



# Polymerase Chain Reaction Versus Blood Culture to Detect *Candida* Species in High-Risk Patients with Suspected Invasive Candidiasis: The MICAFEM Study

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## ABSTRACT

**Introduction:** We evaluated the diagnostic reliability of serum polymerase chain reaction (PCR) versus blood culture, abdominal fluid or both (composite measure) in patients receiving empirical antifungal treatment for suspected invasive candidiasis.

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**Methods:** This observational, prospective, non-interventional, multicentre study in Spain enrolled 176 critically ill patients admitted to the intensive care unit. Separate blood samples for culture and serum PCR were taken before the start of antifungal therapy. Patient assessment was performed according to each site's usual clinical practice. The primary end point was concordance between serum PCR and blood culture. Secondary end points were concordance between serum PCR and a positive abdominal fluid sample or the composite measure. Quality indices included sensitivity, specificity, positive/negative predictive values (PPV/NPV) and kappa indices.

**Results:** Among 175 evaluable patients, rates of *Candida* detection were similar for serum PCR ( $n = 16/175$ , 9.1%) versus blood culture ( $n = 14/175$ , 8.0%). Quality indices for serum PCR relative to blood culture were: sensitivity 21.4%;

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specificity 91.9%; PPV 18.8%; NPV 93.1%; kappa index 0.125. Thirty-two abdominal fluid samples were positive. Quality indices for serum PCR versus abdominal fluid were: sensitivity 31.3%; specificity 83.0%; PPV 15.6%; NPV 92.3%; kappa index 0.100. Quality indices for serum PCR versus the composite measure were: sensitivity 15.8%; specificity 92.7%; PPV 37.5%; NPV 79.9%; kappa index 0.107.

**Conclusion:** The sensitivity of serum PCR for *Candida* detection was low and the rate of concordance was low between serum PCR and the other diagnostic techniques used to identify *Candida* infections. Hospital-based diagnostic tests need optimising to improve outcomes in patients with suspected invasive candidiasis.

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**Keywords:** Abdominal fluid; Blood cultures; *Candida*; *Candida* diagnosis; Empirical antifungal treatment; Invasive candidiasis; Intensive care unit; PCR

## INTRODUCTION

*Candida* species are among the top ten blood-stream pathogens [1] and remain the most common cause of invasive fungal infections worldwide [2–5]. There is wide inter-regional variability in the incidence of blood infections due to *Candida* spp. (candidaemia). One review suggests the incidence may vary between 1.2 and 25 cases per 100,000 inhabitants [6]. Invasive candidiasis, which occurs when *Candida* spp. infects other tissues and organs, causes significant morbidity and high mortality rates [1, 7–9]. Furthermore, the costs associated with invasive candidiasis are substantial because of the need to use additional diagnostic procedures, prolonged hospitalisation and extra medications [10–12].

Patients with haematological malignancies or neutropenia, those undergoing gastrointestinal surgery, premature infants or patients aged > 70 years are at increased risk for acquiring candidaemia during hospitalisation due to an underlying medical condition [13]. Within these high-risk groups, specific factors further increase the risk for invasive candidiasis, for

instance, surgery, the presence of vascular catheters, mucosal colonisation with *Candida* spp., sepsis, length of previous antibiotic therapy and receipt of total parenteral nutrition [13–20]. These risk factors are frequently encountered in critically ill patients in the intensive care unit (ICU) [21] where the incidence of candidaemia is tenfold higher compared with other hospital wards, and mortality rates are between 25 and 50% [22–25].

The difficulties in obtaining an early and accurate diagnosis of invasive candidiasis in critically ill patients have led to the widespread use of empirical antifungal treatment in such patients [25–27]. Empirical antifungal treatment in high-risk patients is currently recommended by the European Society for Clinical Microbiology and Infectious Diseases [28] as well as by the Infectious Diseases Society of America [29]. There is moderate-quality evidence showing that empirical antifungal therapy with an echinocandin can be effective in high-risk settings; therefore, the echinocandins are strongly recommended for empiric treatment in non-neutropenic patients with suspected invasive candidiasis within an ICU setting [30]. However, this strategy may contribute to the overuse of antifungals and could increase the risk of antifungal resistance, which may have implications for the length of hospital stay, lead to adverse patient outcomes and potentially increase healthcare costs [9, 31, 32].

Blood culture is the current ‘gold’ standard for diagnosing invasive candidiasis, despite its numerous limitations, which include delays of up to 48 h until results are available for the majority of patients [33, 34] and possibly longer when information on specific species is required [35]. As it has been shown that delays in initiating appropriate treatment significantly increase the all-cause mortality rate, independently of other risk factors [33, 34], a prompt and accurate diagnosis of invasive candidiasis is crucially important. In addition, blood cultures show positivity in only a minority of cases (especially in patients with deep-seated candidiasis) and often only late during the course of infection [1].

To overcome these difficulties, polymerase chain reaction (PCR)-based assays have been

introduced as an alternative tool for diagnosing invasive candidiasis [25]. The advantages of PCR testing include same-day identification of *Candida* spp. and the potential for relatively fast and continuous monitoring of persistence or resolution of infection [36]. A comprehensive systematic review and meta-analysis of PCR diagnosis for invasive candidiasis has been conducted: 54 studies comprising 4694 patients (of whom 963 had proven, probable or possible invasive candidiasis) were included. The results from this meta-analysis demonstrated that PCR can have a higher diagnostic sensitivity than blood culture, with pooled sensitivity and specificity values of 95% and 92%, respectively. Importantly, PCR allowed the earlier diagnosis of *Candida* infections [36].

