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Original Article

ddPCR enables rapid detection of bloodstream infections in patients on home parenteral nutrition: A prospective cohort study

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KEYWORDS
Central line-associated bloodstream infections;
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Abstract *Introduction:* Chronic intestinal failure patients (CIF) require a central venous access device (CVAD) to administer parenteral nutrition. Most serious complication related to a CVAD is a central line-associated bloodstream infection (CLABSI). The golden standard to diagnose a CLABSI are blood cultures, however, they may require 1—5 days before getting a result. Droplet digital polymerase chain reaction (ddPCR) for the detection of pathogen 16S/28S rRNA is a novel culture-independent molecular technique that has been developed to enhance and expedite infection diagnostics within two and a half hours. In this study, we prospectively compared ddPCR with blood cultures to detect pathogens in whole blood.
Methods: We included adult CIF patients with a clinical suspicion of CLABSI in this prospective single-blinded clinical study. Blood cultures were routinely collected and subsequently two central samples from the CVAD and two peripheral samples from a peripheral venous access point. Primary outcome was the sensitivity and specificity of ddPCR.
Results: In total, 75 patients with 126 suspected CLABSI episodes were included, with 80 blood samples from the CVAD and 114 from peripheral veins. The central ddPCR samples showed a sensitivity of 91% (95%CI 77—98), and specificity of 96% (95%CI 85—99). Peripheral ddPCR

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samples had a sensitivity of 63% (95%CI 46—77) and specificity of 99% (95%CI 93—100).
Conclusion: ddPCR showed a high sensitivity and specificity relative to blood cultures and enables rapid pathogen detection and characterization. Clinical studies should explore if integrated ddPCR and blood culture outcomes enables a more rapid pathogen guided CLABSI treatment and enhancing patient outcomes.

Abbreviations

95% CI	95% confidence interval
C1	First central blood sample
C2	Second central blood sample
CIF	Chronic intestinal failure
CLABSI	Central line-associated bloodstream infection
CRP	C-reactive protein
CVAD	Central venous access device
ddPCR	Droplet digital polymerase chain reaction
DNA	Deoxyribonucleic acid
FN	False negative
FP	False positive
HPN	Home parenteral nutrition
IQR	Interquartile range
NLR	Negative likelihood ratio
NPV	Negative predictive value
P1	First peripheral blood sample
P2	Second peripheral blood sample
PICC	Peripherally inserted central catheter
PLR	Positive likelihood ratio
PN	Parenteral nutrition
PPV	Positive predicted value
SD	Standard deviation
TN	True negative
TP	True positive
TTP	Time-to-positivity
qSOFA	quick Sequential Organ Failure Assessment

Introduction

Patients with chronic intestinal failure (CIF) depend on home parenteral nutrition (HPN), a lifelong treatment that requires the presence of a central venous access device (CVAD). While HPN is a life-saving strategy, it is also associated with potentially life-threatening complications, such as central line-associated bloodstream infections (CLABSIs), resulting in a significant impact on patients and healthcare resources.[1—3]

As for all treatments that require the presence of a CVAD, a rapid and reliable diagnosis of infection-related complications is crucial in HPN care. Although blood cultures are considered the gold standard to diagnose CLABSIs, these come with several drawbacks. For example, culture outcomes are influenced by prior or concomitant antibiotic use, the quantity of blood volume in the culture bottle, and correct (sterile) handling of blood cultures by personnel.[4—6] In addition, the time-to-positivity, which may take up to 1—5 days, hampers antimicrobial guidance. This problem can only be overcome by using broad spectrum antibiotics in the early course of an infection until subsequent tailoring of treatment based on blood culture results.[7] The associated risk for suboptimal treatment of drug-resistant pathogens, induction of microbial resistance, and, in case of negative cultures, unnecessary antibiotic use remain a concern.[8] Finally, early differentiation between bacterial and fungal infections remains difficult, but is crucial given the therapeutic consequences with respect to antimicrobial therapy and CVAD removal.[9]

Several culture-independent techniques have become available that enable rapid pathogen detection. The most common molecular technique being the polymerase chain reaction (PCR), which identifies pathogen DNA from blood cultures and whole blood.[10,11] Yet, detection of pathogens in whole blood remains challenging, mostly due to the limited amount of pathogen DNA in a background of excessively present human DNA, resulting in a low sensitivity and limited clinical applicability.[12,13] To overcome this issue, the more recently developed droplet digital polymerase chain reaction (ddPCR) holds promise.[14] With this technique, the PCR reaction mixture is partitioned into approximately 20,000 minuscule droplets before amplification. Due to sample partitioning, the amount of pathogen DNA in one droplet increases relatively to the background genomic DNA, which may eventually increase the sensitivity of pathogen DNA detection.[15] In our previous pilot study, we compared blood cultures with a broad-spectrum ddPCR test designed to detect pathogen 16S/28S rRNA. Our results showed a sensitivity of 80%, a specificity of 87%, and we were able to detect pathogens or rule out bloodstream infection within 4 h, which makes it a potentially valuable technique for clinical practice.[15]

Here, we prospectively assess the sensitivity and specificity of the 16S/28S rRNA ddPCR compared to routine diagnostics, i.e. using blood cultures, in a robust group of HPN patients with suspected CLABSI. Secondary aim was to evaluate test characteristics of the ddPCR in central and peripheral blood sample subgroups (Gram-positive, Gram-negative and fungi).

