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ORIGINAL ARTICLE

Performance of real-time PCR in suspected haemodialysis catheter-related bloodstream infection: a proof-of-concept study

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ABSTRACT

Background. Catheter-related bloodstream infections (CRBIs) remain a major cause of mortality in haemodialysis (HD) patients with central venous catheters (CVCs), especially because of the non-specific symptomatology and the delay in microbiological diagnosis with possible use of non-optimal empiric antibiotics. Moreover, empiric broad-spectrum antibiotics increase antibiotic resistance development. This study aims to evaluate the diagnostic performance of real-time polymerase chain reaction (rt-PCR) in suspected HD CRBIs compared with blood cultures.

Methods. A blood sample for rt-PCR was collected simultaneously with each pair of blood cultures for suspected HD CRBI. The rt-PCR was performed on the whole blood, without any enrichment stage and with specific DNA primers: 16S (universal bacterial), *Staphylococcus* spp., *Staphylococcus aureus* and *mecA*. Each successive patient with a suspected HD CRBI in the HD centre of Bordeaux University Hospital was included. Performance tests were used to compare the result obtained in each rt-PCR assay with its corresponding routine blood culture.

Results. Eighty-four paired samples were collected and compared for 40 suspected HD CRBI events in 37 patients. Among these, 13 (32.5%) were diagnosed as HD CRBI. All rt-PCRs except *mecA* (insufficient number of positive samples) showed high diagnostic performances within 3.5 h: 16S (sensitivity 100%, specificity 78%), *Staphylococcus* spp. (sensitivity 100%, specificity 97%), *S. aureus* (sensitivity 100%, specificity 99%). Based on the rt-PCR results, antibiotics could be more appropriately targeted, thus cutting anti-cocci Gram-positive therapy from 77% to 29%.

Conclusions. The performance of rt-PCR in suspected HD CRBI events showed fast and high diagnostic accuracy. Its use would improve HD CRBI management with an antibiotic consumption decrease.

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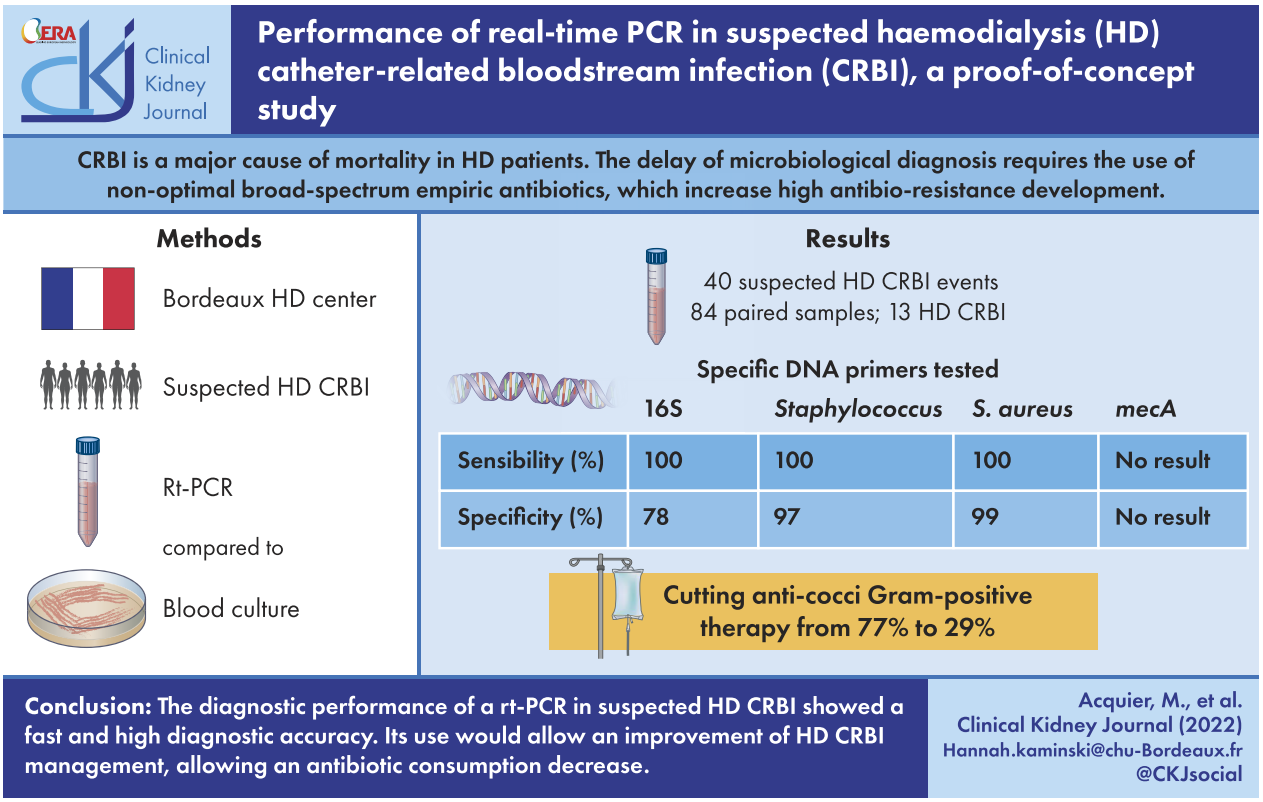
LAY SUMMARY

