Clinical validation of a multiplex real-time PCR assay for detection of invasive candidiasis in intensive care unit patients

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Introduction

In recent years, the incidence of bloodstream infections due to Candida spp. has increased in both adult and paediatric patients. About 30%–50% of all episodes of candidaemia in hospitals occur in patients admitted to the intensive care unit (ICU), and mortality can reach 50%–90%.

Data from autopsy-proven cases of invasive candidiasis (IC) and other forms of deep-seated candidiasis suggest that the sensitivity of blood culture remains inadequate. Moreover, several studies have consistently shown that a delay of 12–48 h in the administration of appropriate antifungal therapy is associated with a significant increase in all-cause mortality that is not related to other mortality risk factors. Alternative strategies for the diagnosis of IC in ICU patients are necessary owing to the low sensitivity of empirical and culture-based antifungal therapy. These approaches include targeted prophylaxis and early treatment based on clinical scores such as the ‘Candida score’. The use of empirical antifungal treatment for high-risk patients is prevalent, leading to increased costs and adverse ecological effects.

The β-D-glucan (BDG) Fungitell Assay (Associates of Cape Cod Incorporated, Falmouth, MA, USA) was approved by the US FDA as an adjunct in the diagnosis of deep-seated mycoses. Although not Candida-specific, the assay has proven to be a promising tool for the diagnosis of IC. In a recent meta-analysis of 16 studies involving 2979 patients (594 with proven and probable invasive fungal infection), serum BDG was effective in detecting patients with invasive fungal infection with a pooled sensitivity of 76.8% and a pooled specificity of 85.3%. The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guideline for the diagnosis and management of Candida diseases 2012 recommended the BDG test for detection of candidaemia in adults and considered it very useful for ruling out infection.
The detection of Candida albicans germ tube antibody (CAGTA; Candida albicans IFA IgG®, Vircell, Granada, Spain) has also been widely used. Preliminary studies showed a sensitivity of 84.4% and a specificity of 94.7%, even in patients with negative blood cultures. Two studies showed a significant decrease in mortality in ICU patients, especially in those with increasing CAGTA values that had been treated with antifungals.

Finally, PCR assays have been developed for the detection of Candida spp. in clinical samples of patients at risk of IC, such as critically ill patients. Fungus-specific PCR assays targeting regions of the 5.8S, 18S and 28S rRNA genes, as well as internal transcribed spacer (ITS) regions, have been evaluated for use with serum or blood samples from various patient groups. Several reports have been published and their pooled sensitivity and specificity were calculated at >85% in a meta-analysis.

An interesting feature of real-time PCR is the possibility of detecting multiple targets (multiplex PCR) as new machines incorporate four to six different detection channels. This technique incorporates probes with different fluorophores but similar hybridization conditions. Consequently, it can simultaneously detect multiple targets in a single reaction, thus reducing the time and cost of performing the reactions separately.

The present study is the result of a collaborative research project between the Mycology Service of the Spanish National Microbiology Center and the Ramón y Cajal Hospital in Madrid. The objectives of this study were to evaluate and validate a new multiplex quantitative real-time PCR (MRT-PCR) for the early detection of IC in clinical samples from critically ill patients and to compare this technique with blood culture, BDG and CAGTA.

Methods

The study was conducted prospectively between January 2010 and June 2012 with funding from the ‘Fondo de Investigaciones Sanitarias’ (Health Research Fund) of the Spanish Ministry of Health (FIS grant, PS09/01528). The institutional review board approved the study protocol and informed consent was obtained from the patients or their representatives.

The study population comprised ICU patients with clinically suspected IC and no prior antifungal treatment. Before antifungal treatment was started (day 0), blood samples were obtained for culture and whole blood samples and serum were obtained for MRT-PCR. These determinations were repeated in samples obtained on days +2, +7, +14 and +21 or until patients were discharged or died. In addition, BDG and CAGTA were determined in serum samples obtained at days 0 and +7 (and in some cases completed with samples from day +2).