The aims of the current study were to evaluate the diagnostic reliability of serum PCR versus either blood culture or abdominal fluid samples (and both as a composite measure) and to assess the clinical response to antifungal therapy in critically ill patients with suspected invasive candidiasis admitted to ICUs across Spain.

## METHODS

### Study Design and Population

This was a prospective, multicentre, observational, non-interventional study (Astellas study no. AST-INF-2012-01) conducted in 27 centres and 9 regions across Spain. The date of first enrolment was 18 March 2013 and the last evaluation was completed on 2 January 2015. An Independent Ethics Committee or an Institutional Review Board reviewed and approved the protocol, protocol amendments and patient information before the start of the study. The "master" ethics committee was the H. Clinico San Carlos committee (a list of all ethics approval bodies that approved the study in the various centres is included in the supplementary material). The study was conducted in accordance with the principles of the 1964 Declaration of Helsinki and its later amendments, Good Clinical Practice guidelines and International Conference on Harmonisation

guidelines. Informed consent was provided by all patients, or their legal representative, before the start of the study.

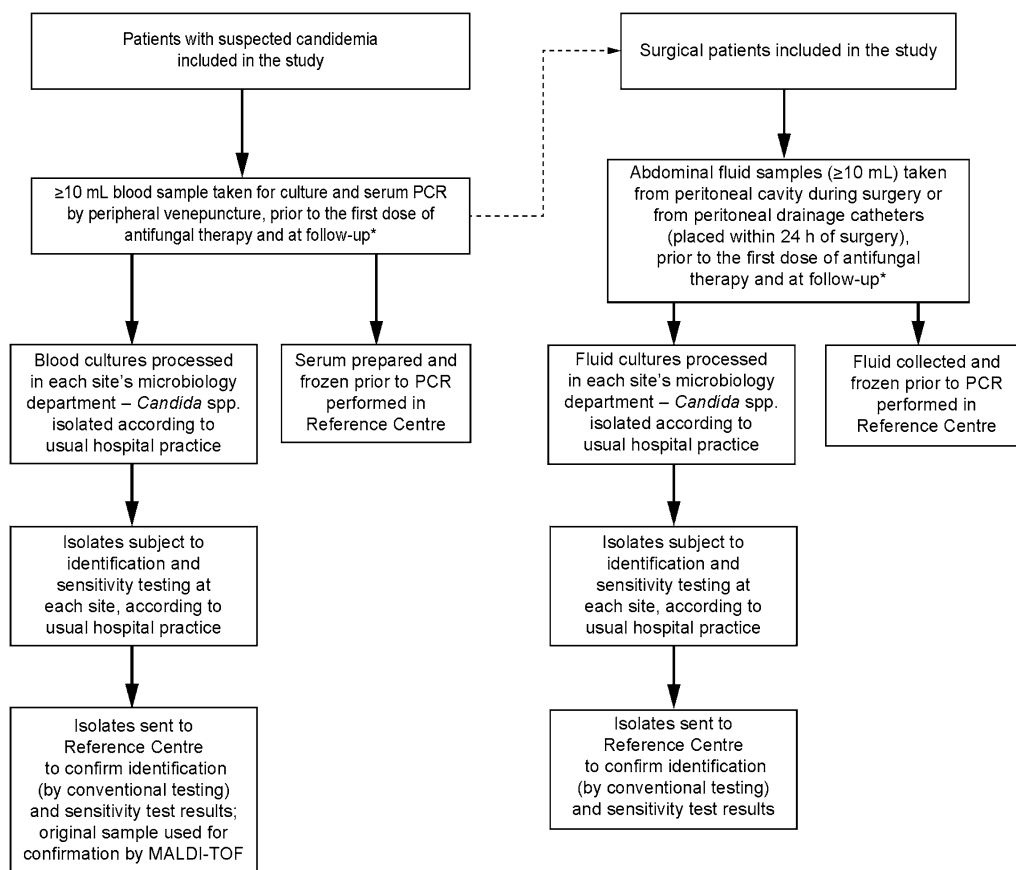
Inclusion criteria were male or female patients aged  $\geq 18$  years admitted to the ICU and requiring empirical antifungal therapy because of a high risk of invasive candidiasis. Patients meeting at least one of the following criteria were considered at high risk: those with a *Candida* score  $\geq 3$  (based on severe sepsis, 2 points; surgery, 1 point; parenteral nutrition, 1 point; *Candida* at multiple sites of infection, 1 point); patients with sepsis and  $\geq 3$  risk factors for invasive candidiasis (from severe pancreatitis, extra-renal depuration, broad-spectrum antibiotic treatment for  $> 7$  days, abdominal surgery, ICU stay of  $> 15$  days, parenteral nutrition, central venous catheterisation, *Candida* at multiple sites of infection, candiduria and Acute Physiology and Chronic Health Evaluation score  $> 15$ ); or immunocompromised patients with sepsis. Patients with documented candidiasis at enrolment, patients taking antifungals at the time of screening or those with any condition that, in the opinion of the investigator, rendered their participation inadvisable were excluded from enrolment.