This study aims to evaluate the diagnostic performance of real-time polymerase chain reaction (rt-PCR) in suspected haemodialysis (HD) catheter-related bloodstream infection (CRBI) compared with blood cultures. For each successive patient with a suspected HD CRBI, a blood sample for rt-PCR was collected simultaneously with each pair of blood cultures. Four DNA primers were tested: 16S (universal bacterial), *Staphylococcus* spp., *Staphylococcus aureus* and *mecA* (for methicillin resistance). Eighty-four paired samples were collected and compared. All rt-PCR except *mecA* (insufficient number of positive samples) showed high diagnostic performances (sensitivity 100%, specificity 78–99%) within 3.5 h. Based on the rt-PCR results, antibiotics could be more appropriately targeted, thus cutting anti-cocci Gram-positive therapy from 77% to 29%. We conclude that the performance showed a fast and high diagnostic accuracy and its use would improve HD CRBI management with an antibiotic consumption decrease.

GRAPHICAL ABSTRACT



Keywords: bloodstream infection, haemodialysis, real-time PCR

INTRODUCTION

Patients treated by haemodialysis (HD) with a central venous catheter (CVC) as vascular access are at risk of infections [1]. Catheter-related bloodstream infections (CRBIs) in HD remains a major cause of morbidity and mortality [2]. It can result in life-threatening complications, including septic shock, endocarditis, septic arthritis, osteomyelitis and epidural abscess [3]. Identifying and treating CRBIs is particularly challenging due to the complex clinical features of such infections, which have a non-specific symptomatology [4]. CRBIs can be caused by Gram-positive and Gram-negative bacteria. The majority of HD CRBIs are caused by *Staphylococcus* spp., and *Staphylococcus aureus* is associated with increased morbidity and mortality [5, 6]. Moreover, patients treated with HD are at high risk of methicillin-resistant *Staphylococcus* (MRS) [7]. The implementation of empiric antibiotic therapy is based on Infectious Diseases Society of America guidelines published in 2010 proposing the combination of molecule targeting of MRS and a beta-lactam active agent on *Pseudomonas aeruginosa* [8]. The diagnostic difficulty leads to extensive use of broad-spectrum antibiotic therapy. Patients treated with HD are exposed to multiple antibiotic courses, thus increasing the risk of infections with multidrug-resistant organisms. Moreover, in patients with non-severe symptoms, a fast negative result for bacteraemia would avoid unnecessary hospitalization and antibiotic use.

Culture-based methods remain the gold standard to identify the causative microorganism in sepsis, with results available

within 6–72 h (longer than an HD session) [9]. Technological developments in rapid non-culture-based infection diagnosis are now seen as crucial for a more rational use of antibiotic therapy [10]. Molecular non-culture approaches based on the detection of circulating bacterial DNA using real-time polymerase chain reaction (rt-PCR) can provide rapid detection of pathogens in blood [11]. Previous studies looking into the use of rt-PCR in bloodstream infections (BSIs) have shown encouraging positive results on mortality reduction and antimicrobial ecology [12]. In recent years, few studies have been published to evaluate the diagnostic performance of non-enriched whole blood rt-PCR in specific populations compared with the reference blood culture test [13–15]. This study aims to evaluate the feasibility, diagnostic performance and potential contributions of rt-PCR in suspected HD CRBIs compared with the standard-of-care blood culture.

