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Update on pathogenesis and diagnosis of intravascular catheter-related infections

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ABSTRACT

Infections associated with the use of intravascular catheters cause a substantial morbidity and mortality. New knowledge in the pathogenesis of catheter related bloodstream infections has led to advances in the prevention and management of these infections. The purpose of the present chapter is to review the most relevant data published recently on pathogenesis and diagnosis of intravascular catheter-related infections. It is focus in four different aspects: *a)* pathogenesis of catheter-related infections and particularly factors affecting biofilm formation and modulation; *b)* pathogenesis of intravascular catheter-related infections caused by *Staphylococcus lugdunensis*; *c)* news on microbiological diagnosis of catheter-related bacteremia; and *d)* evaluation of current use of blood cultures in the era of continuous monitoring blood cultures systems.

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Actualización en la patogénesis y el diagnóstico de las infecciones relacionadas con el uso de catéteres intravasculares

Palabras clave:
Catéter intravascular
Biopelícula
Hemocultivo
Staphylococcus

RESUMEN

Las infecciones asociadas al uso de catéteres intravasculares producen una importante mortalidad y morbilidad. El avance en los conocimientos de la patogenia de las bacteriemias asociadas a su uso nos permitirá desarrollar estrategias adecuadas para su cuidado y prevención. El propósito de esta revisión es destacar los aspectos más relevantes publicados en la literatura científica sobre la patogenia y diagnóstico de las infecciones asociadas a catéteres intravasculares, desarrollando 4 aspectos de interés: *a)* la patogenia de las infecciones asociadas a catéteres intravasculares; *b)* la patogenia de estas infecciones producidas por *Staphylococcus lugdunensis; c)* las novedades en el diagnóstico microbiológico de estas infecciones, y *d)* el uso de hemocultivos en la era de los sistemas de monitorización continua de éstos.

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Introduction

Infections associated with the use of intravascular catheters represent around 10-20% of all nosocomial infections and caused a substantial morbidity and mortality[1]. More than 250,000 intravascular catheter-related bacteremia and fungemia (CR-BSI) occur annually in the USA with a attributable mortality of 12%-25%[2]. New knowledge in the pathogenesis of CR-BSI has led to advances in the prevention and management of these infections[3]. The purpose of this review is to summarize the most relevant data published in the last year on diagnosis and pathogenesis of intravascular infections and particularly those associated with intravascular catheters.

Update on pathogenesis of intravascular catheter-related infections.

The ability of *Staphylococcus epidermidis* to adhere to the surface of a prosthetic biomaterial is a pivotal event in the pathogenesis of biomaterial-related infection. Adherence of *S epidermidis* to biomaterials is a multifactorial process. Fibrinogen-binding protein

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is clearly involved in the pathogenesis of foreign-body infections caused by *Staphylococcus aureus*. Recently, a fibrinogen-binding protein termed Fbe was identified in *S. epidermidis* strains[4]. Guo et al have evaluated the involvement of Fbe from *S. epidermidis* in the pathogenesis of CR-BSI[5]. An *fbe* (gene encoding Fbe protein) mutant was constructed by allelic replacement, wherein an erythromycin resistance gene replaced a portion of the A region of *fbe*. A rat central venous catheter (CVC) infection model was established to assess the importance of Fbe in the pathogenesis of CVC-associated infection due to *S. epidermidis*. A Fbe-positive *S. epidermidis* strain was significantly more likely to cause CVC- associated infection resulting in bacteremia and metastatic diseases than its isogenic Fbe-deficient mutant (100% versus 20%; P <.01). The results obtained in this study confirm the relevance of adherence associated with Fbe in the pathogenesis of CVC-associated infections caused by *S. epidermidis*.