The control group comprised ICU patients with suspected IC in whom IC was discarded and 40 healthy individuals (medical and auxiliary personnel), from whom a single blood sample was obtained and processed for blood culture, Candida PCR, BDG and CAGTA.

Serum and blood cultures were tested blind. With the exception of blood cultures, which were processed immediately, the remaining samples were processed at ~80°C until processed. Blood cultures were processed at the Microbiology Department of Ramón y Cajal Hospital using the BACTEC FX blood culture system (Becton Dickinson Diagnostic Instrument Systems, MD, USA). Fungi were identified using mass spectrometry [matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF), Bruker, Germany] and by PCR of the ITS regions in order to confirm identifications by MALDI-TOF with scores lower than 2.0.

The MRT-PCR assay was performed to detect the six most frequent species of the genus Candida in IC. The technique detected Candida albicans, Candida parapsilosis, Candida tropicalis, Candida glabrata, Candida krusei and Candida guilliermondii using specific molecular beacon probes labelled with different fluorescent dyes: FAM, HEX, ROX and CYAN 500. Primers and probes were designed on the basis of the nucleotide sequences of the ITS ribosomal DNA region from strains belonging to the collection of the Spanish National Center of Microbiology. The probes targeted the ITS1 or ITS2 regions of ribosomal DNA. These regions were chosen as targets because of the possibility of designing a suitable probe for each case. Beacon Designer 5.0 software (Premier Biosoft, Palo Alto, CA, USA) was used to design primers and probes. The assay consisted of two multiplex PCRs: reaction 1 (C. albicans, C. parapsilosis and C. tropicalis), which was performed using the LightCycler Probes Master Kit (Roche Diagnostic, Madrid; Spain); and reaction 2 (C. glabrata, C. krusei and C. guilliermondii), which was performed using the 2x Sensimix Probe Kit (Quantace, Ecogen, Madrid, Spain).

Both PCRs were performed simultaneously in the LightCycler® 480 System (Roche Diagnostics, Mannheim, Germany). DNA from blood and sera was extracted using the QIAamp DNA Mini Kit (Qiagen, Izasa, Madrid, Spain). Eukaryotic DNA was removed by digestion with 50µL of buffer; the PCR was performed with 2µL of DNA extracted from each sample. All samples were performed in duplicate and quantification standards were run in conjunction with each set of samples and negative controls.

BDG was performed using the Fungitell® Assay in Fontlab2000 (Barcelona, Spain) and results >80 pmol/mL were considered positive. A CAGTA assay was performed in the Microbiology Service of Hospital La Fe (Valencia, Spain) and a serum titre >1/160 in at least one sample was considered a positive result. Clinical and laboratory variables were obtained for all patients following a pre-established protocol.

A diagnosis of IC was confirmed based on one of the following criteria: (i) the presence of candidaemia in a patient with consistent clinical manifestations; (ii) isolation of Candida spp. from normal sterile body fluids or peritoneal fluid; only cultures obtained through drainage were considered for diagnosis if samples were obtained at the time of placing the drainage; (iii) ophthalmic examination consistent with Candida endophthalmitis in a patient with clinical sepsis; or (iv) histologically documented candidiasis. Mucosal candidiasis in the absence of clinical and microbiological signs of IC was not considered IC. Colonization was defined as the recovery of Candida species from non-sterile sites (including drainages, see above), independently of the presence of symptoms or signs of systemic disease that were attributable to IC. The Pittet Candida colonization index (number of positive cultures/number of obtained cultures) was obtained and a systematic sample was taken in all patients to calculate the colonization index.