MATERIALS AND METHODS

Study setting

This was a descriptive transversal prospective study performed between October 2018 and October 2019 in Bordeaux University Hospital comparing the diagnostic performance of rt-PCR and blood culture in the diagnosis of HD CRBIs (NCT04026035). All participants gave their verbal consent. A protective committee evaluated and approved the study. This committee stated that our study was a non-interventional study because of the small quantity of blood additionally sampled and allowed us to have solely verbal non-opposition consent.

Participants

The inclusion criteria were patients >18 years old with suspected HD CRBI. Exclusion criteria were age < 18 years and patients passing through the emergency department with antimicrobial therapy received for >8 h. The suspicion of HD CRBI was left to the judgment of the clinician. CVC could be tunnelled or not.

Intervention

After informing the patient, an additional blood sample [7 ml ethylenediaminetetraacetic acid (EDTA) blood collection] was taken concomitantly with those taken as part of the standard management of suspected HD CRBI. The clinical samples were collected in the ward and were sent to both the research laboratory and the routine diagnostic laboratory for processing (Laboratory of Bacteriology of Bordeaux University Hospital). Once collected, the EDTA blood collection was labelled to make it anonymous, then transported to the Aquitaine Microbiology laboratory by staff of the Nephrology Transplantation Dialysis Department of Bordeaux University Hospital. The results of the study technique were compared with the reference technique, i.e. blood culture. The samples used for research were destroyed at the end of the analysis.

Routine diagnostic approach

The standard blood culture sample (15 ml for aerobic and anaerobic bottles) procedure in the routine diagnostic laboratory for suspected HD CRBI is the following: if suspected prior to HD connection, a pair of blood cultures is collected from the venous and the arterial lines of the catheter, associated with a pair of blood cultures from the peripheral vein if possible; if suspected during the session, a pair of blood cultures are collected from the HD circuit [16].

For one suspected CRBI event, one to five pairs of blood cultures were sent to the laboratory and therefore one to five rt-PCRs were performed and compared with the concomitantly collected blood culture pair. In our centre, the microbiological diagnosis of BSI was obtained with blood culture: Gram stain and culture for species identification and antibiotic susceptibility testing.

The blood culture bottles (BacT/Alert FA Plus and BacT/Alert FN Plus) were placed in an automated system (BacT/Alert; all from bioMérieux, Marcy l'Etoile, France) that detects bacterial growth. As soon as the blood culture was positive, a Gram stain was performed and species identification was carried out with mass spectrometry using the MALDI-TOF Biotyper system (Brucker, Bremen, Germany) after a 3-h incubation of the blood culture on a chocolate agar plate. Antibiotic susceptibility was determined using the Phoenix automated system (BD Diagnostics, le Pont de Claix, France) or by agar diffusion, following the recommendations of the Antibiogram Committee of the French Society of Microbiology. The total duration of the technique to obtain the antibiogram depends on the time of positivity of the blood culture, classically between 24 and 96 h.

rt-PCR design in the research laboratory

In parallel with each pair of blood cultures according to the above protocol, a 7-ml EDTA blood tube was collected. From the sample, without a culture or enrichment phase, total DNA was extracted with the QIAamp DNA Blood Mini Kit (Qiagen, Courtaboeuf, France), then the amount of DNA was adjusted with nanodrops in order to reach a DNA concentration of 20 ng/μl (dilution in sterile milliQ water or sterile diethylpyrocarbonate water).

