

Predictive Performance of Gram Staining of Catheter Tips for *Candida* Catheter-Related Bloodstream Infections

Gen Yamada,^{1,✉} Noriko Iwamoto,^{1,✉} Masahiro Ishikane,¹ Ataru Moriya,² Masami Kurokawa,² Kazuhisa Mezaki,² and Norio Ohmagari^{1,✉}

¹Disease Control and Prevention Center, National Center for Global Health and Medicine, Tokyo, Japan, and ²Clinical Laboratory Department, National Center for Global Health and Medicine, Tokyo, Japan

We analyzed 2462 episodes of suspected catheter-related bloodstream infection (CRBSI). The sensitivity, specificity, and positive and negative predictive values for detecting *Candida* CRBSI by gram staining of catheter tips were 66.1%, 99.4%, 84.4%, and 98.4%, respectively. Gram staining may be useful for the early detection of *Candida* CRBSI.

Keywords. CRBSI; *Candida*; catheter tips; gram stain; prediction.

Candida species are important pathogens causing catheter-related bloodstream infections (CRBSIs), accounting for 10%–27% of all cases [1, 2]. Compared with culture tests, gram staining of catheter tips provides more rapid information. Furthermore, it has high specificity for predicting catheter colonization and CRBSI [3, 4]. However, previous studies analyzed microorganisms generally as causative organisms of CRBSIs and included only 7 fungal cases. Thus, the usefulness of gram staining of catheter tips for detecting *Candida* CRBSIs (C-CRBSIs) in clinical practice remains unclear. Thus, we aimed to investigate the predictive performance of gram staining of catheter tips for C-CRBSIs in patients with suspected CRBSIs.

METHODS

Patient Consent

This was a retrospective observational study with secondary use of routine medical records conducted following the Declaration of

Helsinki and was approved by the ethics review committee of the National Center for Global Health and Medicine (approval number: NCGM-S-004402-00). Patients were allowed to withdraw consent for the use of their records for research. Information for opting out of this study is available on the hospital website.

Study Population, Design, and Data Collection

All patients admitted to the National Center for Global Health and Medicine (tertiary care hospital in Tokyo, Japan) between April 1, 2007, and September 21, 2021, with at least 1 central venous catheter (CVC) or peripherally inserted central catheter (PICC) submitted for culture were retrospectively reviewed. Demographic data, intravascular catheter type, results of gram staining and catheter tip culture, and results of blood culture in patients with positive gram staining for yeast-like fungi on the catheter tips were collected.

Laboratory Procedure

The catheter tips were cultured using the roll-plate method described by Maki et al. [5]. The external and internal catheter tip surfaces were rinsed with sterile purified water (0.5 mL). Some of the rinse water was processed with gram stain, and the rest and the catheter tips were together added to thioglycolate (TGC) medium and cultured for 7 days. Gram staining was performed using the following dyeing procedure: crystal violet, iodine solution, ethanol-acetone solution, and fuchsin for 30 seconds, 30 seconds, a few seconds, and 15 seconds, respectively. A catheter with at least 1 yeast-like fungus per 30 oil-immersion fields was considered positive on gram staining. Blood cultures were performed using BACTEC (Becton Dickinson, Sparks, MD, USA). The microbiological laboratory techniques used remained unchanged during the study. All laboratory procedures were performed by skilled microbiologists.

Definitions

(1) Catheter colonization

Maki's roll-plate method with ≥ 15 colony-forming units (CFUs) or TGC medium fluid with $\geq 10^3$ CFU/mL [6].

(2) Catheter-related blood culture

Blood cultures obtained 3 days before to 3 days after catheter removal.

(3) Suspected CRBSI

Patients for whom both catheter tip and catheter-related blood cultures were performed.

(4) C-CRBSIs

Patients with at least 1 positive catheter-related blood culture and also a positive catheter tip culture for the same *Candida* species.

Received 25 August 2022; editorial decision 07 December 2022; accepted 09 December 2022; published online 12 December 2022

Correspondence: Gen Yamada, MD, MPH, Disease Control and Prevention Center, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan (gyamada@hosp.ncgm.go.jp).