Methods

Patient inclusion and blood sample collection

This prospective single-blind cohort study was conducted at the Radboud university medical center, in Nijmegen, the

Netherlands, between May 2019 and February 2022. CIF patients aged >18 years with a clinical suspicion of CLABSI were included after providing written informed consent. Some patients were included repeatedly during different suspected CLABSI episodes in this study. Individuals were excluded in case no paired blood cultures were taken to compare with the 16S/28S rRNA ddPCR, or in case patients received antibiotics ≤7 days before blood culture withdrawal. In this study we used the less strict definition for a line infection (i.e. CLABSI) instead of catheter-related bloodstream infection (CRBSI). A CLABSI was defined according to the definition of Centers for Disease Control and Prevention (CDC) and refers to a bloodstream infection (laboratory confirmed), with an eligible pathogen and a centrale line present that day or the day before.[16] In contrast, a CRBSI refers to a bloodstream infection that is attributed to a central venous catheter by quantitative culture techniques.[17] When a CLABSI was suspected upon presentation (assessment by the treating physician e.g. fever, chills, increased heart rate, inflammatory parameters or general malaise) at the emergency department or during admission on the clinical ward, central and/or peripheral blood cultures (aerobic and anaerobic, approximately 8–10 mL) were routinely collected. Subsequently, these were transported to the department of Medical Microbiology and incubated in the BACTEC system (Becton Dickinson and Company, Franklin Lakes, NJ, USA) until positive result or for a maximum of five days. Isolates were subcultured on Columbia sheep blood and chocolate agar plates (BD Bioscience, Erembodegem, Belgium) and subsequently identified to species level using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF ms, BioTyper; Bruker Daltonics, Bremen, Germany). Directly after drawing blood culture samples, blood samples for the ddPCR were collected in four 6 mL EDTA vials; two from the CVAD (C1 and C2) and two from a peripheral venous access point (P1 and P2). Directly after collection, the ddPCR samples were kept at 4 °C, and within 72 h stored in aliquots of 300 μL at −80 °C until further use.

Data collection

The following data were collected: patient characteristics (sex, age, underlying disease leading to CIF and medical history), physical examination at baseline (body temperature, clinical presentation, laboratory findings), antibiotic use, CVAD characteristics (type of CVAD, lumen, and side and vein used for insertion), and quick Sequential Organ Failure Assessment (qSOFA) at baseline.

Outcomes

Primary aim of this study was to assess the sensitivity and specificity of a 16S/28S rRNA ddPCR compared to blood cultures. Secondary outcomes were additional ddPCR characteristics (predictive values, likelihood ratios), test characteristics specific for central and peripheral ddPCR samples, and for pathogen groups (Gram-positive and Gram-negative bacteria, and fungi).

Peripheral blood samples were drawn from a peripheral access point (e.g. venipuncture or arteriovenous fistula). Blood samples from a subcutaneously tunneled catheter, subcutaneous port system or peripherally inserted central catheter (PICC) were considered central blood samples.

Pathogens cultured from blood cultures were categorized as two groups: non-common commensals, i.e. pathogens that are likely to cause CLABSI, and pathogens defined as common commensals that might have caused false positive results (Fig. 1).[18] DdPCR results and blood culture results (C1, C2, P1, P2) were merged into a final central (C) and a peripheral (P) result, in line with the flowchart depicted in Fig. 2.

Results (central or peripheral blood samples) were considered true positive (TP) in case both the ddPCR and blood culture had a positive final result, or false positive (FP) when the ddPCR was positive and blood culture negative, regardless of the pathogen specific outcomes. A true negative (TN) was defined as both blood culture and ddPCR result were negative, and false negative (FN) was defined as a positive blood culture and negative ddPCR. Concerning the test characteristics for pathogen group specific outcomes (Gram-positive, Gram-negative, and fungi), the ddPCR and blood culture results needed to be concordant as well for a TP result.

DNA isolation and droplet digital PCR procedure

To keep DNA isolation and subsequent pathogen detection as fast as possible a total DNA isolation of whole blood was performed. Therefore prior to DNA isolation, 300 μL whole blood was homogenized with 0.5 mm glass beads in a 2 mL tube (Fisherbrand™, Thermo Fisher Scientific, Waltham, Ma, USA). Blood samples were treated for 70 s using the Bead Mill 4 program 5 (Fisherbrand™) and centrifuged for 30 s at 10.000×*g*. Subsequently, total DNA from the samples was extracted according to the manufacturer's protocol using the High Pure PCR Template Preparation Kit, version 26 (Roche Life Science, Penzberg, Germany). For final elution of samples, 100 μL Elution Buffer was used. Isolated DNA samples were stored at 4 °C until further use.