After the adherence of bacteria to the surface of biomaterials, the later stages of biofilm formation are characterized by multicellular aggregation, which is facilitated through the production of polysaccharide intracellular adhesin (PIA). The role of PIA in early adherence is less characterized and its influence on adherence to orthopaedic biomaterials is unexplored. In a study recently published by Olson et al an in vitro adherence assay to ascertain the adherence of genetically-characterized strains of *S. epidermidis* (wild-type *S epidermidis* strain 1457, strain 1457 M10 isogenic icaA *S. epidermidis* mutant, and a strain of *S. carnosus*, as a negative control) to seven biomaterials (zirconia, ultra-high molecular weight polyethylene (UHMWPE), polymethylmethacrylate (PMMA), cobalt chromium, titanium, silastic intravenous catheters and stainless steel) was developed[6]. The surface area of the biomaterial samples was determined in order to standardize adherence measurements and compare one biomaterial to another. PIA positive *S epidermidis* wild type strain exhibited greater adherence (P <.05) to all biomaterials tested (except titanium and PMMA) compared to the PIA negative *S epidermidis* mutant strain. *S epidermidis* wild type strain exhibited substantially greater adherence (P <.01) to all tested biomaterials compared to the negative control and also *S epidermidis* mutant showed greater adherence (P <.05) to the metallic biomaterials (titanium, stainless steel, cobalt chromium) than the negative control strain. The authors recognized the potential limitations of their contribution as the pathogenesis of prosthetic device related infections is a complex process involving interactions between the pathogen, the biomaterial and the host. This study suggests the critical role of polysaccharide intercellular adhesion in initial adherence of *S epidermidis* to different biomaterials. These findings increased the known functions of PIA, which includes multicellular aggregation, antibiotic resistance and immune evasion. Further studies are warranted to describe the importance of PIA in *in vivo* models of foreign body infections.

Staphylococcus aureus and coagulase-negative staphylococci are the microorganisms most frequently associated with CR-BSI, due to their ability to attach to catheter surfaces and form biofilms. Biofilms bacteria exhibit an altered physiologic state that promotes evasion of host immune defenses and decreases susceptibility to antimicrobial agents at conventional therapeutic levels. For instance, antibiotic concentrations should be 100 to 1,000 times greater to kill sessile bacteria within a biofilm than for planktonic bacteria. Sodium metabisulfite (SMBS) is an inorganic sulfite commonly used as an antioxidant in numerous cosmetic products and several pharmaceuticals, but few studies have examined its antimicrobial properties against human pathogens. Frank et al evaluate the susceptibility of planktonic and sessile bacteria to SMBS[7]. The effect of SMBS on biofilm formation for 3 staphylococcal species: *S. epidermidis* ATCC 35984, *S. aureus* ATCC 35556 and *S. lugdunensis* is also evaluated. All three strains are icaA positive, indicating that are genetically capable of forming biofilm trough at least one well characterized mechanism of biofilm formation. SMBS exhibited bactericidal activity at concentrations of 512, 512 and 1,024 mg/L against *S. aureus*, *S. lugdunensis* and *S. epidermidis*, respectively. A concentration of 720 mg/L inhibited cell growth of all species in a biofilm formation assay. According to these in vitro results, the use o SMBS as a catheter lock may inhibit staphylococcal colonization of catheters, thereby preventing CR-BSI. However, clinical trials would be necessary to definitively determine the safety and efficacy of SMBS for prevention of catheter infections in humans.

Staphylococcus aureus has the capacity to bind to fibronectin and other serum components that coat central venous catheters. In addition, it uses adhesion-receptor interactions to bind to endothelial cells, and is particularly attracted to areas of catheter-related vascular injury. Finally, the clumping factor expressed by the organism appears to make it inherently thrombogenic. Crowley et al have reported a remarkable 71% incidence of associated venous thromboses in a series of patients with CR-BSI due to *S. aureus*[8,9]. In addition, they described how poorly the physical examination is able to distinguish which patients have such thromboses. Ultrasound of the previously catheterized vein, then, should be performed in all patients with CR-BSI due to *S. aureus* before these patients can be considered for a short duration of antimicrobial therapy (i.e., a minimum of 14 days of therapy)[10].

Pathogenesis of catheter-related infections caused by *S. lugdunensis*. Modulation of biofilm formation

Staphylococcus lugdunensis is a coagulase-negative staphylococcal species and has been found to be associated with several types of infection as native valve endocarditis, prosthetic joint infection and intravascular catheter infection. Unlike other coagulase-negative staphylococci, *S lugdunensis* its associated infections tend to mimic those of *S aureus* on the basis of their highly destructive and potential serious nature.

Frank et al have evaluated the production and composition of biofilms in clinical isolates of *S. lugdunensis*[11]. The following aspects were addressed: *a)* classic methods of qualitative and quantitative detection of slime (silicone discs and polystyrene plates) and evaluation of the modulation of its production with glucose, ethanol (0-5-4%) and NaCl (1-5%); *b)* molecular methods - the detection of genes involved in slime formation in other species of Staphylococci (*S. epidermidis*, *S. aureus*); and *c)* the detection of poly-N-acetylglucosamine (PNGA) and other polysaccharides in stationary phase strains and in the preformed slime.