### Treatment and Response Criteria

This was a non-interventional study. Patients were recruited consecutively and managed and treated according to the physicians' usual clinical practice. The decision to prescribe antifungal therapy was made prior to (and independently from) the patient's inclusion in the study. Once the decision had been made to initiate treatment, patients were recruited into the study and the pertinent data and samples were collected at enrolment prior to initiating antifungal treatment, which was then administered according to the physicians' usual practice.

Any other treatments that the patients could receive were according to the treating physician's regular clinical practice and each site's health care protocols.

Investigators were asked to make a clinical assessment of the clinical and microbiological



**Fig. 1** Microbiological analyses. \*Patients meeting the following criteria were considered at high risk: those with a *Candida* score  $\geq 3$  (based on severe sepsis, 2 points; surgery, 1 point; parenteral nutrition, 1 point; *Candida* at multiple sites of infection, 1 point); patients with sepsis and  $\geq 3$  risk factors for invasive candidiasis (from severe acute pancreatitis, extra-renal deputation, broad-spectrum antibiotic treatment for  $> 7$  days, major abdominal surgery, ICU stay of  $> 15$  days, parenteral nutrition, central venous catheterisation, *Candida* at multiple sites of infection, candiduria and Acute Physiology and Chronic Health Evaluation score  $> 15$ ); or sepsis in

immunocompromised patients with neutropenia, haematological disease, solid organ or bone marrow transplantation, treatment with immunosuppressive drugs, chemotherapy or monoclonal antibodies; or human immunodeficiency virus infection. \*\*Blood sample times were as follows: at baseline (before initiating antifungal treatment) and at follow-up (blood cultures were taken according to the site's usual clinical practice). Serum PCR was performed at the same time as the blood culture sample. ICU intensive care unit, MALDI-TOF matrix-assisted laser desorption/ionisation time of flight, PCR polymerase chain reaction

response to empirical antifungal treatment for the suspected invasive candidiasis.

### Microbiological Analyses

Microbiological analyses are summarised in Fig. 1. A blood sample ( $\geq 10$  ml) for culture and serum PCR was taken by peripheral venepuncture, before the first dose of antifungal therapy.

Follow-up blood samples for culture and serum PCR were taken according to the site's usual clinical practice/guideline recommendations 3 and 7 days after the baseline sample. Blood sample collection and management were standardised across the study centres. Samples for PCR were sent to the National Microbiology Centre (CNM).

Blood cultures were processed within each site's microbiology department, according to

usual hospital practice, with identification of isolated strains. When a *Candida* spp. was isolated, identification and sensitivity tests were performed within each site, according to a standardised protocol. The original blood samples were then frozen and sent to the Reference Centre (National Microbiology Centre, Hospital Clínico San Carlos) to confirm the initial identification and sensitivity results. At the Reference Centre, *Candida* isolates were identified, and the sensitivity results determined by the minimum inhibitory concentration to antifungal therapy were confirmed by conventional testing, with the original sample used to confirm identification by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry.

The remainder of the blood sample used for the PCR assay was centrifuged at 3000 rpm for 10 min to separate the serum, which was then frozen at  $-20^{\circ}\text{C}$ . The frozen serum samples were stored at each site's microbiology department until they were sent to the Reference Centre. All the samples were identified by specifically designed labels, and the sites also had specially designed data sheets for accurate sample matching and identification.

Abdominal fluid samples were obtained from the peritoneal cavity during laparotomy or from peritoneal drainage catheters (placed within 24 h of surgery) and were cultured according to each site's regular clinical practice and subjected to sensitivity testing. When *Candida* spp. were identified, isolates were sent to the Reference Centre to confirm the initial identification and sensitivity results by conventional testing.

### Multiplex Real-Time PCR Assay

Deoxyribonucleic acid (DNA) for PCR assays was extracted from blood and sera using the QIAamp<sup>®</sup> DNA Mini kit (Qiagen, Izasa, Madrid, Spain), following the manufacturer's instructions. DNA was eluted in 50  $\mu\text{l}$  of buffer, and 2.2  $\mu\text{l}$  of DNA extracted from each sample was used for subsequent PCR. Two multiplex PCR assays, developed by Fortun et al. (2014), were used to detect six *Candida* species common in IC (*Candida albicans*, *C. parapsilosis*, *C. tropicalis*,