Two sets of primers and probes were used to detect pan-bacterial 16S rRNA (including Gram positive/negative differentiation) and pan-fungal 28S rRNA, as previously described.[15] Primer and probe sequences are presented in Supplementary file A. Pathogen DNA was detected using the QX200 Droplet Digital PCR system (Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol. Sample DNA (2 μL), 1x ddPCR Supermix for Residual DNA Quantification (Bio-Rad), forward and reverse primers (900 nM), probe(s) (250 nM) and DNase-free water were mixed to a final volume of 22 μL. For the reverse 28S primer a 1:1 mixture of 28S Rv a and 28S Rv b was used. After droplet generation, a PCR was performed using an annealing/extension temperature of 61 °C for both the 16S and 28S ddPCR. The QX200 Droplet Reader (Bio-Rad) was used to analyze the droplets with a Poisson analysis, and results were visualized with QuantaSoft software version 1.7.4 (Bio-Rad).[15]

As negative background controls, multiple DNA isolations from (multiple vena punctures) whole blood of a healthy volunteer was used. MilliQ spiked with DNA of different pathogens was used as positive control. All individual ddPCR samples were measured in duplicate. Results were

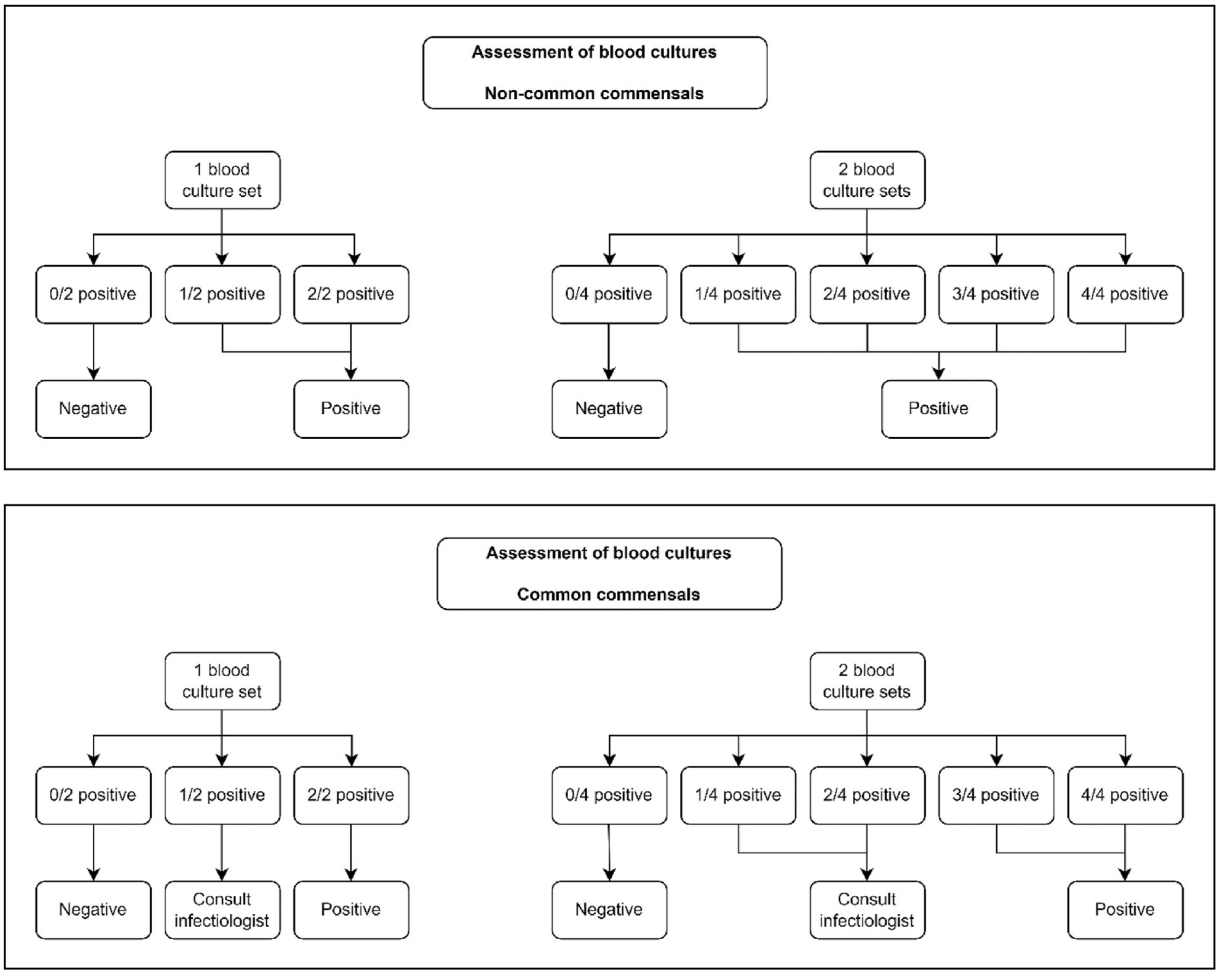

Figure 1. Assesment of blood cultures. Assessment differs between common commensals and non-common commensals due to the risk of contamination with common commensals. One blood culture set comprises two bottles (aerobic and anaerobic).