Statistical analysis

Sensitivity, specificity and predictive values were calculated in four settings: (i) IC; (ii) candidaemia; (iii) deep-seated candidiasis; and (iv) IC in highly colonized patients (Pittet index ≥0.5). Healthy controls were included in all the analyses except the analysis of highly colonized patients. Although no surveillance cultures were available, a low grade of colonization was suspected in the healthy controls. The McNemar χ² test was used to compare sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) between the assays. The univariate analysis of contingency data was performed using a χ² test or Fisher exact test. The analyses were performed using IBM® SPSS® Statistics version 19 (IBM®, Armonk, NY, USA).

Results

We invited 71 ICU patients with suspected IC to participate in the study before starting antifungal treatment. The first eight patients enrolled were excluded because their samples were obtained through venous catheters that had been implanted for several days and the preliminary results of PCR included a high number
of false positives (catheter contamination). From this time, all samples were obtained through direct venipuncture. Therefore, the study sample comprised the remaining 63 patients.

A diagnosis of IC was confirmed in 27 of the 63 patients included in the study. Of these, 21 developed candidaemia. The other six patients had deep-seated (abdominal or urological) IC. The source of candidiasis in the 27 patients with IC was catheter-related candidaemia (16 cases) and deep-seated infection (11 cases, with associated candidaemia in 5). IC was not reported in the remaining 36 patients and an alternative diagnosis was confirmed.

Table 1 shows the characteristics of the study patients. No differences were found between IC and non-IC patients with regard to APACHE score or predisposing factors such as diabetes, renal failure, type of immunosuppression, antibiotics, parenteral nutrition, Candida score or mortality. Significant differences were only observed for the degree of mucosal colonization by Candida spp. (96% versus 64%, *P* = 0.002) and for the Pittet index (0.50 versus 0.34, *P* = 0.013).

Of the 27 patients with IC, 21 had positive blood cultures on day 0 (sensitivity 78%). The time to positivity of the blood culture in this study was 48 h. Ten remained positive on day +2 and 4 on day +7. Candidaemia was not confirmed in any patient on day +14 or +21. Candidaemia was confirmed in 20 (74%). On the other hand, the rates of false-positive results were 77% in whole blood and serum.

The distribution of the Candida species in the 21 patients with candidaemia was as follows: *C. albicans*, *n* = 11; *C. parapsilosis*, *n* = 7; and *C. glabrata*, *n* = 3. The species confirmed by PCR in the remaining six cases of IC were *C. albicans* (*n* = 5) and *C. tropicalis* (*n* = 1).

BDG and CAGTA were performed in samples obtained on days 0 and +7 in the 63 ICU patients. Of the 27 patients with IC, BDG showed a positive result (≥80 pg/mL) in 22 (81%) and CAGTA was positive in 20 (74%). On the other hand, the rates of false-positive results for BDG and CAGTA among the 36 patients without IC were 22.2% (8/36) and 38.9% (14/36), respectively.

Blood cultures and PCR were negative in all 40 healthy controls. By contrast, a high rate of false-positive results was confirmed for BDG (5 cases, 12%) and CAGTA (19 cases, 47.5%).

Table 2 shows the sensitivity, specificity and predictive values of all four techniques (blood culture, MRT-PCR, BDG and CAGTA) for IC, candidaemia, deep-seated candidiasis and IC in highly colonized patients. For IC, deep-seated candidiasis and candidaemia, the control group included all patients admitted to the ICU where IC was finally discarded and healthy volunteers. To calculate the performance of the tests in cases of IC among highly colonized patients, the control group excluded patients admitted to the ICU with a Pittet index <0.5 and all healthy volunteers. The most relevant comparative results are stated below.
MRT-PCR versus blood culture

The combination of both techniques confirmed the diagnosis of IC in 100% of cases. If candidaemia was considered the gold standard for the diagnosis of fungal infection, the specificity and PPV for the MRT assay were 97.3% and 100%, respectively. The sensitivity of the MRT-PCR for the diagnosis of IC was 96.3% overall and 90% in the subgroups (candidaemia, deep-seated candidiasis and highly colonized patients). The size of the series probably prevented differences in sensitivity for blood culture being observed overall (96.3% versus 77.7%, \( P = 0.10 \)), but MRT-PCR showed a clear trend towards superiority in the deep-seated candidiasis subgroup (90.9% versus 45.4%, \( P = 0.06 \)).