*C. glabrata*, *C. krusei*, and *C. guilliermondii*); these PCR assays have been described in detail elsewhere [25]. Briefly, *Candida* spp. were detected using molecular probes with fluorescent dyes (FAM, HEX, ROX, and CYAN 500). Probes and primers were designed based on the ITS1/2 sequences of ribosomal DNA regions of *Candida* strains belonging to the Spanish National Center of Microbiology collection, using Beacon Designer 5.0 software (Premier Biosoft, Palo Alto, CA, USA). Two multiplex PCRs were performed simultaneously in the LightCycler 480 system (Roche Diagnostics, Mannheim, Germany), one using the LightCycler Probes Master Kit (Roche Diagnostic, Madrid, Spain) to detect *C. albicans*, *C. parapsilosis* and *C. tropicalis* and one using the 2  $\times$  Sensimix Probe Kit (Quantace, Ecogen, Madrid, Spain) to detect *C. glabrata*, *C. krusei* and *C. guilliermondii*. Samples were analysed in duplicate and standards were run with each set of samples and negative controls. The following CT values were used to define a positive PCR result: *C. albicans* (20 fg DNA) 34.15–37.44; *C. parapsilosis* (20 fg DNA) 31.45–35.39; *C. tropicalis* (20 fg DNA) 32.34–36.16; *C. glabrata* (200 fg DNA) 31.10–36.24; *C. krusei* (20 fg DNA) 34–38 (200 fg DNA) 30.80–33.80; *C. guilliermondii* (20 fg DNA) 38.40–42.31. The overall result was considered positive if both PCRs for each sample were positive according to the laboratory standard. Sensitivity was determined at the Reference Centre; isolates were identified and the sensitivity results, determined by the minimum inhibitory concentration of *Candida* isolates to antifungal therapy, were confirmed by conventional testing.

Those who conducted the blood/abdominal fluid tests were blinded to the results of the PCR analyses, and vice versa.

A diagnosis of invasive candidiasis was made when a positive blood culture or positive abdominal fluid sample was obtained from a surgical setting or a peritoneal drainage catheter (placed within 24 h of surgery) or when a positive PCR was recorded in samples from patients whose clinical condition improved with antifungal treatment.



## End Points

The primary end point was the degree of concordance between the results of the serum PCR and blood culture. Secondary end points were the degree of concordance between the results for serum PCR and a positive abdominal fluid culture and positivity for blood culture or abdominal fluid culture (assessed as a composite measure).

The relationship between the presence of invasive candidiasis and the risk factors that contribute to a patient's high-risk status (*Candida* score  $\geq 3$ ; sepsis and  $\geq 3$  risk factors for invasive candidiasis; sepsis and immunosuppression) was also investigated.

Data were collected from all patients at the time of enrolment and, if available, when follow-up blood cultures were performed, on discharge from the ICU and at the end of antifungal treatment.

## Statistical Analysis

The sample size was calculated based on the primary end point using estimates of the sensitivity of serum PCR (80%) and blood culture (50%), based on the published literature [35, 37], and the kappa index of concordance between both techniques (0.3) in patients with suspected invasive candidiasis, admitted to ICUs in which empirical treatment was initiated with an antifungal. Taking these estimates into account, a sample size of 179 patients would provide precision of  $\pm 0.1$  to estimate concordance between serum PCR and blood culture diagnoses, with 95% confidence intervals (CIs). Using the assumption that 10% of patients would not be eligible, the planned sample size was 199. The sample size calculation was performed using EpiData software, version 3.1 (The EpiData Association, Odense, Denmark).

The analysis population included all patients who fulfilled the entry criteria and were considered evaluable. Concordance between the results of serum PCR and blood culture (primary end point) was assessed by estimating the kappa index. The kappa index, which assesses inter-test reliability, can range from  $-1$  to  $+1$ , with

higher scores indicating greater concordance [38].

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) and the respective 95% CIs were calculated for serum PCR relative to blood culture.

Positive and negative odds ratios (ORs) and post-test probabilities [positive post-test probability (PPTP) and negative post-test probability (NPTP)] were also determined.

Concordance between the results of serum PCR versus abdominal fluid culture and versus the composite measure were analysed in the same way as the primary end point. The relationship between the presence of invasive candidiasis and patient risk factors (*Candida* score  $\geq 3$ ; sepsis and  $\geq 3$  risk factors for invasive candidiasis; sepsis and immune suppression) was analysed using a Chi-squared test. Binary logistic regression models were used to determine which risk factors had the greatest weight, according to the microbiological test used. These models were executed hierarchically and with the stepwise backward method. Data were presented as ORs and 95% CIs.

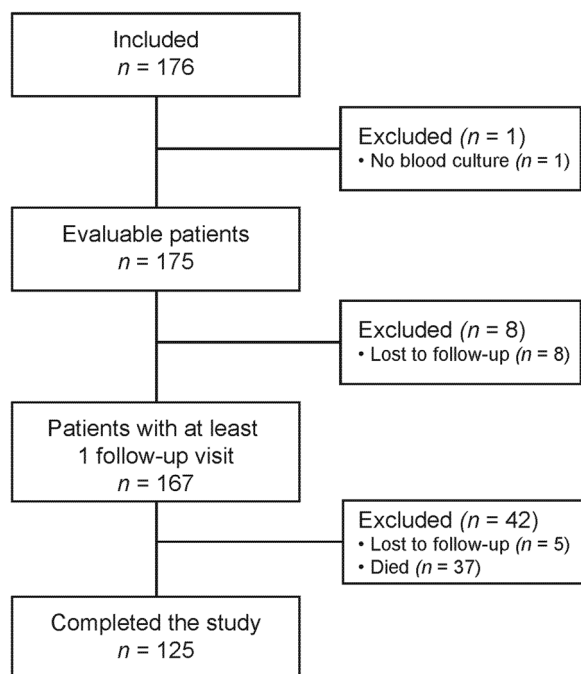
An adjusted binary logistic regression model (with a bilateral significance level of 0.10, Chi-squared test) was used to evaluate which of the individual risk factors were related to the presence of invasive candidiasis according to blood culture results. All calculations were performed with STATA for Windows, version 12 (<http://www.stata.com/>).

