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## Sonication did not provide reliability to Maki technique for catheter related bloodstream infection diagnosis

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### ABSTRACT

**Objective.** The aim of our study was to analyze sonication and Maki techniques for diagnosis of catheter tip colonization and catheter-related bloodstream infection (CRBSI) on patients admitted to ICU.

**Material and methods.** Observational and prospective study in one Intensive Care Unit. Patients with some central venous catheter (CVC) at least for 7 days and catheter-related infection (CRI) suspicion (new episode of fever or sepsis) were included. We performed Maki technique followed by sonication of catheter tip. We compared area under the curve (AUC) of Maki, sonication, and techniques combination to diagnosis catheter tip colonization and CRBSI.

**Results.** We included 94 CVC from 87 CRI suspicion episodes. We found 14 cases of catheter tip colonization and 10 cases of CRBSI. Of the 14 catheter tip colonization cases, 7 (50.0%) were detected by Maki and sonication techniques, 6 (42.9%) were detected only by Maki technique, and 1 (7.1%) was detected only by sonication technique. Of the 10 CRBSI, 6 (60.0%) were detected by Maki and sonication techniques, 4 (40.0%) were detected only by Maki technique, and any only by sonication technique. We found higher AUC in Maki technique than in sonication technique to diagnosis of CRBSI ( $p=0.02$ ) and to diagnosis of catheter tip colonization ( $p=0.03$ ). No significant differences were found in AUC between Maki technique and combination techniques for diagnosis of catheter tip colonization ( $p=0.32$ ) and of CRBSI ( $p=0.32$ ).

**Conclusion.:** Sonication did not provide reliability to Maki technique for diagnosis of catheter tip colonization and CRBSI.

**Keywords:** Sonication, Maki, colonization, bloodstream infection

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### La sonicación no proporciona rentabilidad a la técnica de Maki para el diagnóstico de bacteriemia relacionada con catéter

#### RESUMEN

**Objetivo.** El objetivo de nuestro estudio fue analizar las técnicas de sonicación y Maki para el diagnóstico de la colonización de la punta del catéter y la bacteriemia relacionada con el catéter (CRBSI) en pacientes ingresados en UCI.

**Material y método.** Estudio observacional y prospectivo en una Unidad de Cuidados Intensivos. Se incluyeron pacientes con algún catéter venoso central (CVC) insertado al menos durante 7 días y sospecha de infección relacionada con el catéter (IRC) (nuevo episodio de fiebre o sepsis). Se realizó técnica de Maki y posteriormente sonicación de la punta del catéter. Comparamos áreas bajo la curva (AUC) de Maki, sonicación y combinación de técnicas para el diagnóstico de colonización de la punta del catéter y de CRBSI.

**Resultados.** Se incluyeron 94 CVC de 87 episodios de sospecha de IRC. Encontramos 14 casos de colonización de la punta del catéter y 10 casos de CRBSI. De los 14 casos de colonización de la punta del catéter, 7 (50,0%) fueron detectados por Maki y técnicas de sonicación, 6 (42,9%) fueron detectados solo por la técnica de Maki y 1 (7,1%) fue detectado solo por la técnica de sonicación. De los 10 CRBSI, 6 (60,0%) fueron detectados por técnicas de Maki y sonicación, 4 (40,0%) fueron detectados solo por la técnica de Maki, y ninguno solo por la técnica de sonicación. Encontramos mayor AUC con Maki que en la sonicación para el diagnóstico de CRBSI ( $p=0.02$ ) y para el diagnóstico de colonización de la punta del catéter ( $p=0.03$ ). No encontramos diferencias significativas en AUC entre Maki technique y combinación de técnicas para el diagnóstico de CRBSI ( $p=0.32$ ) y para el diagnóstico de colonización de la punta del catéter ( $p=0.32$ ).

**Conclusiones.** La sonicación no proporcionó rentabilidad a la técnica de Maki para el diagnóstico de colonización de la punta del catéter y CRBSI.

**Palabras clave:** Sonicación, Maki, colonización, bacteriemia.

## INTRODUCTION

The use of a central venous catheter (CVC) may be needed due to different motives, such as the administration of fluids, blood products, parenteral nutrition, medications, or the monitoring of hemodynamic status. However, the use of those devices has different risks such as catheter related bloodstream infection (CRBSI), which leads to an increase of morbidity, mortality and assistant costs [1-4].