The strains showed different capacities for slime formation. No correlation between the origin of the strain and the degree of slime formation was demonstrated. There was a greater formation of slime in the presence of glucose and a lower degree of formation with NaCl and ethanol. Homologues of the *icaADBC* (PNAG) gene were characterised in all strains. No PGNA was detected in the biofilm, which was only susceptible to the action of proteases.

Despite the presence of the *icaADBC* gene, the biofilm formed by *S. lugdunensis* is essentially of proteinaceous nature. It is necessary to ascertain which genes are responsible for the adherence and formation of slime in this species and to determine its regulation.

Staphylococci are a major cause of CR-BSI because of their ability to form biofilms on indwelling polymeric devices. Changes in environmental conditions are known to affect staphylococcal biofilm formation, and it has been reported that intravenously infused drugs like catecholamine inotropes and heparin, stimulate biofilm formation by some staphylococci. *S. lugdunensis* is responsible for biofilm-related infections, but factors that influence biofilm formation by this species have not been determined. Frank et al evaluated the effects of catecholamines and heparin on biofilm formation of a collection of *S. lugdunensis* isolates and other staphylococcal species[12]. Using a microtiter plate biofilm formation assay, dopamine stimulated biofilm formation in two-thirds of *S.*

lugdunensis isolates, whereas dobutamine prevented nearly all *S. lugdunensis* isolates from adhering to polystyrene. The effect of catecholamines on biofilm formation by other species was variable and was dependent on the concentrations of the drugs and on the strain. Heparin markedly reduced biofilm formation by 87% of *S. lugdunensis* isolates. Preformed biofilms of *S. lugdunensis, S. aureus, S. epidermidis* and a *Staphylococcus carnosus* strain that produces the biofilm matrix polysaccharide poly-*N*-acetylglucosamine/polysaccharide intercellular adhesin detached from polystyrene after exposure to heparin at concentrations used in catheter locks. A statistically significant amount of biofilm detachment by treatment with 3,600 U/mL heparin was achieved with 85% (17/20) of the organisms tested. These data suggest that intravenous pharmaceuticals may influence staphylococcal biofilm formation on and detachment from intravascular catheters, enabling biofilm cells to disperse, to travel through the bloodstream and to colonize additional sites. Although this study demonstrates that exposure to dobutamine and heparin prevented or reduced biofilm formation by *S. lugdunensis* at concentrations that contact the catheter lumen after intravenous administration, other authors have demonstrated stimulation of *S. aureus* biofilms in response to heparin[13]. Differences in growth media, the type of surface used, and incubation time may contribute to variations in the biofilm formation abilities of individual strains and species. Future studies are needed in order to examine the effect of cathecolamines and heparin on biofilm formation and detachment under physiologic conditions.

The same group of scientists have evaluated the activity of both inhibitory and subinhibitory concentrations of ten antistaphylococcal drugs (cefazolin, daptomycin, linezolid, moxifloxacin, nafcillin, rifampin, quinupristin-dalfopristin, tetracycline, trimethoprim-sulfamethoxazole and vancomycin) against planktonic bacteria and biofilms of clinical isolates of *S. lugdunensis*[14]. In order to assess the antimicrobial susceptibility of *S. lugdunensis* biofilms, a modified protocol originally designed for determining the antimicrobial susceptibility of *P aeruginosa* biofilms was used. The effect of subinhibitory concentrations of antimicrobial agents on biofilm formation was measured by using the conventional microtiter plate biofilm assay. Planktonic isolates were susceptible to all agents tested but the biofilm resistance profiles were not uniform. Several antimicrobial agents assayed produced varied responses in this test. Moxifloxacin was able to kill 11 of 15 isolates growing in biofilms at <0.5 mg/L. The most pronounced and pervasive effect occurred with nafcillin, which significantly increased biofilm formation, in terms of change in cell density, in 14 of 15 (93%) of *S. lugdunensis* isolates, whereas tetracycline and linezolid significantly decreased biofilm formation in 14/15 (93%) and 12/15 (80%) of isolates, respectively. An expected outcome of MBC testing was the observation that vancomycin was not bactericidal against 93% of *S. lugdunensis* isolates, suggesting widespread vancomycin tolerance in this species. The results of the present study suggest that the heterogeneity of the biofilm response displayed by individual *S. lugdunensis* isolates may result from differential gene regulation patterns utilized among strains living in biofilms. In our opinion the high rate of vancomycin tolerance among *S. lugdunensis* isolates is an observation that justifies *in vivo* studies of vancomycin against *S. lugdunensis* biofilm infections. The present study identified several conditions that warrant further studies to delineate whether the observed effects are of clinical relevance.

