagnosis [3]. Despite major advances in the diagnosis of mycobacterial infections, microscopic examination for AAFB remains a primary tool because it identifies infections with positive-AAFB smear cases. However, it is of less utility for paucibacillary disease such as in pediatric patients, HIV and those with extrapulmonary disease. Mycobacterial culture has a high sensitivity and is the test of choice for the microbiological diagnosis of tuberculosis. Although the specificity of NAAT is very high, sensitivities vary widely and depend on both the AAFB smear status of the specimen (positive or negative) and the origin of the sample. The results take 1-2 days. Its use is indicated when the degree of suspicion is

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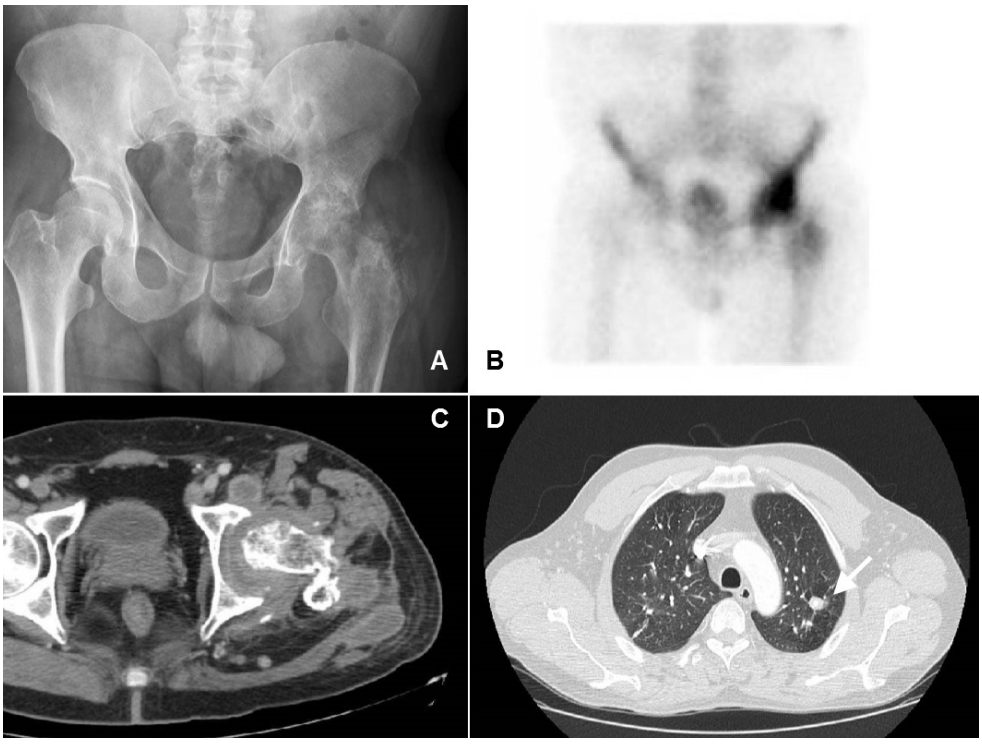

Figure 1 | **(A) Hip X-ray indicating destruction of the left proximal femur and loss of the joint space. (B) Tc99m-scintygraphy revealing chronic inflammation in acetabulum and femoral head. (C) Destruction with loss of bone density in the left proximal femur and collections in the thigh root in a hip CT-scan. (D) Chest CT-scan revealing an upper left lobe 15 mm nodule with central calcification, consistent with a tuberculous granuloma (arrow).**

moderate to high [4]. A few studies have shown the effectiveness of NAAT in the diagnosis of extrapulmonary tuberculosis, in which the reported sensitivities ranged from 87.3% to 97 % (positive ZN) to 68% (negative ZN) and the specificity was over 97% [4]. Early determination of the identity and sensitivy of the mycobacteria is very important not just from a public health and epidemiological perspective, but also to guide the treatment and management [5].

Current Spanish consensus document about the treatment of tuberculosis recommend a 9-month standard treatment for tuberculous spondylitis and there is no specific pattern for tuberculous osteoarthritis of other location [6]. In addition, there is no optimal duration of therapy for patients with bone and soft tissue tuberculosis in which all caseous material cannot be removed with surgery and all studies about antituberculous treatment duration for osteoarticular tuberculosis have been performed in spinal tuberculosis. Some authors recommend to extent antituberculous treatment beyond the recommended 6-9 months in patients with a significant burden of disease, like in our case [2,7]. A logical approach would be to assess the effectiveness of the treatment by periodic blood tests and a CT-scan. PET-CT could be a useful tool to monitor the effectiveness of the treatment, and there is an increasing evidence about it [8–10]. In any case, it is not usually necessary to use such a long treatment pattern and most cases could benefit from a shorter treatment.

We believe that this may be an illustrative case of this extrapulmonary manifestation of tuberculosis, with serious consequences in terms of severity of infection and subsequent physical disability.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

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None to declare

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