The semiquantitative technique of Maki et al is considered the reference standard to demonstrate catheter tip colonization due to its simplicity [5]. However, a potential disadvantage lies is that as it consists in rolling the catheter tip across the agar then could detect microorganism of external catheter tip surface but could not detect microorganism of internal catheter tip surface. Thus, Maki's technique could give false negative of catheter tip colonization for patients with colonization by an endoluminal mechanism. The possible superiority of quantitative techniques (sonication and vortexing) to catheter tip colonization diagnosis in respect to Maki technique lies of their potential ability to detect catheter tip colonization by exoluminal and also by endoluminal mechanism [6-9]. However, all quantitative methods are time-consuming and due to this its use has not widespread established in clinical microbiology laboratories.

There are scarce data about the reliability comparison between Maki's semiquantitative technique and sonication quantitative method for detection of CRBSI [10-13]. Some studies concluded that Maki and sonication methods exhibited similar reliability [10-12] and in one study was found the potential benefit of sonication jointly with Maki method [13].

Recent guidelines for the diagnosis of intravascular catheter-related infection (CRI) recommended that semiquantitative catheter culture by Maki technique and quantitative catheter segment culture by sonication have the same strength of the recommendations and quality of the evidence, which is of A-II [14,15].

Previous studies analyzing sonication and Maki techniques have included CVC from any patient admitted to the hospital and CVC removed due to any motive [10-13]. However, there has been not analyzed sonication and Maki techniques including only CVC from patients admitted to ICU, and CVC removed for catheter-related infection (CRI) suspicion after at least 7 days with that CVC. Therefore, the novel objective of our study was to analyze sonication and Maki techniques including only CVC from patients admitted to ICU, in whom CVC was removed for CRI suspicion, and remained at least 7 days with that CVC.

## MATERIAL AND METHODS

**Design and subjects.** A prospective and observational study was carried out between June 2020 and March 2021 after the approval by the Institutional Ethic Review Board of the Hospital Universitario de Canarias (Tenerife, Spain). The requirement of

written informed consent was waived due to the patient visits prohibition by the public health policy of Spanish Government in the COVID-19 pandemia context and due to the only change of our daily clinical practice by the study was the sonication technique (which is a procedure for CRBSI diagnosis that is internationally accepted).

We included patients admitted to ICU and removing CVC for CRI suspicion after at least 7 days with that CVC. CRI suspicion was established when a patient developed a new episode of sepsis or fever. We defined sepsis according to Sepsis-3 Consensus criteria of 2016 [16]. We considered fever when temperature was  $\geq 38^{\circ}\text{C}$ .

**Variables recorded.** We recorded the following variables for each patient: Sex, age, admission diagnostic, diabetes mellitus, asthma, chronic liver disease, smoking, chronic obstructive pulmonary disease (COPD), human immunodeficiency virus, hematological tumor, solid tumor. Also, we registered the use of renal replacement therapy, parenteral nutrition, corticosteroids or immunosuppressive therapy previously to admission. In addition, we recorded the use of corticosteroids, immunosuppressive therapy, parenteral nutrition, propofol or renal replacement therapy at moment of CRI suspicion. Finally, we also registered site of CVC, time of CVC, and death at 30 days.

**Sample collections.** The following samples were collected from each patient: paired blood samples, catheter-tip and other clinical samples. Paired blood samples were taken from peripheral vein, with 10 ml blood sample in each one and separated by 15 minutes. Catheter-tip sample was taken after scrubbing the skin surrounding the insertion site with 2% chlorhexidine and cutting off the tip (distal 5-cm segment) using sterile scissors. First, we performed catheter-tip culture using the Maki's technique and then sonication. Maki's semi-quantitative technique was performed by rolling each catheter tip to a blood agar plate [5]. Sonication quantitative technique was performed by placing small fragments of catheter tip in 1 mL of brain-heart infusion broth, then vortexing, sonicating for 1 min (at 35 000 Hz and 125 W), and vortexing for 15 seconds. Finally, 0.1 mL of the sonicated broth was streaked onto sheep blood agar plates [13]. Patients without blood culture, Maki's technique and sonication technique were excluded of the analysis.