## RESULTS

### Patient Population

Overall, 176 patients were recruited into the study; of these, 175 patients were regarded as evaluable and included in the analysis set.

Of the total evaluable patients, 125 (71.4%) completed the study period and 50 (28.6%) withdrew early. Among the patients who withdrew early, 37 died (21.1% of the total evaluable patients). The most common reasons for death were multi-organ failure and septic shock [ $n = 17$  (9.7%) and  $n = 10$  (5.7%), respectively].



**Fig. 2** Flow chart of the study population

A flow diagram detailing patient disposition is presented in Fig. 2.

Demographic data and clinical characteristics are summarised in Table 1. The mean age was 67 years and most patients were male. The mean duration of stay in the ICU was 6.3 days. A total of 132 (75%) patients had a *Candida* score  $\geq 3$  and 169 (96.0%) had sepsis. In total, 99 patients (56.3%) had concomitant infections and 167 (94.9%) were receiving concomitant antibiotic treatment. The median number of days that patients were in the ICU before initiation of antifungal treatment was 5 days (interquartile range, 1–12 days).

### Empirical Antifungal Treatment

In patients with available data ( $n = 116$ ), the mean (SD) duration of antifungal treatment was 21.72 (17.50) days. For participants who received  $\geq 1$  follow-up visit ( $n = 167$ ), the microbiological response was non-evaluable for the majority of patients ( $n = 122/167$ , 73.1%). According to clinical criteria, 34 (20.4%) of patients were cured, 76 (45.5%) were improved and 57 (34.1%) experienced treatment failure.

*Candida* eradication was achieved in 28/167 (16.8%) patients, but persisted (at the end of antifungal treatment) in 17/167 (10.2%) patients.

### Primary End Point

Rates of detection of *Candida* infection were similar for blood culture and serum PCR [ $n = 14/175$  (8.0%) with blood culture and  $n = 16/175$  (9.1%) with serum PCR]. *C. albicans* was the most common pathogen and was detected with a greater frequency with serum PCR (14/16, 87.5%) than with blood culture (8/14, 57.1%). Other pathogens that were detected using both techniques were *C. glabrata* (12.5% and 42.9%, respectively) and *C. krusei* (12.5% and 7.1%, respectively). *C. tropicalis* and *C. parasilopsis* were detected in one sample each using blood culture.

Concordance rates and quality indices for the results of blood culture versus serum PCR are shown in Table 2. The sensitivity of serum PCR relative to blood culture was 21.4% and the specificity was 91.9%; the PPV was 18.8% and the NPV was 93.1%, while the PPTP and NPPTP were 18.8% and 6.9%, respectively, indicating that the concordance of serum PCR using blood culture as a reference was very low, as confirmed by the kappa value (0.125) (Table 2).

### Secondary End Points

Thirty-two patients had positive results for *Candida* spp. in the abdominal fluid. The most common species were *C. albicans* [ $n = 18$  (56.3%)] and *C. glabrata* [ $n = 9$  (28.1%)].

Concordance rates and quality indices for the results for serum PCR versus abdominal fluid samples are also shown in Table 2. The sensitivity and specificity of serum PCR relative to abdominal fluid were 31.3% and 83.0%, respectively. The PPV and NPV were 15.6% and 92.3%, respectively; PPTP was 17.0% and NPPTP was 7.7%. The kappa index was 0.100, also indicating a low rate of concordance between serum PCR and abdominal fluid samples.

When the results of serum PCR versus the composite measure of blood culture plus

**Table 1** Demographics and clinical characteristics at baseline

Patient characteristic	Mean (SD), unless specified (N = 176)
Gender, n (%)	
Male	115 (65.3)
Female	61 (34.7)
Age (years)	66.6 (12.7)
BMI (kg/m <sup>2</sup> )	28.0 (6.4)
Reason for ICU admission, n (%)	
Medical	88 (50.0)
Surgical	88 (50.0)
Abdominal	81 (46.0)
Chest	4 (2.3)
Cardiovascular	4 (2.3)
Orthopaedic and trauma surgery	1 (0.6)
Other	2 (1.1)
Duration of ICU stay (days)	6.3 (9.9)
<i>Candida</i> score	3.10 (1.16)
Patients with <i>Candida</i> score, n (%)	
< 3	44 (25.0)
3	56 (31.8)
4	67 (38.1)
5	9 (5.1)
Patients with sepsis, n (%)	169 (96.0)
Severe	43 (24.4)
Septic shock	103 (58.5)
Patients considered at high risk, n (%)	
<i>Candida</i> score $\geq 3$	132 (75.0)
Serious sepsis (2 points)	147 (83.5)
Prior abdominal surgery (1 point)	116 (65.9)
Parenteral nutrition (1 point)	108 (61.4)
Multiple colonisation (1 point)	27 (15.3)