MRT-PCR versus BDG

The sensitivity of BDG was not significantly lower than that of MRT-PCR (\( P = 0.7 \)). BDG revealed a high number of false positives (12% in healthy controls and 22% in ICU patients), thus explaining the significantly lower PPV of BDG than MRT-PCR (and blood cultures) overall and in the subgroups.

MRT-PCR versus CAGTA

The sensitivity for CAGTA was no higher than 75% for any of the settings. The frequency of false positives for the CAGTA assay was 47.5% in healthy controls and 39% in ICU patients. Specificity and PPV for the CAGTA assay were significantly lower than for MRT-PCR (and blood cultures).

Blood cultures were negative in 6 of the 27 patients with IC who were enrolled. Table 3 shows the characteristics of these six patients. They all had abdominal collections and Candida spp. was documented. A favourable response was documented for all except one patient, who died (necropsy not performed). IC was ruled out in two patients with a positive PCR result (false-positive MRT-PCR; Table 4).

Discussion

MRT-PCR has a promising role in the management of ICU patients with suspected IC. The MRT-PCR assay analysed in this study performed better than blood culture; however, differences were not

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Table 2. Performance of diagnostic procedures in patients with IC, candidaemia and deep-seated candidiasis (analysis per patient)

<table>
<thead>
<tr>
<th></th>
<th>IC (cases, 27; population, 103)</th>
<th>Candidaemia (cases, 21; population, 97)</th>
<th>Deep-seated candidiasis (cases, 11; population, 87)</th>
<th>IC among highly colonized patients (Pittet index &gt;0.5) (cases, 16; population, 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sensitivity</td>
<td>77.7% (21/27)</td>
<td>—</td>
<td>45.4% (5/11)</td>
<td>87.5% (14/16)</td>
</tr>
<tr>
<td>specificity</td>
<td>100% (76/76)</td>
<td>—</td>
<td>100% (76/76)</td>
<td>100% (14/14)</td>
</tr>
<tr>
<td>PPV</td>
<td>100% (21/21)</td>
<td>—</td>
<td>100% (5/5)</td>
<td>100% (14/14)</td>
</tr>
<tr>
<td>NPV</td>
<td>92.7% (76/82)</td>
<td>—</td>
<td>92.7% (76/82)</td>
<td>87.5% (14/16)</td>
</tr>
<tr>
<td>RT-PCR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sensitivity</td>
<td>96.3% (26/27)</td>
<td>95.2% (20/21)</td>
<td>90.9% (10/11)</td>
<td>93.7% (15/16)</td>
</tr>
<tr>
<td>specificity</td>
<td>97.3% (74/76)</td>
<td>97.3% (74/76)</td>
<td>97.4% (74/76)</td>
<td>100% (14/14)</td>
</tr>
<tr>
<td>PPV</td>
<td>92.8% (26/28)</td>
<td>90.9% (20/22)</td>
<td>83.3% (10/12)</td>
<td>100% (15/15)</td>
</tr>
<tr>
<td>NPV</td>
<td>98.7% (74/75)</td>
<td>98.7% (74/75)</td>
<td>98.7% (74/75)</td>
<td>93.3% (14/15)</td>
</tr>
<tr>
<td>BDG (≥80 pg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sensitivity</td>
<td>81.5% (22/27)</td>
<td>95.2% (20/21)</td>
<td>63.6% (7/11)</td>
<td>81.2% (13/16)</td>
</tr>
<tr>
<td>specificity</td>
<td>82.9% (63/76)</td>
<td>85.5% (65/76)</td>
<td>82.9% (63/76)</td>
<td>50.0% (7/14)</td>
</tr>
<tr>
<td>PPV</td>
<td>62.8% (22/35)</td>
<td>64.5% (20/31)</td>
<td>35.0% (7/20)</td>
<td>65.0% (13/20)</td>
</tr>
<tr>
<td>NPV</td>
<td>92.6% (63/68)</td>
<td>98.5% (65/66)</td>
<td>94.0% (63/67)</td>
<td>70.0% (7/10)</td>
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<tr>
<td>CAGTA (≥1/160)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sensitivity</td>
<td>74.1% (20/27)</td>
<td>71.4% (15/21)</td>
<td>72.7% (8/11)</td>
<td>75.0% (12/16)</td>
</tr>
<tr>
<td>specificity</td>
<td>56.5% (43/76)</td>
<td>53.9% (41/76)</td>
<td>53.9% (41/76)</td>
<td>35.7% (5/14)</td>
</tr>
<tr>
<td>PPV</td>
<td>37.7% (20/53)</td>
<td>30.0% (15/50)</td>
<td>18.6% (8/43)</td>
<td>57.1% (12/21)</td>
</tr>
<tr>
<td>NPV</td>
<td>86.0% (43/50)</td>
<td>87.2% (41/47)</td>
<td>93.2% (41/44)</td>
<td>55.5% (5/9)</td>
</tr>
</tbody>
</table>