**Definitions.** European Centre for Disease Prevention and Control (ECDC) criteria were used to define infections [17]. We considered catheter-tip colonization as a significant growth of a microorganism on the CVC tip by the semi-quantitative method of Maki et al ( $\geq 15$  colony-forming units) [5] or by the quantitative method of sonication ( $\geq 100$  colony-forming units) [13]. CRBSI was defined as a positive blood culture by recognized pathogen, CVC tip colonization with the same microorganism and no other apparent infection source. We defined bloodstream infection of unknown origin (BSIUO) as bloodstream verified during survey and no source found. Primary bloodstream infection (PBSI) includ-

ed CRBSI and BSIUO; therefore, some PBSI had a positive CVC tip colonization (by a semi-quantitative or quantitative method) and others not.

**Statistical analysis.** We reported categorical variables as frequencies and percentages, and continuous variables as medians and percentiles 25–75. We used chi-square test to compare categorical variables between group, and Mann-Whitney T test to compare continuous variables. Concordance between Maki and sonication techniques for the diagnosis of catheter

tip colonization and CRBSI were determined using Cohen's Kappa test, and the percentages of agreement and disagreement between both techniques were calculated. We carried out receiver operating characteristic (ROC) analyses to diagnosis of catheter tip colonization and of CRBSI by Maki, sonication and combination of both techniques. Comparison of area under the curve (AUC) of ROC curves was carried out using the method of DeLong et al. [18]. We considered a difference as statistically significant when p-values were <0.05. We carried out statistical analysis with SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

<b>Table 1</b>		<b>Characteristics of CVC developing or not catheter-related bloodstream infection (CRBSI) and developing or not primary bloodstream infections (PBSI).</b>				
Data	Non CRBSI (n=84)	CRBSI (n=10)	P-value CRBSI vs non	Non PBSI (n=71)	PBSI (n=23)	P-value PBSI vs non
Time of CVC (days); median (p 25-75)	9 (7-12)	10 (9-13)	0.31	9 (7-12)	9 (8-12)	0.75
Site of CVC; n (%)			0.71			0.83
Subclavian	18 (21.4)	3 (30.0)		15 (21.1)	6 (26.1)	
Jugular	45 (53.6)	4 (40.0)		37 (52.1)	12 (52.2)	
Femoral	21 (25.0)	3 (30.0)		19 (26.8)	5 (21.7)	
Age; years (p 25-75)	65 (54-72)	64 (52-71)	0.74	64 (54-72)	64 (52-72)	0.80
Sex female; n (%)	23 (27.4)	0	0.11	20 (28.2)	3 (13.0)	0.17
Admission diagnostic; n (%)			0.38			0.07
Medical	63 (75.0)	9 (90.0)		51 (71.8)	21 (91.3)	
Surgical	14 (16.7)	0		14 (19.7)	0	
Traumatology	7 (8.3)	1 (10.0)		6 (8.5)	2 (8.7)	
Diabetes mellitus; n (%)	23 (27.4)	4 (40.0)	0.47	23 (32.4)	4 (17.4)	0.20
Renal replacement therapy previously to admission ;n (%)	3 (3.6)	1 (10.0)	0.37	2 (2.8)	2 (8.7)	0.25
COPD; n (%)	10 (11.9)	0	0.59	7 (9.9)	3 (13.0)	0.70
Asthma; n (%)	4 (4.8)	1 (10.0)	0.44	3 (4.2)	2 (8.7)	0.59
Chronic liver disease; n (%)	4 (4.8)	0	0.99	4 (5.6)	0	0.57
Smoking; n (%)	14 (16.7)	1 (10.0)	0.99	11 (15.5)	4 (17.4)	0.99
Parenteral nutrition previously to admission; n (%)	1 (1.2)	0	0.99	1 (1.4)	0	0.99
Corticosteroids previously to admission; n (%)	3 (3.6)	0	0.99	3 (4.2)	0	0.99
Immunosuppressive therapy previously to admission; n (%)	4 (4.8)	1 (10.0)	0.44	4 (5.6)	1 (4.3)	0.99
Hematological tumor; n (%)	0	1 (10.0)	0.11	0	1 (4.3)	0.25
Solid tumor; n (%)	1 (1.2)	0	0.99	1 (1.4)	0	0.99
Human Immunodeficiency Virus; n (%)	1 (1.2)	0	0.99	1 (1.4)	0	0.99
Corticosteroids at sepsis; n (%)	12 (14.3)	0	0.35	8 (11.3)	4 (17.4)	0.48
Immunosuppressive therapy at sepsis; n (%)	2 (2.4)	0	0.99	2 (2.8)	0	0.99
Parenteral nutrition at sepsis; n (%)	14 (16.7)	2 (20.0)	0.68	10 (14.1)	6 (26.1)	0.21
Propofol at sepsis; n (%)	34 (40.5)	4 (40.0)	0.99	31 (43.7)	7 (30.4)	0.33
Renal replacement therapy at sepsis; n (%)	7 (8.3)	1 (10.0)	0.99	7 (9.9)	1 (4.3)	0.67
Deaths at 30 days; no. (%)	23 (27.4)	3 (30.0)	0.99	20 (28.2)	6 (26.1)	0.99