**Table 1** continued

Patient characteristic	Mean (SD), unless specified (N = 176)
Sepsis in patients with $\geq 3$ risk factors	163 (92.6)
Serious acute pancreatitis	24 (13.6)
Extrarenal depuration	56 (31.8)
Broad-spectrum antibiotics > 7 days	105 (59.7)
Major abdominal surgery	116 (65.9)
Admission to ICU > 15 days	51 (29.0)
Parenteral nutrition	109 (61.9)
Central venous catheter	162 (92.0)
Colonisation in 2 or more locations	27 (15.3)
Candiduria	21 (11.9)
APACHE on admission > 15	120 (68.2)
Diabetes mellitus	39 (22.2)
Major burns (serious burns)	0 (0)
Sepsis in patients with immunosuppression	36 (20.5)
Neutropenia	5 (2.8)
Haematological disease	4 (2.3)
Solid organ or bone marrow transplantation	9 (5.1)
Treatment with immunosuppressive drugs, chemotherapy agents or monoclonal antibodies	29 (16.5)
Human immunodeficiency virus	5 (2.8)

*BMI* body mass index, *ICU* intensive care unit, *SD* standard deviation

abdominal fluid were examined, the quality indices remained comparable (sensitivity 15.8%; specificity 92.7%; PPV 37.5%; NPV 79.9%). The kappa index was 0.107, again indicating a very low rate of concordance between serum PCR and the composite measure.



**Table 2** Quality indices for serum PCR, blood culture, abdominal fluid and the composite measure in patients with suspected invasive candidiasis

Parameter	PCR vs. blood culture ( <i>n</i> = 175)	PCR vs. abdominal fluid ( <i>n</i> = 175)	PCR vs. composite measure (blood culture and abdominal fluid) ( <i>n</i> = 175)
True positive, <i>n</i>	3	5	6
False negative, <i>n</i>	11	27	32
False positive, <i>n</i>	13	11	10
True negative, <i>n</i>	148	132	127
Sensitivity, % (95% CI)	21.4 (0.0–42.9)	31.3 (8.5–54.0)	15.8 (4.2–27.4)
Specificity, % (95% CI)	91.9 (87.7–96.1)	83.0 (77.2–88.9)	92.7 (88.3–97.1)
PPV, % (95% CI)	18.8 (0.0–37.9)	15.6 (3.0–28.2)	37.5 (13.8–61.2)
NPV, % (95% CI)	93.1 (89.1–97.0)	92.3 (87.9–96.7)	79.9 (73.6–86.1)
PLR, ratio (95% CI)	2.65 (0.86–8.22)	2.03 (0.37–2.68)	2.16 (0.84–5.57)
NLR, ratio (95% CI)	0.85 (0.63–1.17)	0.91 (0.88–1.13)	0.91 (0.75–1.10)
PPTP, % (95% CI)	18.8 (0.0–37.9)	17.0 (4.0–20.0)	37.5 (13.8–61.2)
NPTP, % (95% CI)	6.9 (3.0–10.9)	7.7 (3.3–10.0)	20.1 (13.9–26.3)
Kappa index (95% CI)	0.125 (0.00–0.33)	0.100 (0.00–0.26)	0.107 (0.00–0.26)

CI confidence interval, NLR negative likelihood ratio, NPTP negative post-test probability, NPV negative predictive value, PCR polymerase chain reaction, PLR positive likelihood ratio, PPTP positive post-test probability, PPV positive predictive value

### Risk Factors for Invasive Candidiasis

Results of the analysis of the relationship between the presence of invasive candidiasis and patient risk factors are summarised in Table 3. The only significant relationships were between the presence of invasive candidiasis as determined using abdominal fluid/the composite measure and a candidiasis score  $\geq 3$  (both  $P = 0.006$ ).

According to the logistic regression analyses (Table 4), patients with a *Candida* score  $\geq 3$  were seven times more likely to present with invasive candidiasis than those with a lower *Candida* score when assessed by blood culture, but this result was non-significant ( $P = 0.100$ ). They were also 4.15 times more likely present with invasive candidiasis when assessed using the composite measure ( $P = 0.030$ ). When assessed using serum PCR, none of the three risk

factors influenced the probability of being positive for *Candida* spp. (Table 4).

Patient risk factors that were positively but not statistically significantly associated with the presence of invasive candidiasis according to blood culture results, using the adjusted binary logistic regression model, were abdominal surgery (OR 4.342;  $P = 0.068$ ) and an ICU stay  $> 15$  days (OR 2.560,  $P = 0.090$ ) (Table 5). There was also a 44% relative reduction in the risk of invasive candidiasis in non-immunosuppressed versus immunosuppressed patients (OR 0.556), but this was not statistically significant ( $P = 0.419$ ) (Table 5).

### DISCUSSION

The primary objective of this study was to examine the concordance between the results for serum PCR and blood culture for the

**Table 3** Relationship between presence of invasive candidiasis and patient risk factors

	<i>Candida</i> score $\geq 3$					Sepsis with $\geq 3$ risk factors					Sepsis in patients with immunosuppression				
	Yes		No		<i>P</i> <sup>a</sup>	Yes		No		<i>P</i> <sup>a</sup>	Yes		No		<i>P</i> <sup>a</sup>
	<i>n</i>	%	<i>n</i>	%		<i>n</i>	%	<i>n</i>	%		<i>n</i>	%	<i>n</i>	%	
Serum PCR															
Negative	119	90.8	40	90.9	0.989	147	90.7	12	92.3	> 0.05	35	97.2	124	89.2	> 0.05
Positive	12	9.2	4	9.1		15	9.3	1	7.7		1	2.8	15	10.8	
Blood culture															
Negative	118	90.1	43	97.7	0.106	149	92.0	12	92.3	> 0.05	33	91.7	128	92.1	> 0.05
Positive	13	9.9	1	2.3		13	8.0	1	7.7		3	8.3	11	7.9	
Abdominal fluid															
Negative	101	77.1	42	95.5	0.006	131	80.9	12	92.3	> 0.05	33	91.7	110	79.1	> 0.05
Positive	30	22.9	2	4.5		31	19.1	1	7.7		3	8.3	29	20.9	
Blood culture + abdominal fluid															
Negative	96	73.3	41	93.2	0.006	125	77.2	12	92.3	> 0.05	32	88.9	105	75.5	> 0.05
Positive	35	26.7	3	6.8		37	22.8	1	7.7		4	11.1	34	24.5	