A panel of four sets of primers was used in the PCR reaction: 16S rDNA [17], *Staphylococcus* DNA [18], *S. aureus* DNA [19] and *mecA* DNA [20]. The primers used were ordered from Eurofins MGW Operon (Luxembourg) and are referenced. A total of 15 μl of the reagent mix was placed in each rt-PCR tube, then a 5-μl DNA sample was deposited for a final volume of 20 μl per tube. The individual tubes were deposited into the wells of the CFX 96 thermal cycler (Bio-Rad, Marnes-la-Coquette, France). Once the program was completed, the amplification and melting curves obtained were analysed using the CFX 96 software. It is therefore a SYBR Green technique with the use of melting curves to improve the specificity of the technique. The melting temperature (T_m) for the *Staphylococcus* spp. primers was 75°C. The T_m of the expected products with the *S. aureus* and *mecA* primers was 77–78°C. For the pair of primers targeting the bacterial 16S rDNA, the T_m was 85°C. A duplicate was systematically performed. The following sequence was used for each rt-PCR run: 98°C for 2 min once, 98°C for 5 sec, 56°C for 30 sec × 40 cycles. For the melting curve, the temperature was increased from 65° to 95°C with an increment of 0.5°C per 5 sec.

For each sample, a negative control was analysed adding water to the reaction mix. This manipulation also allowed us to check the non-contamination of the reaction mixes. A positive control of each rt-PCR was performed using a strain from an American Type Culture Collection (Manassas, VA, USA) for each patient series performed at the same time.

Table 1: Baseline characteristics.

Characteristics	*n*
Patients	37
Suspicion of HD CRBI	40
Sample for rt-PCR	84
BSI	14
HD CRSI	13
Positive blood cultures with diagnosis of contamination	3
Empiric antimicrobial therapy	19
Empiric coverage for Gram-negative organism	18
Empiric coverage for methicillin-resistant *Staphylococcus* spp.	12

Positive cut-off for rt-PCR results

The rt-PCR results were expressed as an exponential curve of amplicon expansion. The cycle threshold (Ct) of positivity was set, depending on our analysis, ≤28 cycles. Considering the different curves obtained from the negative controls, where the expansion always started after 30 cycles, and the results of the rt-PCR of the samples, which were either <30 cycles or equal to 28 cycles, we fixed the threshold to 28 cycles. This setting allowed at least two cycles to differentiate the positive samples from the negative ones.

Statistical analysis

The results of both diagnostic methods were compared. A full-blind/blinded sample analysis was carried out without any knowledge of the results obtained in the routine diagnostic laboratory. In addition, the time necessary to obtain results in both approaches was assessed.

The sample size was not defined beforehand, as this was a proof-of-concept study. Qualitative variables are described in terms of numbers and percentages and quantitative variables are described in terms of number, mean, standard deviation (SD), median and quartiles. Diagnostic performances were evaluated by calculating sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) and by analysing the interrater reliability between rt-PCR and blood culture. The reference method was the blood culture. The interrater reliability between the two tests was assessed with the κ coefficient.

RESULTS

Patient demographics and study feasibility

A total of 40 suspected HD CRBI events were identified in 37 patients (3 patients had 2 different events). During the course of those 40 suspected HD CRBIs, 84 pairs of blood cultures were collected and 84 concomitant samples were analysed by rt-PCR. Among the 84 blood cultures, 27 (32%) were positive. Among the 40 suspected events, 23 (57.5%) had at least one positive blood culture. Three had only one positive blood culture for coagulase-negative *Staphylococcus* and were judged by the clinician to be contamination (single positive blood culture with negative controls, a growth time >48 h and a clinical improvement of patient without antibiotic treatment) and one BSI from the urinary tract was diagnosed. A total of 13 (32.5%) diagnoses of HD CRBI were made: 12 were related to a tunnelled catheter and 1 was related to a non-tunnelled catheter. The baseline characteristics of the patients are reported in Table 1. Identified microorganisms are described in the supplementary material.

Table 2: Evaluation of rt-PCR for 16s.

Result	Blood culture positive	Blood culture negative	Total
rt-PCR positive	27	13	40
rt-PCR negative	0	44	44
Total	27	57	84

Table 3: Evaluation of rt-PCR for *Staphylococcus* spp.

Result	Blood culture positive	Blood culture negative	Total
rt-PCR positive	13	2	15
rt-PCR negative	0	69	69
Total	13	71	84

Table 4: Evaluation of rt-PCR for *Staphylococcus aureus*.

Result	Blood culture positive	Blood culture negative	Total
rt-PCR positive	6	1	7
rt-PCR negative	0	77	77
Total	6	78	84

Diagnostic performance of rt-PCR versus blood culture

The median time needed to obtain results was 3.5 h (IQR: 3 h 10 min–3 h 50 min) for rt-PCR and 12 h (IQR 9.75–21) for blood culture Gram stain.