CVC = central venous catheter; COPD = Chronic Obstructive Pulmonary Disease

	Maki +	Maki -	Total
Sonication +	7/6	1/0	8/6
Sonication -	6/4	80/84	86/88
Total	13/10	81/84	94/94

## RESULTS

We included 94 CVC from 87 patients with CRI suspicion. We found 23 PBSI, 10 (43.5%) were CRBSI and 13 (56.5%) were BSUO. We no found significant differences between group of CVC developing CRBSI (n=10) and no developing it (n=84) in rate of death ( $p=0.99$ ), time of CVC, site of CVC, and in other variables (Table 1). Neither we found significant differences between group of CVC developing PBSI (n=23) and no developing it (n=71) in rate of death ( $p=0.99$ ), time of CVC, site of CVC, and in other variables (Table 1).

We found 14 cases of catheter tip colonization of which 10 were cases of CRBSI. Of the 14 catheter tip colonization cases, 7 (50.0%) were detected by Maki and sonication techniques, 6 (42.9%) were detected only by Maki technique, and 1 (7.1%) was detected only by sonication technique (Table 2). Of the 10 CRBSI, 6 (60.0%) were detected by Maki and sonication techniques, 4 (40.0%) were detected only by Maki technique, and any only by sonication technique (Table 2).

The AUC to diagnosis of CRBSI was for Maki technique of 98% (95% CI = 93%-99%;  $p<0.001$ ), by sonication technique of 79% (95% CI = 69%-87%;  $p<0.001$ ) and by techniques combination of 98% (95% CI = 92%-99%;  $p<0.001$ ). We found higher AUC in techniques combination than in sonication technique ( $p=0.02$ ) and in Maki technique than in sonication technique ( $p=0.02$ ) to diagnosis of CRBSI. No significant differences were found in AUC between Maki technique and combination techniques ( $p=0.32$ ).

The AUC to diagnosis of catheter tip colonization was for Maki technique of 96% (95% CI = 90%-99%;  $p<0.001$ ), by sonication technique of 79% (95% CI = 69%-86%;  $p<0.001$ ) and by techniques combination of 100% (95% CI = 96%-100%;  $p<0.001$ ). We found higher AUC in techniques combination than in sonication technique ( $p=0.002$ ) and in Maki technique than in sonication technique ( $p=0.03$ ) to diagnosis of catheter tip colonization. No significant differences were found in AUC between Maki technique and combination techniques ( $p=0.32$ ).

The agreement between Maki and sonication techniques for catheter tip colonization was 92.6%, and Maki technique showed 1/94 (1.1%) false negatives (Cohen's Kappa: 0.63 (95% CI: 0.38-0.88);  $P< 0.001$ ) The agreement between Maki and sonication techniques for CRBSI was 95.7%, and Maki technique showed 0/94 false negatives (Cohen's Kappa: 0.73 (95% CI: 0.48-0.98);  $P< 0.001$ ).

We found that *Staphylococcus epidermidis* was the most frequent microorganism responsible of catheter tip colonization (Table 3) and CRBSI (Table 3).