PCR polymerase chain reaction

<sup>a</sup> Chi-squared test

detection of *Candida* spp. in patients admitted to ICUs in Spain and in whom empirical antifungal treatment was initiated because of suspected invasive candidiasis. Although a meta-analysis of studies evaluating PCR diagnosis for invasive candidiasis was published in 2011 [36], the patient populations assessed in this study were highly heterogeneous in terms of country of origin, age, underlying conditions, etc. In contrast, the current study provides information on a specific group of patients from Spain. It is important determine the issues that lead physicians to initiate antifungal therapy [39] and to perform PCR simultaneously with blood culture to avoid storage problems.

In this study, the rates of *Candida* detection in blood samples were low, whether using serum PCR (16/175, 9.1%) or blood culture (14/175, 8.0%). *Candida* spp. appeared to be more readily detected in the abdominal fluid (32/49,

65.3%). As could be expected, *C. albicans* was the most frequently detected species, although *C. glabrata*, *C. krusei*, *C. tropicalis* and *C. parapsilosis* were also identified. These five species represent the most common *Candida* pathogens [20, 30].

The sensitivity of serum PCR relative to blood culture for the detection of *Candida* spp. was 21.4%. While the results for PCR relative to abdominal fluid were marginally higher (31.3%), the sensitivity of serum PCR relative to the composite measure (*Candida* positivity in blood culture or abdominal fluid) decreased even further (15.8%) (Table 2).

While the specificity and NPV values for PCR obtained in the current study were similar to those previously reported in other studies [25, 36, 40], the sensitivity and PPV values achieved (Table 2) were much lower overall. This may be due to the use of serum samples for

**Table 4** Logistic regression models for risk factors

Risk factor	Effect	OR	95% CI	P
<i>Candida</i> infection according to blood culture				
<i>Candida</i> score $\geq 3$	Yes vs. no	7.00	(0.69–71.31)	0.100
Sepsis with $\geq 3$ risk factors	Yes vs. no	0.51	(0.04–5.93)	0.594
Sepsis in patients with immunosuppression	Yes vs. no	1.62	(0.37–7.03)	0.521
<i>Candida</i> infection according to serum PCR				
<i>Candida</i> score $\geq 3$	Yes vs. no	0.71	(0.20–2.49)	0.588
Sepsis with $\geq 3$ risk factors	Yes vs. no	0.39	(0.03–5.26)	0.481
Sepsis in patients with immunosuppression	Yes vs. no	0.14	(0.01–1.77)	0.128
<i>Candida</i> infection according to blood culture + abdominal fluid				
<i>Candida</i> score $\geq 3$	Yes vs. no	4.15	(1.15–14.98)	0.030
Sepsis with $\geq 3$ risk factors	Yes vs. no	1.36	(0.14–12.93)	0.789
Sepsis in patients with immunosuppression	Yes vs. no	0.62	(0.19–2.05)	0.432

CI confidence interval, OR odds ratio, PCR polymerase chain reaction

**Table 5** Risk factors associated with the presence of invasive candidiasis according to blood culture (adjusted binary logistic regression model with a bilateral significance level of 0.10, Chi-squared test)

Risk factor	Effect	OR	95% CI	P
<i>Candida</i> infection according to blood culture				
Abdominal surgery	Yes vs. no	4.342	(0.898–20.998)	0.068
Admission to ICU	> 15 days vs. $\leq 15$ days	2.560	(0.793–8.263)	0.090
Sepsis to patients with some immunosuppression criterion	Yes vs. no	0.556	(0.134–2.308)	0.419

CI confidence interval, ICU intensive care unit, OR odds ratio

our PCR assays rather than whole blood, according to each site's usual practice; greater sensitivity has been reported when PCR is performed in whole-blood samples [36; the CANDINEO Study [40].

The results from our analyses showed a very low concordance rate between the results of serum PCR and blood culture (kappa index: 0.125). The degree of concordance was not enhanced by using the composite measure, since the kappa index for serum PCR versus the composite measure was 0.107, again indicating a very low degree of concordance between serum PCR and the other diagnostic techniques used in this study.

Logistic regression analyses of the risk factors that, in clinical terms, may be considered to contribute to the probability of developing a *Candida* infection indicated a direct relationship between a *Candida* score  $> 3$  and a higher probability of invasive candidiasis (as diagnosed using blood culture or the composite measure). None of the other risk factors showed a particular association with the risk of developing invasive candidiasis.