analyzed by two independent research technicians, blinded for diagnosis and treatment of the patients and merged into one final central or peripheral outcome. Thresholds were manually set, above the bar of negative droplets cloud. A sample was screened positive if a cloud of clustered positive droplets gave a higher concentration (copies/μL) and number of droplets than the negative background controls, and was higher than the minimal detection limit of ddPCR being 0.25 copies/μL. The ddPCR was repeated in case of doubt, inconsistency between the research

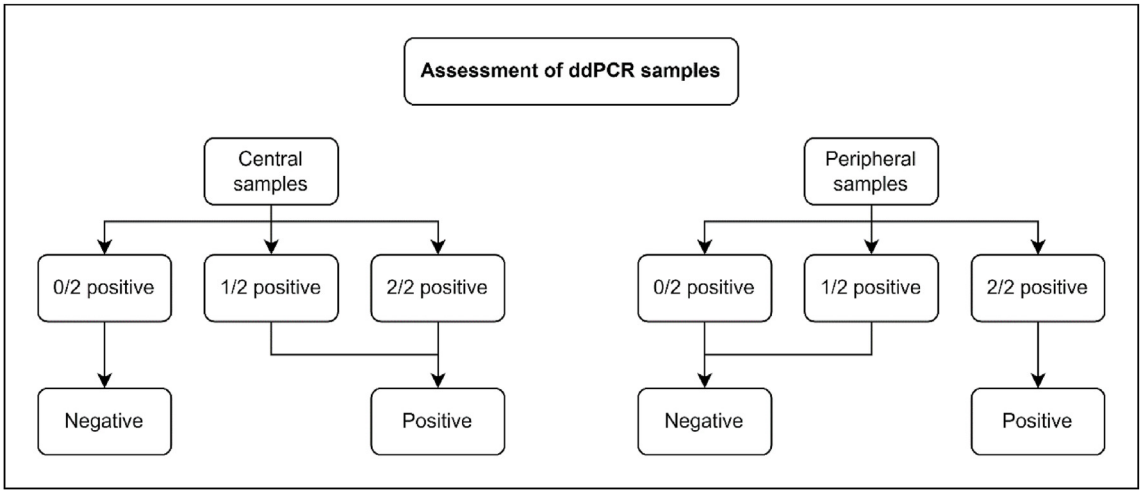

Figure 2. Assessment of droplet digital PCR (ddPCR) samples. Separate outcomes for central and peripheral blood samples.

technicians or technical problems (Supplementary file B). DdPCR results were reported for each individual sample.

Statistical methods

Baseline characteristics were analyzed using descriptive statistics. Continuous variables were expressed as means with standard deviation (SD), median with interquartile range (IQR), or numbers with percentages.

Test characteristics were descriptive in nature and expressed as percentages with 95% confidence interval (95% CI). A sensitivity analysis was performed with only the first event of every patient to rule out bias of multiple inclusions of a patient (Supplementary file C). The time-to-positivity (TTP) of the TP and FN samples were compared to analyze if the TTP influenced the ddPCR outcome. All analyses were performed using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp. Armonk, NY, USA).

Ethical approval

This study was reviewed and approved by the research ethics committee of the Radboudumc in Nijmegen, the Netherlands (reference number 2019–5342) and registered in the 'Nederlands Trial Register' (ID number NL7716).

Results

Study population

In total, 75 patients who developed 126 episodes of suspected CLABSIs were included. Twenty-nine patients were enrolled more than once. The majority of patients were female (69%) with a mean age of 55 years. Patients presented with a mean body temperature of 38.3 °C, median C-reactive protein (CRP) of 24 mg/L and most patients (91%) had a low sepsis severity score (qSOFA score of <2). Baseline characteristics are presented in Table 1.

Table 1 – Baseline characteristics.

Patient characteristics	n = 75
Female - no. (%)	52 (69)
Age - mean (±SD)	55 (17)
Pathological mechanism of CIF – no. (%)	
Short bowel syndrome	24 (32)
Gastrointestinal motility disorder	39 (52)
Extensive small bowel mucosal disease	2 (3)
Intestinal fistula	1 (1)
Mechanical obstruction	3 (4)
Other	6 (8)
Suspected bloodstream infection episode	n = 126
Physical examination at baseline	
Days of fever before admission - median (range)	0 (0–20)
Body temperature °C - mean (±SD)	38.3 (1.2)
Fever - no. (%)	
Developing <1 h after start HPN	15 (12)
Developing >1 h after start HPN	26 (20)
Not during infusion of HPN	21 (16)
No fever	8 (7)
Other	3 (3)
Unknown	53 (42)
Heart rate - mean per min (±SD)	97 (22)
Systolic blood pressure - mean (±SD)	119 (21)
Diastolic blood pressure - mean (±SD)	70 (16)
Respiratory rate - mean (±SD)	18 (5)
Glasgow coma scale – no. (%)	
15	126 (100)
<15	0 (0)
Chills - no. (%)	65 (52)
qSOFA - no. (%)	
0	75 (59)
1	40 (32)
2	7 (6)
Unknown	4 (3)
Inflammatory signs exit-site - no. (%)	
Yes	39 (31)
No	78 (62)
Unknown	9 (7)
Laboratory examination	
Leukocytes x10^9/L (median IQR)	8.6 (6–12)
CRP mg/L (median IQR)	24 (7–60)
Lactate mmol/L (median IQR)	1.5 (1.2–2)
CVADs	
Type of CVAD - no. (%)	
Tunneled catheter	81 (64)
Subcutaneous port system	24 (19)
Non-tunneled catheter	1 (1)
PICC	15 (12)
Arteriovenous fistula	5 (4)
CVAD lumen - no. (%)	
Single lumen	90 (71)
Multi lumen	10 (8)
Unknown	1 (1)
Not applicable	25 (20)
Site of vein insertion - no. (%)	
Left	57 (45)
Right	68 (54)
Unknown	1 (1)
Vein used for insertion - no. (%)	
Jugular	72 (57)
Subclavian	21 (17)
Femoral	12 (9)
Other	20 (16)
Unknown	1 (1)