The diagnostic performance of each rt-PCR is summarized in Tables 2–4. Supplemental Fig. 1 shows examples of three different results and their interpretation. Unfortunately, performances of the *mecA* amplification could not be evaluated due to the small number of positive blood cultures for MRS ($n = 1$).

The 16S rt-PCR showed excellent sensitivity (100%), with no false negatives, resulting in an excellent NPV of 1. On the other hand, the presence of 10 false positives resulted in a decreased specificity (77%) and a positive predictive value of 0.72. The κ coefficient representing the interrater reliability of the two methods was 0.72, leading to strong agreement.

The *Staphylococcus* spp. rt-PCR showed excellent diagnostic performance, with sensitivity and NPV at 1, as well as a very good specificity (98%) and PPV (0.92). The κ coefficient was 0.95, which corresponds to very strong agreement.

The *S. aureus* rt-PCR also had a sensitivity of 1 and an NPV of 1. There were no false negatives in the 64 samples with blood cultures that did not contain *S. aureus*. The specificity was excellent at 0.98, with a slightly poorer PPV at 0.86 in view of the few *S. aureus* found in total ($n = 6$). The κ coefficient was 0.91, corresponding to very strong agreement.

Interestingly, when analysing the agreements by events and not by samples, 34 of 40 events had 100% agreement between rt-PCR and blood culture. Among three events with two samples, the two pairs of blood cultures were discordant but the rt-PCRs were concordant with their paired blood culture. For three events with a negative blood culture, 16S rt-PCR had a false-positive result (one of two samples for the first event, one of three samples for the second event and one of five samples for the last event).

Table 5: Comparison of the use of antimicrobial therapy with or without rt-PCR.

Empiric therapy	Clinician judgment	Based on rt-PCR
No use of empiric antimicrobial therapy, *n*	21	18
In cases of no BSI, *n*/*N* (%)	18/21 (86)	18/18 (100)
In cases of BSI, *n*/*N* (%)	3/21 (14)	0/18 (0)
Empiric antimicrobial therapy, *n*	19	22
In cases of no BSI, *n*/*N* (%)	8/19 (42)	8/22 (36)
In cases of BSI, *n*/*N* (%)	11/19 (58)	14/22 (64)
Empiric coverage for Gram-negative organism, *n*	18	15
In cases of no Gram-negative BSI, *n*/*N* (%)	12/18 (67)	8/15 (53)
In cases of Gram-negative BSI, *n*/*N* (%)	6/18 (33)	7/15 (47)
Empiric coverage for Gram-positive BSI, *n*	13	7
In cases of no Gram-positive BSI, *n*/*N* (%)	10/13 (77)	2/7 (29)
In cases of Gram-positive BSI, *n*/*N* (%)	3/13 (23)	5/7 (71)

Potential added value of rt-PCR

We first focused on the potential contribution of rt-PCR regarding the time needed to obtain results, because it should, in theory, be shorter than the HD session and the sensitivity of the technique. Indeed, in three cases of HD CRBI suspected cases, the clinician did not initiate probabilistic antibiotic therapy, although the final diagnosis was HD CRBI. With the use of rt-PCR, all HD CRBIs would have been diagnosed before the end of the HD session, avoiding any delay in treatment.

Second, based on the rt-PCR results, antibiotics would be more appropriately targeted, thus cutting anti-cocci Gram-positive therapy from 77% to 29%. Indeed, with a positive result for 16S rt-PCR, combined with a negative result for *Staphylococcus* spp. and *S. aureus* rt-PCR, clinicians can safely avoid antibiotics targeting MRS.

DISCUSSION

We reported the first systematic diagnostic accuracy study of an rt-PCR assay versus blood culture in the specific setting of suspected HD CRBI. The study population was composed of 37 patients, with 40 suspected cases of HD CRBI, and we were able to analyse 84 samples. This first study demonstrated the feasibility of rt-PCR for suspicion of HD CRBI.