An additional binary logistic regression model was used to determine which factors are most related to the presence of candidiasis, according to the blood culture result. Abdominal surgery and a longer ICU stay ( $> 15$  days)

showed a positive trend towards being risk factors for the presence of *Candida* spp. in blood cultures. These findings are consistent with previous studies, which have also found that abdominal surgery and length of stay at the ICU are associated with higher risk of invasive candidiasis [1, 41]. Conversely, we cannot explain why a very low concordance was found between blood culture and PCR; it may be necessary to perform PCR at the same time as blood culture.

The apparent lack of relationship between risk factors and the presence of invasive candidiasis using PCR is somewhat surprising, especially when this technique has proved to be very sensitive in similar ICU settings [25]. However, in another study the diagnostic accuracy of PCR was also low because the tests were performed in frozen samples [40]; this factor may be applicable to our study because of the freezing of samples prior to dispatch to the Reference Centre. In addition, there was no significant relationship between invasive candidiasis (as assessed using blood culture) and a number of known risk factors. This may also be attributable to the low sensitivity of blood cultures to diagnose *Candida* infections and the broad definition of a 'high-risk' population. Patients with just one of three positive blood cultures might have shown a negative PCR because of a low burden of *Candida* spp. Likewise, regarding the origin of candidaemia, abdominal fluid from a peritoneal catheter may present a higher *Candida* burden than an intra-abdominal infection and therefore diminish the sensitivity of PCR. Difficulties with distinguishing *Candida* colonisation from intra-abdominal invasive candidiasis may contribute to the low sensitivity of PCR in some patients [30].

Due to the non-interventional nature of this study, all patient examinations and follow-up visits were performed in line with the investigators' usual clinical practice. The low cure rate is of note, but it is not possible to speculate on the reasons for this as information relating to treatment strategies is not available. The lack of use of follow-up PCR is also notable, and the possible reasons for this may include discharge of patients to other units and therefore loss to follow-up or improvement of their clinical

condition and therefore no additional samples or investigations were needed.

In a high proportion of cases, a follow-up blood culture was not conducted, i.e. the physicians in this study appeared to rely on signs and symptoms to determine whether the *Candida* infection had resolved. Other recent studies that have evaluated PCR as a diagnostic tool for invasive candidiasis have reported conflicting results [25, 40, 42]. In a Spanish study amongst critically ill patients admitted to the ICU, PCR appeared to have a high sensitivity (96.3%), specificity (97.3%) and strong positive predictive value (92.8%), especially in patients with deep-seated disease (sensitivity 90.9% versus 45.4% for blood culture) [25], and therefore the authors concluded that PCR may be a useful test for confirming a diagnosis of invasive candidiasis [25]. However, in our study of high-risk patients, the incidence of candidaemia was strikingly lower than in the previous study [25]. Similarly, in another recent Spanish study, PCR had a low discriminating capacity (sensitivity 84.00%; PPV 26.9%), whether used alone or in combination with other antibody-based diagnostic tests [40]. In the study by Nguyen et al. [42], *Candida* PCR testing was more sensitive than blood cultures among patients with deep-seated candidiasis (88% versus 17%, respectively), and it significantly enhanced the ability of blood culture to detect invasive candidiasis by increasing overall sensitivity to 98% when used in combination with blood culture [42].

Nonetheless, significant shortcomings for PCR testing still remain, for instance, a relatively low sensitivity because of the low quantity of *Candida* cells in the blood [1], sample susceptibility to contamination [25] and other issues that need to be overcome before it can be used routinely and effectively in clinical practice (e.g. the lack of a standardised assay) [40]. Also, the lack of commercially available methods that are easy-to-use is still limiting [1]; different methodologies between centres performing these assays may also affect the consistency of clinical data which are generated.

Another limitation of the study was that information on patients who failed screening

was not collected; for this reason, the possibility of selection bias cannot be excluded. Due to its observational nature, additional limitations of this study may have included variations in physicians' clinical practice among all 27 participating centres. In addition, a second PCR that might provide information as to whether the initial negative results were truly negative was performed in very few patients. Furthermore, a number of pre-specified secondary end points (such as the prognostic value and diagnostic sensitivity of serum PCR in invasive candidiasis) were not assessed as planned because of low patient numbers or a low prevalence of invasive candidiasis in our samples. It would also be interesting to evaluate whether the outcomes of patients diagnosed by PCR and blood culture differ, but this was beyond the scope of the current study.

## CONCLUSIONS

In this observational, prospective, multicentre study amongst critically ill patients at a high risk of invasive candidiasis who had been admitted to an ICU, there was a low rate of concordance between serum PCR and blood culture, and serum PCR and culture of abdominal fluid samples, for the diagnosis of *Candida* infections. Thus, the PCR methodology used in the current study would not provide a clear diagnostic advantage in these patients. Within this patient population, there was a direct and statistically significant association between a *Candida* score > 3 and a higher probability of invasive candidiasis. Other evidence has substantiated the need for rapid and accurate methods to detect *Candida* spp., but this study highlights the importance of refining and validating such tests as well as establishing the risk factors for identification of critically ill patients who require early therapeutic intervention.

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**Data Availability.** All relevant data are included in this manuscript. The protocol and complete data sets generated and/or analysed during the current study are not publicly available, but are available from the corresponding author on reasonable request. Access to anonymized individual participant level data will not be provided for this trial as it meets one or more

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