Diagnosis of intravascular catheter-related infections.

The diagnosis of intravascular CR-BSI is a matter of intense clinical research. The accuracy of many microbiological methods generates important debates among experts and no clear consensus about the gold standard has been established. Additionally the variability in definitions used for years has not facilitated the understanding of the different studies. Nevertheless, in the last year a few studies have been published providing data and conclusions of interest in this matter.

Rapid information to clinicians within 24 h of suspecting an episode of bloodstream infection is critical for antimicrobial choice or modification of therapeutic approaches, and for morbidity and mortality[1]. Rapid diagnosis of CR-BSI by direct catheter-tip staining may help physicians to choose antimicrobial agents and to rule out the catheter as the cause of sepsis immediately after withdrawal.

Bouza et al[15] described a prospective evaluation of direct Gram and acridine orange staining of the external surface of central venous catheters for rapid detection of catheter-associated infection, obviating the need for awaiting conventional bacterial culture results. The results were compared with the semiquantitative culture method. Both staining techniques were randomly assigned to be performed either before (group A) or after (group B) rolling on the agar plate. Of the 425 catheter tips processed, 25.7% were significantly colonized and 6.1% were from patients with CR-BSI. The yield of group A was superior to that of group B for the prediction of colonization (sensitivity, 94.3%/69.6%; specificity, 92.4%/96.2%; positive predictive value (PPV), 80.6%/86.7%; negative predictive value (NPV), 98.0%/90.0%; and full concordance, 91.9%/89.3%). The values for the prediction of CRBSIs were as follows for group A and group B: sensitivity, 100% vs. 69.2%; specificity, 96.3% vs. 100%; PPV, 86.7% vs. 100.0%; NPV, 100% vs. 93.9%; and full concordance, 97.0% vs. 94.7%.

In this study, Bouza et al, in agreement with previous studies, showed the superiority of sensitivity of acridine orange staining (84,6%) over Gram staining (73,1%) for the detection of CRBSI. The staining procedure is easy to perform, is inexpensive, and can be applied to all types of catheter tips. However, the introduction of this method in microbiology laboratory routine is time consuming and only catheters from patients with suspected bacteremia should be studied by this staining method.

This same group compared prospectively 3 techniques (paired quantitative blood cultures, differential time to positivity (DTP), and superficial cultures) for the diagnosis of CR-BSI without catheter withdrawal in adult patients without neutropenia who had short-term central venous catheters and were admitted to intensive care units[16]. The authors studied 204 episodes of suspected CR-BSI, and 28 of the episodes were confirmed to be CRBSI.

The reference standard for the evaluation of the 3 conservative methods for diagnosis of CR-BSI was paired quantitative blood cultures with positive results (colony count ratio of ≥5:1) and/or conventional cultures of peripheral blood and semiquantitative catheter-tip culture positive for the same microorganism (≥15 colony-forming units).

In a recent meta-analysis, Safdar et al[17] evaluated 8 methods for the diagnosis of CR-BSI and concluded that, among the techniques studied, paired quantitative blood culture was the most accurate test (sensitivity, 0.87 [95%CI, 0.83-0.91]; specificity, 0.98 [95%CI, 0.97-0.99]). The data from Bouza et al are consistent with this meta-analysis. They reported that paired quantitative blood cultures with a quotient of ≥5 represented the best specificity (97.7%) in the diagnosis of CR-BSI and had a sensitivity of 71.4% , a positive predictive value of 83.3%, a negative predictive value of 95.6%, and an accuracy of 94.1%. Paired quantitative blood culture methods are now regarded as the reference standard for the diagnosis of CRBSI if catheter removal is undesirable. However, this method is not routinely used in clinical practice because of their cost and complexity.