**P value**

<table>
<thead>
<tr>
<th></th>
<th>PCR versus blood culture</th>
<th>PCR versus BDG</th>
<th>PCR versus CAGTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se</td>
<td>0.10</td>
<td>0.004, PPV: 0.006</td>
<td>0.004, PPV: 0.001</td>
</tr>
<tr>
<td>Sp</td>
<td>0.004, PPV: 0.006</td>
<td>0.017, PPV: 0.049</td>
<td>0.005, PPV: 0.01</td>
</tr>
</tbody>
</table>

Se, sensitivity; Sp, specificity.
IC: all controls and cases were included for analysis.
Candidaemia: all controls were included; in cases, patients with IC and negative blood culture were excluded.
Deep-seated candidiasis: all controls were included; in cases, patients with IC from catheter-related bloodstream infection were excluded.
IC among highly colonized patients: all patients with a Pittet index <0.5 were excluded (including all control patients).
<table>
<thead>
<tr>
<th>Age (years)</th>
<th>APACHE index</th>
<th>ICU (days)</th>
<th>Hospitalization (days)</th>
<th>Fortun et al. (Pittet et al.) Candida score</th>
<th>Antifungal therapy</th>
<th>Dead</th>
<th>BDG</th>
<th>CAGTA</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>#27</td>
<td>75</td>
<td>13</td>
<td>11</td>
<td>10</td>
<td>0.2 (1/5)</td>
<td>1</td>
<td>no</td>
<td>negative</td>
<td>1/1280</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>liposomal amphotericin B</td>
<td>7 days, fluconazole 7 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>#29</td>
<td>84</td>
<td>10</td>
<td>6</td>
<td>7</td>
<td>0.4 (2/5)</td>
<td>1</td>
<td>no</td>
<td>negative</td>
<td>1/160</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>fluconazole 14 days</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>#35</td>
<td>57</td>
<td>24</td>
<td>3</td>
<td>46</td>
<td>1 (3/3)</td>
<td>2</td>
<td>no</td>
<td>positive</td>
<td>1/1280</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>liposomal amphotericin B</td>
<td>22 days, fluconazole 14 days</td>
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<td>#48</td>
<td>67</td>
<td>23</td>
<td>8</td>
<td>23</td>
<td>0.3 (2/6)</td>
<td>3</td>
<td>no</td>
<td>positive</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>fluconazole 14 days</td>
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<tr>
<td>#56</td>
<td>72</td>
<td>19</td>
<td>2</td>
<td>4</td>
<td>1 (6/6)</td>
<td>4</td>
<td>yes</td>
<td>negative</td>
<td>1/320</td>
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<tr>
<td></td>
<td></td>
<td></td>
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<td>caspofungin 6 days, fluconazole 6 days</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>#57</td>
<td>55</td>
<td>12</td>
<td>2</td>
<td>63</td>
<td>0.5 (2/4)</td>
<td>4</td>
<td>no</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>caspofungin 7 days</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Deep-seated IC is characterized by a low fungal load and short duration of candidaemia, thus explaining the low sensitivity of BDG. BDG (Fungitell) generates, even if two positive determinations are required. Major limitation of BDG is the large number of false positives it presents. However, determination of BDG itself was useful for monitoring the response in patients with IC. A very important finding of this study is the superiority of MRT-PCR over BDG and CAGTA, especially in terms of specificity and MRT-PCR over BDG and CAGTA, especially in terms of specificity. However, MRT-PCR over BDG and CAGTA, especially in terms of specificity.
Quantification of biomarkers seems to be the best strategy for both early detection of infection and reduction of empirical treatments. The 2012 ESCMID guideline for the diagnosis and management of *Candida* diseases recommends determination of some biomarkers (e.g. BDG and mannan) to rule out infection and reduce the prophylactic and empirical misuse of antifungal compounds. In this study MRT-PCR had a sensitivity of 96%, but sensitivity on day 0 was reduced to 81.4% and sensitivity on day 7 was 92.5%. Considering the high NPV of MRT-PCR (93.3%–98.7%) and in order to choose an optimal strategy to reduce empirical therapy in these patients, a possible recommendation would be to start antifungal therapy in cases of suspected IC and remove antifungal therapy if MRT-PCR remains negative in a second determination (~7 days later). If serum has been used for RT-PCR on day 0 and the result is negative, according to the results of this study and in order to increase the NPV of the test, whole blood can be recommended for a second MRT-PCR determination.