## DISCUSSION

Previous studies analyzing Maki and sonication method for the diagnosis of catheter tip colonization have included CVC from any patients admitted to the hospital and CVC removed due to any motive [10-13]. Some of those studies concluded that Maki and sonication methods exhibited similar reliability [10-12] and in one study was found the potential benefit of sonication jointly with Maki method [13]. In the study by Guembe et al [13] were included 252 CVCs and the authors found a colonization rate of 14.3% (36/252) and a CRBSI rate of 5.9% (15/252). Of the 36 CVC colonizations, 21 (58.3%) were detected by Maki and sonication, 6 (16.7%) only by Maki technique, and 9 (25.0%) only by sonication technique. Of 15 CRBSI, 11 cases (73.3%) were detected by Maki and sonication, and 4 cases (26.7%) only by sonication technique [13]. The authors concluded that both techniques are complementary and they recommended sonicating fragments

Microorganism	Total	Both techniques positives	Maki positive only	Sonication positive only
<i>Staphylococcus epidermidis</i>	8/5	1/1	6/4	1/0
<i>Enterococcus faecalis</i>	1/1	1/1	0/0	0/0
<i>Escherichia coli</i>	1/1	1/1	0/0	0/0
<i>Klebsiella spp.</i>	2/2	2/2	0/0	0/0
<i>Enterobacter cloacae</i>	1/1	1/1	0/0	0/0
<i>Pseudomonas aeruginosa</i>	1/0	1/0	0/0	0/0
TOTAL	14/10	7/6	6/4	1/0

of patients with bacteremia of unknown origin and a negative catheter tip culture by the Maki technique [13].

We only found one catheter tip colonization by sonication that was not detected by Maki technique, and this colonization was not responsible of CRBSI. We found higher AUC in Maki technique than in sonication technique for diagnosis of catheter tip colonization and of CRBSI, and no significant differences were found in AUC between Maki technique and combination techniques for diagnosis of catheter tip colonization and of CRBSI. Thus, in our study, the use of sonication no added any rentability in the diagnosis of CRBSI by Maki technique.

The different results obtained between Gembe et al [13] and our study would be explained because in that study, CVC were collected from a general population (which included ICU and non-ICU adult patients) and CVC had different catheter duration (short and long-term). However, in our study CVC were collected from ICU adult patients and were mainly short term (which have mainly an extraluminal colonization). As sonication is more reliable to detect intraluminal colonization (which appears over all in long-term catheters), it may have no impact at all in the present study, which only included CVC from ICU adult patients, that were mainly short term, which most would be most detected by Maki technique.

Recent guidelines for CRI diagnosis recommended that semiquantitative catheter culture by Maki technique and quantitative catheter segment culture by sonication have the same strength of the recommendations and quality of the evidence [4,15]. We think that the greater simplicity of Maki's semiquantitative technique, the results of our study and the results of other studies makes Maki procedure as the technique of choice for routine work in the microbiology laboratory, and that the use of sonication technique did not provide profitability to the Maki technique for the diagnosis of CRBSI. Skin-colonizing microorganisms (as coagulase-negative staphylococci) are more likely to colonize the external surface of catheter and are the most isolated microorganism in the series, and this fact would explain the absence of profitability of sonication in ICU patients.

Some limitations must be recognized in our study. First, we have not taken other quantitative techniques (as vortexing) to compare its profitability for CRBSI diagnosis with Maki technique and sonication. Second, we have not reported what proportion of CVC were excluded due to have not all culture (blood, Maki technique and sonication technique). Third, sonication was performed after Maki technique in all catheter tip; thus, Maki technique could cause a great loose of microbial load (as bacteria were already discharged by Maki) and sonication would be in disadvantage. Fourth, the sample size of our study could be relatively low; however, it was enough to find that higher AUC in techniques combination than in sonication technique and in Maki technique than in sonication technique for diagnosis of catheter tip colonization and of CRBSI. The sample size to find higher significant AUC in techniques combination than in Maki technique was of 220 CVC for diagnosis of catheter tip colonization and of 5,235 CVC for diagnosis CRBSI.

The novel aspect of our study was that we analyzed sonication and Maki techniques including only CVC from patients admitted to ICU, in whom CVC was removed for CRI suspicion, and remained at least 7 days with that CVC. In our study, sonication did not provide reliability to Maki's technique for CRBSI diagnosis.

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## CONFLICTS OF INTEREST

All the authors state that they have no conflicts of interest

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