Blot et al[18], by using a continuous blood culture–monitoring system, have described a new method that is based on DTP of qualitative blood cultures drawn simultaneously from both a catheter hub and a peripheral vein. The authors found that cultures of catheter-drawn blood that yielded positive results ≥120 min earlier than cultures of peripheral blood were accurate in detecting CR-BSI

(specificity, 91%; sensitivity, 94%). Although findings by other investigators have shown lower sensitivities and specificities of DTP than those found by Blot et al, the results published in this issue by Bouza et al (sensitivity, 96.4%; specificity, 90.3%; positive predictive value, 61.4%; negative predictive value, 99.4%; and accuracy, 91.2%) are comparable to those reported by Blot and colleagues. Because many laboratories now use automated continuous blood culture–monitoring systems, they will be able to use these systems for the diagnosis of CR-BSI by the DTP. This method is easier to perform and is less costly than the paired quantitative blood cultures method, and has recently shown to be a practical and feasible diagnostic method of CR-BSI in cancer patients in a real practice setting[19]. However, the major disadvantage of culture of blood collected through a central venous catheter for the diagnosis of CR-BSI is a higher rate of contamination, compared with that for culture of blood obtained through a peripheral venipuncture.

Although the paired quantitative blood cultures and the DTP methods have excellent sensitivity and specificity, the contribution of the study by Bouza et al[16] is to demonstrate that the surveillance skin and hub cultures performed well for diagnosis of CR-BSI (sensitivity, 78.6%; specificity, 92.0%; positive predictive value, 61.1%; negative predictive value, 96.4%) in adult patients with short-term catheters who were admitted to intensive care units. The authors did not find differences in accuracy between the 3 techniques that they evaluated.

A possible limitation of the study by Bouza et al is that, as part of the reference standard definition of CR-BSI, the authors used catheter-tip culture performed according to the roll-plate semiquantitative method, and microorganisms that colonized the internal lumen of the catheter could be not recovered. However, these authors have previously demonstrated that quantitative techniques of sonication or vortexing were not superior to the semiquantitative method[20].

Bouza et al recommend a realistic approach for routine diagnosis of CR-BSI in clinical microbiology laboratories by means of the combination of semiquantitative superficial cultures and conventional peripheral blood cultures. These methods are easy to perform and low in cost. In addition, if Gram staining of swabs of the skin insertion site and the inner surface of the hub is performed, this could provide a rapid and inexpensive conservative method for the diagnosis of CRBSI. Negative results of Gram staining and culture of both superficial swabs could practically rule out the catheter as the source of infection, thereby avoiding many unnecessary catheter removals. Further studies of this method for the diagnosis of CR-BSI, including Gram staining and semiquantitative cultures of hubs and skin insertion sites, are needed. Nevertheless, in cases in which CR-BSI is suspected, a prudent attitude must be taken, because a small percentage of CR-BSIs due to hematogenous seeding yield superficial cultures with negative results.

An important limitation of DTP is that laboratory personnel should be available 24 h a day to process blood samples. Unfortunately, a 24-hour microbiological laboratory service is not feasible in most hospitals. Schwetz et al[21] investigated in vitro whether storage of blood samples influences the time to positivity used for the calculation of DTP and the results of the Gram stain-acridine orange leukocyte cytospin (AOLC) test. They concluded that 24-h storage of blood samples at room temperature may lead to false-negative DTP and false-positive Gram satin-AOLC test results, whereas storage at 4ºC does not. They concluded that in daily clinical practice, blood samples drawn for the DTP test and/or the Gram stain-AOL test that cannot be processed within 8 h should be stored at 4ºC to obtain correct results in suspected cases of CR-BSI.

Recent practice guidelines for the diagnosis of CR-BSI describe as an "unresolved issue" the number of lumens from which blood culture specimens should be drawn to make a conservative diagnosis of CRBSI. Bouza et al have recently revised how many CRBSI episodes would be missed if not all catheter lumens were sampled[22]. They performed a retrospective study (1 January 2003-31 May 2009) in patients with microbiologically proven CR-BSI in which all available catheter lumens (those that did not contain clots) were used to draw blood culture samples. They calculated the number of episodes that would have been missed in double- and triple-lumen catheters if the culture of samples obtained from 1 lumen had been eliminated. They studied 171 episodes of proven CRBSI in 154 patients. Overall, if 1 lumen-associated culture had been eliminated for both double-lumen and triple-lumen catheters, 27.2% and 15.8% of episodes of CRBSI, respectively, would have been missed. If they had eliminated 2 cultures for triple-lumen catheters, 37.3% of episodes would have been missed. These authors conclude that samples for blood culture should be obtained through all catheter lumens to establish a diagnosis of CRBSI.