CIF: chronic intestinal failure; CRP: C-reactive protein; CVAD: central venous access device; HPN: home parenteral nutrition; PICC: peripherally inserted central catheter; qSOFA: quick Sequential Organ Failure Assessment; SD: standard deviation. Missing laboratory values (CRP: 3, leukocytes: 3, Lactate: 18). Reference values: (CRP: <10 mg/L, leukocytes: 4–11*109/L, lactate: 0.8–2.1 mmol/L).

The 126 suspected CLABSI episodes resulted in 194 blood samples; 80 central and 114 peripheral. In total, 75 blood samples yielded positive blood culture results, and 60 ddPCR samples showed positive ddPCR results, as shown in Fig. 3. The detected and missed pathogens by blood culture or ddPCR are shown in Supplementary file D.

Test characteristics

Central and peripheral ddPCR results were analyzed separately and compared to blood cultures. The central ddPCR results had a sensitivity of 91% (95%CI 77—98) and specificity of 96% (95%CI 85—99), whereas peripheral ddPCR results had a sensitivity and specificity of 63% (95%CI 46—77) and 99% (95%CI 93—100), respectively. Combined results showed a sensitivity of 76% (95%CI 65—85) and a specificity of 97% (95%CI 93—99). Remaining test characteristics are summarized in Table 2 and Supplementary file E.

Test characteristics per pathogen group

In Table 2, test characteristics are shown for Gram-positive bacteria, Gram-negative bacteria and fungi. In total, 75 blood cultures were positive; resulting in 52 Gram-positive, 26 Gram-negative, and three fungi-positive blood samples. In comparison, 60 positive ddPCR samples resulted in 40 Gram-positive, 23 Gram-negative, and two fungi positive samples (of two different patients). Only specific non-common commensals were discussed with an infectiologist, like the *Carnobacterium divergens*, given the limited pathogenic potential of this pathogen.

False positive and false negative blood samples

In total, three ddPCR results were FP and 18 FN. Central ddPCR results showed two FP and three FN samples. In contrast, peripheral ddPCR results showed one FP and 15 FN