A recent meta-analysis examined the yield of PCR techniques applied directly to blood samples in the diagnosis of IC. More than 4600 patients were analysed in 54 studies using standard PCR, nested PCR and real-time PCR. When controls were patients at risk for IC, sensitivity and specificity values were 93% and 95%, respectively. In this meta-analysis, the sensitivity of blood cultures in the diagnosis of IC was only 38%. Nguyen et al recently evaluated the role of PCR and BDG in patients with IC. A species-specific TaqMan real-time PCR assay was used with alignments of available ITS1 and/or ITS2 sequences for five *Candida* species (*C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis* and *C. parapsilosis* complex). The sensitivity of PCR (serum or plasma) was higher than that of BDG and blood culture for the diagnosis of IC. Alam et al compared the detection of mannan, anti-mannan antibodies, BDG and a semi-nested PCR in patients with culture-proven or suspected candidaemia, women with *C. albicans* vaginitis and healthy controls. In candidaemia (culture-proven or clinically suspected), the positivity of the tests was 53% for PCR, 29% for BDG, 16% for mannan and 29% for anti-mannan antibodies.

The present study also shows the superior performance of the PCR-based technique in comparison with blood cultures and BDG, mainly for deep-seated candidiasis. The results confirm the clinical utility of PCR-based assays to detect and rule out infection. Prospective clinical studies including external validation and harmonization of assays should be performed before recommendations can be made; however, the excellent performance of PCR-based approaches seems to make them very useful clinical tools.