Candida albicans CR-BSI are difficult to treat and often require removal of the device for cure, although this may be technically difficult and associated with significant risk. Moreover, in many cases *C. albicans* infections are associated with biofilms that can be up to 1000-fold more resistant to antifungal agents, making the infections very difficult to control[23]. Under these circumstances, the ability to identify a biofilm device infection before catheter removal may obviate unnecessary removals of catheters. Nett et al demonstrated that cell wall changes (specifically, increased β-1,3 glucan) was associated with biofilm, in contrast with planktonic *C. albicans*, and used two catheter biofilm models (in vitro and in vivo) to determine whether biofilm cells secreted more β-1,3 glucan and whether these differences could be used to discern the presence of a *Candida* biofilm infection with 3 species (*C. albicans, C. glabrata*, and *C. parapsilosis*)[24]. A commercialized limulus lysate assay was used to quantify β-1,3 glucan in supernatants from planktonic or biofilm cultures and in the serum of rats with an intravascular catheter biofilm infection or disseminated candidiasis. The secreted polysaccharide β-1,3 glucan was detected from both in vitro and in vivo models from each condition. However, the concentrations of β-1,3 glucan from the biofilm condition were 4-10 fold greater in vitro (P <.001) and were 10-fold greater in vivo (P <.001), despite equal or fewer numbers of cells in the biofilm conditions. The magnitude of the difference varied by species. Biofilm infection with *C. glabrata* was associated with the highest serumβ-1,3 glucan content. The *C. parapsilosis* and *C. albicans* biofilm infections produced 2-8 fold higher serum β-1,3 glucan concentration, compared with the respective disseminated models. The authors conclude that β-1,3 glucan may serve as a useful tool for the diagnosis of *Candida* biofilm and device-associated infections. If the β-1,3 glucan is a biofilm marker for *Candida*, patients with candidemia and a negative biofilm test would be able to avoid device removal. However, this is only an experimental study and requires further investigation of the assay in patients with *Candida* biofilm infections in order to determine whether this assay may be able to diagnose a biofilm or device-associated infection in a heterogeneous patient population.

Current use of blood cultures in the era of continuous-monitoring blood culture systems (CMBCSs)

Conventional blood cultures are still highly useful for diagnosis of bloodstream infections included CR-BSI. Studies reported in the 1970s established that two to three blood cultures from adults obtained during a 24-h period detect >99% of all bloodstream infections[25]. The number of blood cultures when continuous-monitoring blood culture systems are used (CMBCSs) is still controversial. Although several reports have shown that two to three 20-ml blood cultures are adequate for the detection of bacteremia an fungemia in adults, a recent study[26] found that two blood cultures detected only 80% of bloodstream infections and that three blood cultures detected 96% of episodes. Lee et al have reviewed the data at two university hospitals to confirm this finding[27]. They assessed all

blood cultures obtained from adult inpatients in 2004 and 2005. All instances in which ≥3 blood cultures per patient were obtained during a 24-h period were included and the clinical significance of positive blood cultures was determined. Of 629 monomicrobial episodes with ≥3 blood cultures obtained during a 24-h period 73.1% were detected with the first blood culture, 89.7% with the first two blood cultures and 99.8% with the first three blood culture. Of 351 monomicrobial episodes with ≥4 blood cultures, 96.9% were detected with the first three blood cultures and 99.7% with the first four blood cultures. The authors conclude that two blood cultures in a 24-h period will detect approximately 90% of bloodstream infections in adults and a third blood culture set may be needed. The previous held axiom that virtually all bloodstream infections can be detected with two blood cultures may no longer be valid but may also depend on the definition of the "first" blood culture obtained that differs in several studies.

"The higher the volume of blood cultured the higher the yield of blood cultures" has been a well-accepted dictum since J. A. Washington II performed his classic work[25]. This rule has not been questioned in the era of highly automated blood culture machines, nor has it been correlated with clinical variables. Bouza et al[28] made a prospective analysis of the relationship between blood volume, the yield of blood cultures, and the severity of clinical conditions in adult patients with suspected bloodstream infections (BSI).