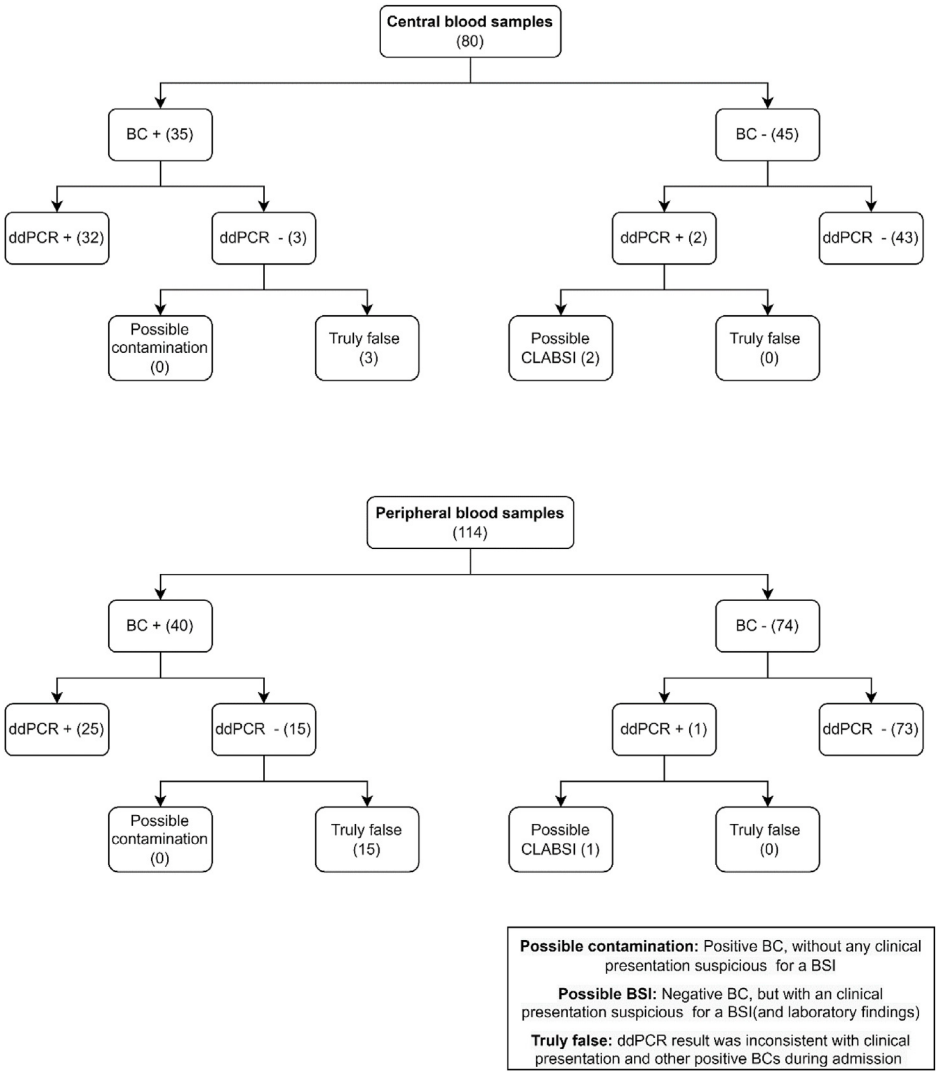

Figure 3. Flowchart central and peripheral blood samples, Possible contamination: Positive blood cultures without any clinical presentation suspicious for a (CLABSI); Possible CLABSI: Negative BC, but with an clinical presentation suspicious for a CLABSI (including laboratory findings); Truly false: ddPCR result was inconsistent with clinical presentation and other positive BCs during admission. BC: blood culture; CLABSI: central line-associated bloodstream infection; ddPCR: droplet digital polymerase chain reaction.

Table 2 ddPCR test characteristics.

	Sensitivity - % (95%CI)	Specificity - % (95%CI)	PPV - % (95%CI)	NPV - % (95%CI)	PLR	NLR
Overall test results						
Central	91 (77—98)	96 (85—99)	94 (80—98)	93 (83—98)	21 (5—80)	0.09 (0.03—0.27)
Peripheral	63 (46—77)	99 (93—100)	96 (78—99)	83 (77—88)	46 (7—329)	0.4 (0.3—0.6)
Combined	76 (65—85)	97 (93—99)	95 (86—99)	87 (80—92)	30 (10—93)	0.3 (0.2—0.4)
Per pathogen group						
Central						
Gram-positive	83 (61—95)	100 (94—100)	100 (NA)	93 (85—97)	NA	0.2 (0.07—0.4)
Gram-negative	92 (64—100)	94 (85—98)	75 (53—89)	98 (91—100)	15 (6—41)	0.08 (0.01—0.5)
Fungi[a]	50 (1—99)	99 (93—100)	50 (8—92)	99 (95—100)	39 (4—426)	0.5 (0.1—2)
Peripheral						
Gram-positive	66 (46—82)	98 (92—100)	90 (70—97)	89 (83—93)	28 (7—112)	0.4 (0.2—0.6)
Gram-negative	46 (19—75)	99 (95—100)	86 (44—98)	93 (90—96)	47 (6—357)	0.5 (0.3—0.9)
Fungi[a]	0 (0—98)	100 (97—100)	NA	99 (99—99)	NA	1 (1—1)

[a] Large confidence intervals of the sensitivity and NPV are due to the small numbers of fungal cases.
95%CI: 95% confidence interval; Central: central blood samples; ddPCR: droplet digital polymerase chain reaction, Gram-positive: Gram-positive bacteria; Gram-negative; Gram-negative bacteria; NA: not applicable; NLR: negative likelihood ratio; NPV: negative predictive value; Peripheral: peripheral blood samples; PLR: positive likelihood ratio; PPV: positive predictive value.

results. An extensive overview of all FP and FN cases and associated outcomes is shown in Supplementary file F. In order to explore if pathogen load influenced outcomes of the ddPCR, the TTP of blood cultures FP and FN ddPCR samples are shown in Fig. 4. Central blood cultures had a median TTP of 8 and 18 h for the TP and FN ddPCR results, respectively. The TTP for peripheral blood cultures was 17 and 15 h for the TP and FN ddPCR results, respectively.