**Table 4. Positive PCR in the absence of IC (false-positive PCR)**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>APACHE</th>
<th>ICU (days)</th>
<th>Hospitalization (days)</th>
<th>Candida score</th>
<th>Antifungal therapy</th>
<th>Antifungal treatment</th>
<th>Dead</th>
<th>BDG</th>
<th>CAGTA</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>#20</td>
<td>50</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>0.17 (1/6)</td>
<td>fluconazole 3 days</td>
<td>no</td>
<td>negative</td>
<td>1/640</td>
<td>laparoscopic cholecystectomy, fever with antibiotic response</td>
</tr>
<tr>
<td>#59</td>
<td>28</td>
<td>17</td>
<td>14</td>
<td>1</td>
<td>0.25 (1/4)</td>
<td>fluconazole 5 days</td>
<td>no</td>
<td>negative</td>
<td>1/640</td>
<td>cardiac surgery, fever and leucocytosis, refractory to antibiotic therapy, 10 days after catheter removal (culture tip: <em>Staphylococcus epidermidis</em>)</td>
</tr>
</tbody>
</table>

PCR for detection of candidiasis in ICU patients
made us more demanding in our analysis. Therefore, a second population that did not comprise carriers of catheters and whose samples had been obtained from direct venipuncture was included. In fact, the work analyses the different techniques and MRT-PCR in both settings (Table 2): including only ICU controls (last column of Table 2) and including all controls (the rest of the columns). The yields of MRT-PCR are similar, once guaranteed that samples for MRT-PCR analysis were obtained from direct venipuncture, regardless of the type of control used. Second, no gold standard is available for the diagnosis of IC. In the current study, the criteria applied in the definition of IC were very strict and had been validated in rigorous studies. Third, in the absence of candidaemia, it is not easy to demonstrate the presence of IC and it is not easy to rule out IC in highly colonized patients with refractory fever. Colonization is generally accepted as the principal factor limiting the specificity of PCR. Although colonization was significantly higher in the group of patients with IC in the present series, the degree of colonization was also very high (64%) in patients without IC and all but two had a negative PCR result. Finally, this MRT-PCR assay detects C. albicans, C. parapsilosis, C. tropicalis, C. glabrata, C. krusei and C. guilliermondii. The recent CANDIPOP population-based study conducted in 29 Spanish hospitals collected a total of 781 isolates (from 767 patients, 14 of them having mixed fungaemia) during 2010 and 2011. The species found most frequently were C. albicans (44.6%), C. parapsilosis (24.5%), C. glabrata (13.2%), C. tropicalis (7.6%), C. krusei (1.9%) and C. guilliermondii (1.7%). All of these species could be detected using the MRT-PCR assay. In addition, Candida lusitaniae (1.3%) and other Candida and non-Candida species accounting for ~5% of the isolates in the CANDIPOP study would not be likely to be diagnosed by this test.

In conclusion, our results show MRT-PCR to be a very effective tool for the management of IC in critically ill patients. Although blood cultures remain an irreplaceable diagnostic procedure owing to their specificity, ease of use and efficiency, MRT-PCR can serve as a complementary diagnostic assay, thus reducing the disproportional use of empirical treatment without affecting mortality. Further multicentre clinical studies are warranted, as is external validation of PCR-based assays.

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Transparency declarations

In the last 5 years, J. F. has received grant support from Astellas Pharma, Gilead Sciences, Merck Sharp and Dohme, Pfizer and Instituto de Salud Carlos III. He has been paid for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas Pharma and Schering-Plough. In the past 5 years, J. P. has received grant support from Astellas Pharma and Pfizer. He has been paid for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer and Astellas Pharma. In the past 5 years, M. C.-E. has received grant support from Astellas Pharma, bioMérieux, Gilead Sciences, Merck Sharp and Dohme, Pfizer, Schering-Plough, Soria Melguizo SA, Ferrer International, the European Union, the ALBAN programme, the Spanish Agency for International Cooperation, the Spanish Ministry of Culture and Education, the Spanish Health Research Fund, Instituto de Salud Carlos III, the Ramon Arce Foundation and the Mutua Madrileña Foundation. He has been an advisor/consultant to the Pan American Health Organization, Astellas Pharma, Gilead Sciences, Merck Sharp and Dohme, Pfizer and Schering-Plough. He has been paid for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas Pharma and Schering-Plough. All other authors: none to declare.

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