The accuracy of our diagnostic method is high. The *Staphylococcus* spp. and *S. aureus* rt-PCRs proved to have excellent performances and are promising for this use. Regarding the universal 16S bacterial probe, rt-PCR showed excellent sensitivity (100%) and a high NPV. However, the method seems to be too sensitive, as 10 false-positive results were observed. These characteristics are reassuring regarding its potential future application. Compared with other studies developing an rt-PCR diagnostic method directly from whole blood without any enrichment stage, we found a higher sensitivity [14, 15]. The hypotheses explaining this difference include

a different DNA extraction method, without a prior enrichment step; the use of a simplex rt-PCR, which minimizes the risk of error together with increasing the sensitivity of the technique [21, 22] and the larger bacterial burden. Indeed, the sample is taken directly at the source of the infection (on the catheter or on the circuit with a high blood flow).

The diagnostic method with these four rt-PCRs on whole blood without enrichment also made it possible to extract the DNA directly from whole blood in an EDTA blood tube, resulting in a shorter amount of time needed for result delivery (median of 3.5 h). This methodology allowed us to collect important information in <4 h, which is the usual duration of an HD session. To our knowledge, this is the simplest method to acquire those results in such a short time. Thanks to this reduced time, a major challenge for the clinician confronted with a suspicion of HD CRBI can be addressed: the decision to introduce the most accurate antibiotic treatment and avoid broad-spectrum antibiotics.

It is known that exposure to antibiotic therapy is one of the main risk factors for the appearance and spread of multiresistant bacteria. Multiresistant bacteria are also associated with significant adverse events. In 2006, the National Health Safety Network reported antibiotic use in 32 ambulatory HD centres [23]. During the 12-month study period, 977 introductions were recorded, amounting to a pooled average of 3.5 introductions per 100 patient-months. Chronic HD patients with catheters had the highest rate of antibiotic therapy introduction. Vancomycin accounted for 73% of all initial antibiotic therapy prescriptions. Later, Snyder *et al.* [24] prospectively followed 278 HD patients over a 12-month study period. Overall, 29.8% of doses were classified as inappropriate, and the most common inappropriate antibiotic used was vancomycin. With our approach, the rt-PCR results could have resulted in significant antibiotic economy, namely a reduced use of Gram-positive anti-cocci therapy. However, rt-PCR performances are not flawless, and the severity criteria excludes the restriction of antibiotic therapy.

Our study has several limitations. First, the diagnostic method developed here does not attempt to distinguish HD CRBI from BSI related to another source or from a contamination. Indeed, the development of this diagnostic method is only appropriate in a complementary role to blood cultures: microbiological diversity makes it impossible to reach a BSI diagnosis with only rt-PCR, and the analysis of the antibiogram is crucial in the subsequent management of the disease to de-escalate treatment to a targeted antibiotic therapy.

Second, our population of patients was small. The study was carried out in a single centre, due to the constraints of delivering the samples to the laboratory performing the rt-PCR, which made it difficult to carry out a multicentre study. This small number of patients made it impossible to test the diagnostic performance of the *mecA* rt-PCR, preventing the provision of this crucial information to the clinician. Also, the interpretation of the results should be considered. The negativity of the sample is based on the negative control, water, which shows a curve

expansion after 30 cycles in a systematic way (background noise due to SYBR Green fluorescent molecules). All curves showed either a similar curve expansion (Ct >30 cycles) or a shorter curve expansion (≤28 cycles of Ct). The reproducibility of these two results led us to set these two cut-offs. These cut-offs are therefore based on local expertise and not on the literature (non-existent in the field). The boundary we have drawn between a positive and a negative result will have to be validated on a replication cohort.

In conclusion, this study shows that rt-PCR is a promising diagnostic method for suspected HD CRBI, with high diagnostic performance. This innovative and robust rt-PCR approach should allow clinicians to better tailor the initial empirical antimicrobial therapy, crucial for both a good outcome for these vulnerable patients and for a reduction of antibiotic resistance risk. With blood culture remaining the reference method for HD, the use of rt-PCR bedside could lead to a crucial optimization of HD patient care.

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

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AUTHORS' CONTRIBUTIONS

M.A., A.Z., V.D., F.M., C.C. and H.K. were responsible for the conception and design and the analysis and interpretation of data. M.A., V.D., F.M., S.R., Y.D., V.D.P., R.D.L.F., C.C. and H.K. were responsible for drafting or revising the article.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared upon reasonable request to the corresponding author.

CONFLICT OF INTEREST STATEMENT

None declared.

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