Discussion

Early detection of CLABSIs and pathogen identification is of key importance in CIF patients to improve their outcomes, including CVAD salvage. In this prospective cohort study, 16S/28S rRNA ddPCR was compared with blood cultures and showing for central ddPCR samples a sensitivity of 91% and specificity of 96%, and for peripheral ddPCR samples a sensitivity of 63% and specificity of 99%. The outcomes of the central ddPCR samples seem promising, especially given the fact that ddPCR samples were only obtained after two 10 mL blood samples had been drawn for blood culturing, thus not interfering with the gold standard for detection of CLABSIs. This most likely results in a lower pathogen DNA load for the subsequent ddPCR blood samples. Moreover, the short time to diagnosis of the ddPCR (two and a half hours) compared to blood cultures (up to five days) enables the clinician to adjust treatment in a timely manner. Especially in CIF patients a short time to diagnosis seems key to improve CVAD salvage outcomes and ddPCR may guide decisions with respect to catheter removal in clinical emergencies in these patients who

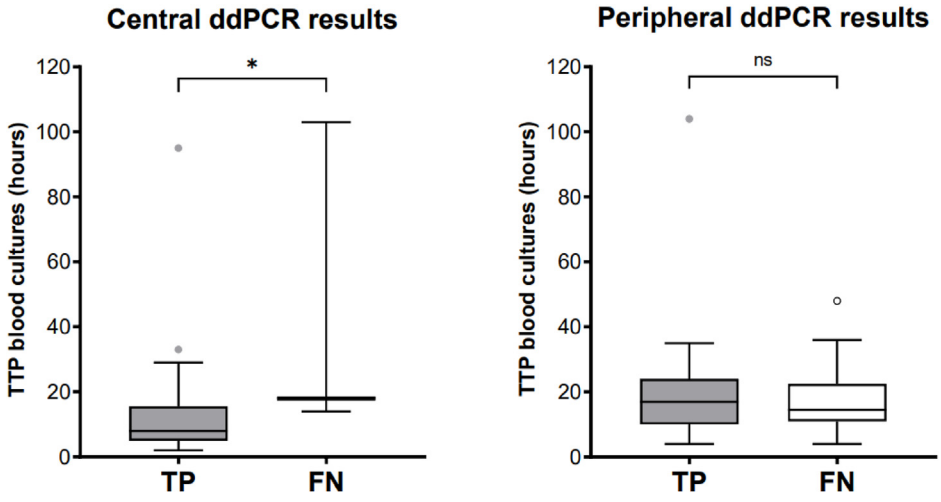

Figure 4. Time-to-positivity of positive blood cultures relative to ddPCR results. True positive (TP): both ddPCR and blood culture were positive; False negative (FN): positive blood culture and negative ddPCR results; TTP: Time-to-positivity in hours. The central blood samples included 32 and 3 cases for TP and FN, respectively. The peripheral blood samples included 25 and 15 cases for TP and FN, respectively.

heavily depend on their CVADs. In addition, an early diagnosis may reduce the hospital admission period, antibiotic use and possibly antibiotic resistance.[19,20]

To our knowledge, this is the first study reporting separate outcomes for centrally and peripherally obtained ddPCR samples. So far, most studies on ddPCR merely focused on specific pathogen detection or on laboratory data.[13,21] A few semi-comparable studies describe peripheral data, and only one study from Wu et al. combines both central and peripheral blood samples, but does not differentiate the outcomes.[22,23] This issue is obviously important as substantial differences were found in pathogen detection between central and peripheral ddPCR samples (sensitivity 91% and 63%, respectively), which probably reflects the difference in microbial concentrations that originate from the intraluminal biofilm that develops over time in the CVAD of these CIF patients.[24] The previously mentioned studies reported a higher sensitivity and lower specificity than seen in our peripheral ddPCR samples. Wu et al. describes in an intensive care unit setting a sensitivity of 73% and specificity of 63%[23] and a recent study of Lin et al. describes a sensitivity of 75% and specificity of 75%.[25] Two other studies had a calculated sensitivity of 73—90% and specificity of 18—39%.[22,26] A possible explanation for the lower peripheral sensitivity in our study may be the patient setting with a lower septic profile score, which may reflect a lower pathogen load and a higher chance of FN outcomes. In this respect it is important to note that our CIF patients are instructed to immediately contact the hospital when suspecting a CLABSI, which probably is also reflected by the low qSOFA score: 91% of our study population had a score of 0—1 which indicated no high risk for in-hospital mortality compared to a SOFA score of 7—11.3 in previously mentioned studies with a high risk of in-hospital mortality.[22,23,26] An explanation for the higher test specificity in our cohort compared to a study by Hu et al. is the fact that recent antibiotic use was an exclusion criterion for enrollment in our investigation, whereas in the former study 91% of patients received antimicrobial drugs before blood culture and ddPCR sampling.[22] It is known that antibiotic use decreases the sensitivity of the blood cultures which may increase the chance of false positive results of molecular testing. However, it leaves the question whether reported negative blood culture results in the work by Hu et al. may in fact have rather been false negatives with the ddPCR results being true positive. Moreover, it is important to acknowledge that mentioned studies all use different primers and probes, a factor that may account for variations in sensitivity and specificity.