The prospective study was carried out during a period of 6 months. They randomly selected one of every two episodes of significant BSI (patients) and approximately the same number of episodes of sepsis with negative blood cultures (controls). They included in the study only episodes with three sets of blood cultures drawn, which is the official recommendation. In the case of microorganisms of doubtful significance (*Bacillus* spp., nonhemolytic *Streptococcus* spp., *Propionibacterium acnes*, *Corynebacterium* spp., *Clostridium* spp., and coagulase-negative *Staphylococcus* spp.), they considered to be clinically relevant only those with evidence of clinical manifestations of infection not explained by other causes, in which the microorganisms were isolated in two or more different blood cultures. For the few patients with recurrent bacteremia, only the first episode was included.

Overall, 298 patients with significant BSI and 303 patients with sepsis and negative blood cultures were studied. The mean volume of blood cultured in patients with BSI (30.03 ± 14.96 ml [mean ± standard deviation]) was lower than in patients without BSI (32.98 ± 15.22 mL [*P* <.017]), and more episodes of bacteremia were detected with <20 mL (58.9%) than with >40 mL (40.2%) of blood cultured (*P* <.022). When patients were stratified according to the severity of their underlying condition, patients with BSI had higher APACHE II scores, and higher APACHE II scores were related to lower sample volumes (*P* <.001). A multivariate analysis showed that in the group of patients with APACHE II scores of >18, higher volumes yielded higher rates of bacteremia (odds ratio [OR]: 1.04 per ml of blood; 95% confidence interval [CI], 1.001-1.08). They conclude that the higher yield of blood cultures inoculated with lower volumes of blood reflects the conditions of the population cultured. Washington's dictum holds true.

Another recent study[29] tried to determine whether health care professionals are aware of the current evidence-based recommendations for blood culture collection. An anonymous survey of employees qualified to collect blood cultures was conducted in an urban tertiary care facility. Surveys were distributed to and collected from health care professionals across all shifts. The survey was conducted (July-October 2004) throughout the patient care areas of the emergency department, medical and surgical intensive care units, and general medical and surgical wards. The survey asked "What volume of blood should be collected in one bottle for a blood culture?".

Of the 360 employees of the hospital surveyed, 355 returned evaluable answers for blood culture volume collected. Overall, 79% (95%CI, 74%-83%) answered less than 10 mL, and 44% (95%CI, 39%-49%) answered less than 5 mL of blood. When examined by occupation, 90% (95%CI, 86%-94%) of nurses, 97% (95%CI, 91%-100%) of technicians, and 55% (95%CI, 46%-64%) of physicians answered less than 10 mL; 52% (95%CI, 45%-59%) of nurses, 63% (95%CI, 46%-79%) of technicians, and 26% (95%CI, 18%-35%) of physicians answered less than 5 mL. Of all respondents, 21% (95%CI, 17%-25%) answered 1 mL or less.

They findings reveal that a high percentage of health care personnel do not know the optimal volume of blood recommended for collection. Because volume remains the most important determinant for the optimal yield of organisms, these findings raise an important quality assurance issue.

The study has some limitations. This is a single-center study, and blood culture collectors and practice may vary, depending on the hospital system. The fact that the hospital system currently recommends collection of 5 mL rather than 10 mL per bottle may cause some confusion. They attempted to compensate for this controversial departure by reporting results based on both the 5-mL and the 10-mL standard.

The matter of the volume of blood is particularly controversial in pediatric patients. Connell et al[30] has developed an study to determine the volume of blood submitted for culture in routine practice and to establish the proportion of blood cultures with a blood volume inadequate for reliable detection of bacteremia. The volume of blood samples submitted for culture from pediatric patients (up to 18 years) was measured over a 6-month period. Blood cultures were deemed adequate submission if the contained an appropriate (age-related) volume of blood and were submitted in the correct bottle type. During the study, an educational intervention designed to increase the proportion of adequate blood culture submission was undertaken. A total of 1,358 bottles from 783 patients were analyzed. In the preintervention period, 46.0% of bottle contained an adequate blood volume and only 35.4% were adequate submission on the basis of selection of the correct bottle. After the intervention, these figures were 63.9% and 51.2%, respectively. Overall, blood cultures with an adequate blood volume were more likely than those with a an inadequate volume to yield positive blood cultures (5.2% versus 2.1%) and adequate submission were more likely than inadequate submission to yield positive blood cultures (5.1% versus 2.8%). They conclude than in routine clinical practice, a negative blood culture result is almost inevitable for a large proportion of blood cultures because of the submission of an inadequate volume of blood.