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<https://doi.org/10.1093/ofid/ofac667>

Outcome

The primary outcome was the diagnostic performance (ie, sensitivity, specificity, positive predictive value [PPV], and negative predictive value [NPV]) of gram staining of catheter tips for C-CRBSI diagnosis.

Statistical Analysis

Summary statistics of patients' background factors and *Candida* subspecies were calculated. The mean (SD), median (interquartile range), or percentage (%) was used as appropriate. In patients with suspected CRBSI, sensitivity, specificity, PPV, and NPV were calculated with 95% CIs to predict C-CRBSIs. Cases in which the catheter tip culture was positive for *Candida* species but no subspecies were identified were considered attributable to the same *Candida* species as that detected in the blood culture. An additional

episode of catheter tip culture 24 hours after the previous episode was considered a separate episode. Moreover, based on previous studies, a period other than ± 3 days of catheter removal was selected for catheter-related blood culture, and predictive performance was recalculated in the same way: (i) blood cultures obtained 7 days before to 2 days after catheter removal [7] and (ii) blood cultures obtained 2 days before to 1 day after catheter removal [8]. PPV is a particularly useful diagnostic test measure obtained from gram staining of catheter tips. To estimate the 95% CI of ± 0.1 , 61 cases with positive gram staining of the catheter tip were needed, estimated using the normal approximation to the binomial distribution [9]. Our center receives approximately 10–20 cases of C-CRBSI annually; thus, we determined that a 14-year study period would provide sufficient cases. Statistical analyses were performed using SAS software (version 9.4; SAS Institute, Cary, NC, USA).

Table 1. Characteristics of Intravenous Catheters With Positive Gram Staining for Yeast-Like Fungi

Characteristic	
No. of catheters	90
Male sex, No. (%)	58 (64.4)
Age, median (IQR), y	76 (65–81)
Catheter type, No. (%)	...
CVC	86 (95.6)
PICC	4 (4.4)
No. of catheter-related blood cultures, No. (%)	...
2 or more sets	75 (83.3)
1 set	15 (16.7)
Time to obtain blood cultures, No. (%)	...
–3 d	5 (5.6)
–2 d	14 (15.6)
–1 d	22 (24.4)
0 d	46 (51.1)
1 d	3 (3.3)
2 d	0 (0.0)
3 d	0 (0.0)
Timing of antifungal treatment initiation, No. (%)	...
Before blood culture	10 (11.1)
After blood culture	67 (74.4)
No antifungal treatment	10 (11.1)
No data	3 (3.3)
<i>Candida</i> species, No. (%)	...
<i>Candida albicans</i>	34 (39.5)
<i>Candida parapsilosis</i>	16 (18.6)
<i>Candida glabrata</i>	19 (22.1)
<i>Candida tropicalis</i>	8 (9.3)
<i>Candida lusitanae</i>	1 (1.2)
<i>Candida guilliermondii</i>	1 (1.2)
<i>Candida krusei</i>	2 (2.3)
<i>Candida metapsilosis</i>	1 (1.2)
<i>Candida paratropicalis</i>	1 (1.2)
<i>Candida</i> species	3 (3.5)
<i>Candida</i> catheter colonization, No. (%)	81 (90.0)
C-CRBSI, No. (%)	76 (84.4)

Abbreviations: C-CRBSI, *Candida* catheter-related bloodstream infection; CVC, central venous catheter; IQR, interquartile range; PICC, peripherally inserted central catheter.

RESULTS

During the study, 2462 episodes of catheter tip and catheter-related blood cultures from 2381 patients were submitted, excluding 6 episodes for which data on bacterial quantity were missing. CVC and PICC accounted for 97.8% and 2.2% of the cases, respectively.

Ninety episodes of yeast-like fungus detection on gram staining from 88 patients were identified. Of the patients included, 64% were men; the median age was 76 years (Table 1). Of the 90 episodes of positive gram staining, *Candida* colonization was identified in 81 (90%); 5 episodes showed 2 *Candida* species on catheter tip culture; 76 episodes (84%) were accompanied by positive catheter-related blood culture and categorized as C-CRBSI. Overall, 2372 episodes were negative for yeast-like fungi by gram staining. *Candida* colonization was identified in 48 of these episodes (2%), and 39 (1.6%) were categorized as C-CRBSIs.