We used three probes to differentiate between Gram-positive, Gram-negative and fungi as described in our previous study.[15] The sensitivity for both Gram-positive and Gram-negative pathogen groups confirmed that our primers and probes are well-designed. The few cases of fungal infections precluded any conclusions about the sensitivity for this group. However, the fungal specificity for both central and peripheral ddPCR samples seems high (99—100%), which is important for clinical decision making. A fungal infection is an indication for CVAD removal, with often a delay in diagnosis and treatment due to the long TTP of blood culture results. Our current results suggest that a fungal infection might be ruled out within two and a half hours. In the current study we analyzed data at pathogen group level. However, we also learned that the binding efficiency of probes varies resulting in differences of the amplitude of a droplet cloud. This notion often allows discrimination between different species within one pathogen group and sometimes resulted in detection of additional species next to the findings in the blood culture.[15]

Blood cultures and ddPCR are mechanistically different techniques as may be reflected in different outcomes and which hampers comparisons between both diagnostic tests (e.g. competition plays a role during blood culturing). For this reason, we assessed all FP and FN cases, and categorized these as possibly contaminated, possible CLABSI or truly false blood samples (Fig. 3). All three FP ddPCR results were categorized as a possible CLABSI, because clinical signs suggested a CLABSI infection, despite negative blood culture results. However, the 18 FN ddPCR samples seemed truly false. Most FN were encountered in peripheral samples, but were still detected in central samples. This may be explained by biofilm formation in the CVAD as described earlier. In addition, the findings in central FN ddPCR results may be explained by the longer TTP that is related to a lower pathogen load or results from the fact that ddPCR samples are taken after blood culture samples were taken, probably resulting in a lower pathogen DNA load (Fig. 4). Finally, some FN cases may be explained by poor binding of the probe. However, a disadvantage is that lower binding efficiency results in a lower droplet cloud amplitude and may result in an overlap between the negative droplet bar (and low background signal) and a pathogen cloud (e.g. *Corynebacterium* species), especially in ddPCR samples with a low pathogen load. Furthermore, two pathogens were detected but categorized to the wrong Gram-stain group. For example, *Corynebacterium amycolatum* and *Leifsonia* were detected as Gram-negative, but are in reality Gram-positive bacteria (supplementary file F). The latter results from the fact that the DNA sequence of these pathogens seems more compatible with the Gram-negative probe when compared with the Gram-positive alternative. The clinical relevance of this finding seems limited since infections based on these particular microbes, at least in our population, are rare.

Stringent criteria for interpretation of blood culture results in CLABSI exist[16]; future studies should also focus on developing interpretation criteria for molecular diagnostics for CLABSI (Supplementary file E). In this study the central ddPCR result was coined as positive in case one of two central ddPCR samples yielded a positive result, whereas the peripheral ddPCR result was categorized positive only in case both peripheral ddPCR samples were positive (Figs. 1 and 2).

DNA isolation is a crucial step in ddPCR analysis for which several options exist. Given that time-to-diagnosis is one of the major advantages of ddPCR over blood culture analysis, we focused on a simple yet effective isolation method to save time. In this vein for this current article, we have optimized our DNA isolation protocol using beads instead of enzymatic digestion, which may results in a lower rate of FN test results in the Gram-positive spectrum (unpublished results).[13,15] In the future it would be interesting to explore options to increase the sensitivity of the ddPCR. An option would be to compare 2 μL DNA with 4 μL, especially for the

peripheral samples. Another possibility would be to firstly eliminate human DNA and then isolate microbial DNA so only DNA of these pathogens can be added to the ddPCR mixture in order to reduce viscosity and increase sensitivity. This isolation of DNA however will take approximately 1 h longer and may result in longer intervention times.

Strengths of this study include its prospective design, the robust amount of patients and blood samples of this specific CIF population, and the differentiation between centrally and peripherally obtained blood samples. To our knowledge, our research group is the first to use pan-bacterial and pan-fungal rRNA primer/probe sets.[15] Prior studies used six assay panels or assessed a single pathogen.[21,22,25,26] One study also used pan-bacterial 16S rDNA, but only to detect *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pneumonia* and compare their results with the outcome of specific probes for the aforementioned pathogens.[27] Also, we further optimized our 16S/28S rRNA ddPCR and designed a flowchart to enable assessment of individual ddPCR samples, which has not been described previously. A limitation of our study inherent to the use of this molecular technique is the fact that we did not obtain an antibiogram. However, in the future, these former two points may be solved by measuring more fluorescent channels. This enables the development of more specific primers and/or probes for common resistance genes and for the most important and clinically relevant pathogens besides the broad spectrum 16S primers. In addition the 16S and 28S probes enable detection of a broad range of bacteria and fungi, but some low-amplitude pathogen DNA clouds may have been missed because of an overlap with the negative droplet bar (and low background noise). Finally, this study had a low number of patients who developed fungal infections, which precluded any conclusions regarding the sensitivity of our fungal primers and probes. Obviously, our results need to be confirmed in prospective clinical studies with simultaneous drawing and processing of ddPCR and blood culture samples to assess patient outcomes, length of hospital stay and antibiotic use.

In conclusion, the 16S/28S rRNA ddPCR is a promising technique for an early detection of CLABSI and shows a high sensitivity and specificity relative to blood cultures, especially in centrally drawn blood samples.

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Data sharing

Data described in the manuscript, code book, and analytic code will be made available upon request pending approval.

Conference presentation

Oral presentation ESPEN Vienna (2022) and World Congress for Vascular Access Athens (2022).

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2024.03.003.