Because of the decrease in the incidence of anaerobic bacteremia demonstrated in several studies, a debate about whether and when anaerobic blood cultures should be routinely performed has been ongoing for >15 years. In a study by Lassman et al[31], the authors report the reemergence of anaerobic bacteremia at the Mayo Clinic (Rochester, USA). They compared 2 periods (1993-1996 vs. 2001-2004) and observed a mean incidence of 53 cases per year during the first period, in comparison to 91 cases per year during the second, which implies an overall increase of 74% in the incidence of positive blood culture results for obligate anaerobes per 100,000 patient-days, which was highly significant (*P* <.001). In addition, there was a 30% increase in the number of positive anaerobic blood culture results per 1000 cultures performed during the 12-year period (*P* =.002), with a proportional decrease in positive culture results for aerobic and facultative anaerobic organisms. Organisms from the *Bacteroides fragilis* group, other species of *Bacteroides*, and *Clostridium* species were the most commonly isolated. These results are in contrast to the 45% decrease in the incidence of anaerobic bacteremia during 1974-1988, which was reported from the same institution. The authors consider several factors as the cause for this increasing trend, including a change in culture media and type, a potential shift in the distribution of organisms, and underlying clinical conditions. Although none of those factors reached statistical significance, the

authors conclude that the increasing number of patients with complex underlying diseases could be the cause for the increase of the frequency of anaerobic bacteremia, and recommend that anaerobic blood cultures should be routinely performed in medical centres with a patient population similar to theirs. There are several reasons why laboratories should employ routine anaerobic blood cultures: the increasing frequency of antibiotic resistant and/or highly virulent anaerobic organisms, worse outcomes when an appropriate antibiotic is not given, the isolation of unusual anaerobic species, the high degree of association between anaerobic bacteremia and immunosuppressed patients, and the lack of predictability of the "typical" clinical contexts for anaerobic bacteremia due to changes in demographic characteristics of patients during recent years. These data have been confirmed in another study developed in France by Grohs et al[32]. Using the BacT/Alert automated system, they conducted a 1-year retrospective study on blood cultures focusing on the relevance of routine use of anaerobic bottle. The rate of patients with positive blood cultures was 19.7%. Among these, 13.5% had a positive anaerobic bottle in the absence of any aerobic bottle, and 2/3 of these grew with non-obligate anaerobes. These patients were hospitalized in 20 out of 26 wards of the hospital. For 65.4% of the monomicrobial-positive blood cultures growing Enterobacteriaceae, the anaerobic bottle detected growth earlier than in the corresponding aerobic bottles. The authors suggest that the use of anaerobic bottle is still relevant in their institution.

The incubation time for blood cultures is still a matter of debate. Unexplained fever in children with CVCs may be a sign of CR-BSI; usually the children receive antibiotics until bacteremia is ruled out. When can blood cultures for such children be considered sterile and antibiotics be stopped? Investigators recently conducted a retrospective cohort study at the Children's Hospital of Philadelphia to answer this question[33]. The researchers reviewed data relating to children who presented to the ambulatory services between 2000 and 2003 with a CVC and suspected infection, whose blood culture from the CVC grew a recognized pathogen. All blood cultures had been incubated using the BacT/Alert system, which monitors for microbial growth every 10 min. Two hundred patients (median age, 5.5 years) with a variety of underlying conditions and CVC types were selected for study. Median time to blood-culture positivity was 14 h. The probability of a positive culture, after adjustment for age, catheter type, and recent antibiotic use, was 85.2% at 24 hours and 96.6% at 36 hours. Culture positivity was faster for bloodstream infections caused (entirely or in part) by Gram-negative bacteria than for those caused by other pathogens. Only 10 cultures became positive after 48 hours, and 8 of these grew organisms unlikely to be pathogens. The authors suggest that in clinically stable children, discontinuation of empirical antibiotic treatment may be warranted if blood cultures are negative after 24–36 hours of incubation. Today, many children with suspected CVC-associated bacteremia receive empirical antibiotics for longer periods (usually 48 or 72 h), but continuously monitored automated blood-culture systems may permit more-rapid detection of bacteria than did earlier systems. A weakness of this study is that peripheral blood cultures were not frequently obtained in patients with CVCs and were not studied.

Conflict of interest

The authors declare they have not any conflict of interest.

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