The sensitivity, specificity, PPV, and NPV for C-CRBSI detection from gram staining of the catheter tip were 66.1% (95% CI, 56.7%–74.7%), 99.4% (95% CI, 99.0%–99.7%), 84.4% (95% CI, 75.3%–91.2%), and 98.4% (95% CI, 97.8%–98.8%), respectively (Table 2). Similar results were obtained after varying the period beyond ± 3 days of catheter removal according to the definition of catheter-related blood culture (Supplementary Table 1).

Table 2. Correlation Between Catheter Tip Gram Stains and C-CRBSIs

	C-CRBSI Present	C-CRBSI NOT Present	Total
Gram stain
Positive	76	14	90
Negative	39	2333	2372
Total	115	2347	2462

Sensitivity, 76/115 (66.1%); specificity, 2333/2347 (99.4%), positive predictive value, 76/90 (84.4%); negative predictive value, 2333/2372 (98.4%).

Abbreviation: C-CRBSI, *Candida* catheter bloodstream infection.

Supplementary Table 2 shows the characteristics of 14 false-positive episodes. Two or more sets of blood cultures were obtained in 12 episodes and 1 in 2 episodes. Thirteen episodes had a negative blood culture. One episode had a positive blood culture for *Candida* species and was treated for possible C-CRBSI. Detailed information on antifungal treatment was available for 11 episodes. None of them had antifungal treatment preceding the blood culture examination.

DISCUSSION

Our study demonstrated that gram staining of the catheter tip afforded a good PPV (84.4%) for C-CRBSI detection in patients with suspected CRBSIs. Gram staining of a catheter tip is a simple and rapid test. Previously, the predictive performance of gram staining for CRBSI detection was investigated for all microorganisms, including *Candida* species, and sensitivity, specificity, PPV, and NPV, respectively, of 73%, 98%, 90%, and 94% were obtained [10]. These results are generally similar to those of the present study. However, the study included only 7 catheters colonized by *Candida* species. To our knowledge, our study is the first large study to investigate the feasibility of using gram staining to detect C-CRBSIs.

Routine empirical antifungals in the initial CRBSI treatment are associated with side effects, the emergence of antifungal resistance, and high costs [11]. However, as the incubation time until *Candida* species detection is several days [12], considering the high mortality in candidemia [13], a delay in initiating appropriate antifungal therapy is associated with adverse outcomes [14, 15]. The high PPV of gram staining of catheter tips in this study supports the validity of initiating antifungal therapy for suspected CRBSIs as soon as yeast-like fungi are identified by gram staining of the removed catheter tip.

This study had several limitations. First, other apparent sources of bloodstream infection were not ruled out, which may have led to the inclusion of infections other than C-CRBSI. Second, because of the retrospective nature of the study, the submission of catheter tips for culture was at the attending physician's discretion. Therefore, not all CRBSI cases were included in the present study. Third, in 87 of the 90 episodes with positive gram staining, we could mention the presence or absence of prior antifungal therapy but not for all the 2462 episodes included in this study; thus, some C-CRBSI cases may have been missed because of false-negative results. Finally, whether the results of this study will improve the prognosis of C-CRBSI remains unclear, and further studies are needed.

In conclusion, we found that gram staining of catheter tips had good specificity, PPV, and NPV for detecting C-CRBSIs and may be useful for the early detection of C-CRBSIs.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

Financial support. This research was supported by the National Center for Global Health and Medicine Intramural Research Fund (grant number: 20A05).

Potential conflicts of interest. All authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Author contributions. All authors have read and approved the final manuscript and contributed significantly to the work. Conceptualization: G.Y., N.I., M.I., A.M., M.K., K.M., and N.O. Data curation: G.Y., A.M., M.K., and K.M. Formal analysis: G.Y. Methodology: G.Y., N.I., M.I., and M.I. Project administration: N.I., M.I., and N.O. Resources: A.M., M.K., and K.M. Supervision: N.I., M.I., K.M., and N.O. Writing—original draft: G.Y., N.I., M.I., A.M. Writing—review and editing: K.M., K.M., and N.O.

Data availability. Data are not publicly available.

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