PCR diagnosis of invasive candidiasis: systematic review and meta-analysis
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CRD summary
The authors concluded that direct polymerase chain reaction (PCR)-test using blood samples may have higher sensitivity for the diagnosis of invasive candidiasis than conventional blood cultures. However, the review contained no comparative accuracy data for PCR versus conventional blood cultures, so the authors’ conclusion that PCR had a higher sensitivity compared with conventional blood culture is purely speculative.

Authors’ objectives
To assess the diagnostic accuracy of direct polymerase chain reaction (PCR)-based methods to detect invasive candidiasis directly in blood samples.

Searching
PubMed, LILACS, NLM Gateway, and KoreaMed were searched up to July 2009. Conference proceedings of the European Congress of Clinical Microbiology and Infectious Diseases, and Inter-science Conference on Antimicrobial Agents and Chemotherapy were searched from 2000 to 2009. Searches were performed with no language or publication status restrictions. Search terms were reported. Reference lists of included studies and relevant reviews were scanned.

Study selection
Eligible for inclusion in the review were prospective or retrospective cohort and case-control studies that assessed the diagnostic accuracy of PCR-based methods for detecting Candida species. Studies had to report true positives, false positives, true negatives and false negatives.

The index tests included any PCR based method used for the identification of Candida species to generic or species level, including standard, nested, real time, or reverse transcriptase PCR, using single or multiple assays. All target genes and primers were accepted. The reference standard was based on established criteria for the definition of invasive candidiasis in neutropenic patients (EORTC criteria) and definitions used in recent clinical trials for non-neutropenic patients.

True positives were categorised into three groups. True positive I corresponded to candidaemia. True positive II corresponded to proven or probable invasive candidiasis defined for neutropenic patients by host, clinical and microbiological criteria, and for non-neutropenic patients by the isolation of Candida species from blood or other normally sterile sites in the presence of at least one indication of infection (such as inflammation at site of infection; elevated or subnormal temperature on two occasions at least four hours apart; and systolic blood pressure of ≤100 or ≥ 30mmHg below baseline) within four days prior to treatment initiation. True positive III corresponded to proven, probable or possible invasive candidiasis as indicated by host and clinical criteria without microbiological documentation for neutropenic patients, and for non-neutropenic patients by sepsis responsive to antifungal treatment with one or more risk factors for invasive candidiasis without microbiological documentation. True negative individuals were categorised into two groups: "True negative healthy" (healthy individuals) and "True negative at risk" (patients at risk of invasive candidiasis who did not fulfil the criteria for True positive III patients). Studies of PCR testing of blood cultures after incubation or after the identification of growth were excluded.

Included studies were published from 1993 to 2009. Approximately 33% of studies assessed adults alone, 15% children alone, and 15% assessed both adults and children; the remaining studies did not specify the age of the patient population. Nearly 50% the studies used standard PCR, 25% used nested PCR and 25% used real time PCR. Approximately 25% of studies used serum, whilst the remaining studies used whole blood samples (fresh or frozen stored blood samples). Most studies used rRNA genes (mostly 18S rRNA) as targets for PCR. In approximately 50% of studies, the PCR sample-processing time ranged from four to twelve hours, which allowed for the reporting of results within one working day. PCR sampling was performed prospectively in all studies.

Two reviewers independently performed study selection and disagreements were resolved by discussion and consultation with a third reviewer.
Assessment of study quality
Two reviewers independently assessed study quality using the QUADAS (Quality Assessment of Diagnostic Accuracy Studies) tool. Disagreements were resolved by discussion and consultation with a third reviewer.

Data extraction
Data on true positives, true negatives, false positives and false negatives were extracted to calculate sensitivity and specificity. Where studies reported multiple data sets, data were extracted separately for each index test and reference standard.

Data extraction was performed by two independent reviewers. Any disagreements were resolved by discussion or by consultation with a third reviewer.

Methods of synthesis
Meta-analysis was performed using a hierarchical logistic regression model to calculate pooled sensitivity and specificity values, diagnostic odds ratios (DORs), likelihood ratios, and their associated 95% confidence intervals (CIs). The authors reported to have used the ‘metandi’ STATA command to generate this model, usually used for bivariate models, which would not give pooled likelihood ratios directly. However, this reporting error did not impact on the validity of what the authors did.

Random-effects meta-regression was performed to assess the effects of moderators (study methods and infection characteristics) on sensitivity and specificity separately.

Subgroup analyses were performed based on the results of the meta-regression and on studies performed after 2000 (those using recent technology).

Results of the review
Fifty-four studies were included in the review (4,694 patients); there were 16 case-control studies, 36 prospective cohort studies, and two retrospective cohort studies. Study quality varied in the recruitment of consecutive patients, the timing of PCR in relation to blood cultures, the testing of all patients with reference tests, and the description of clinical information available at the time PCR was conducted.

True Positive I individuals (with candidaemia) versus True Negative healthy individuals (15 case-control studies): In all 15 studies specificity was 100%. Sensitivity was 100% in 12 studies, and ranged from 77 to 93% in three studies. Sensitivity and specificity were 100% in all studies that used whole blood samples. The study with the lowest sensitivity used the CA1/CA2 Candida actin gene as the target gene. All other studies used rRNA or P450 genes. No summary receiver operating characteristic analysis was performed.

True Positive I individuals (with candidaemia) versus True Negative at-risk individuals (49 studies; 14 case-control studies, 35 cohort studies): Summary sensitivity was 95% (95% CI 88 to 98) and summary specificity was 92% (95% CI 88 to 95). The positive likelihood ratio was 12.3 (95% CI 7.9 to 19.3) and the negative likelihood ratio 0.05 (95% CI 0.02 to 0.14). A higher positive likelihood ratio was obtained when the analysis was restricted to studies performed after 2000 (positive likelihood ratio 15.4, 95% CI 9 to 26.4). Meta-regression showed that higher sensitivity was associated with in vitro detection limit of ≤10CFU/ml and the use of a PCR procedure other than the commercial SeptiFast kit (multiplex realtime PCR) (p<0.01).

True Positive II individuals (proven/probable/possible invasive candidiasis) versus True Negative at-risk individuals (17 studies; six case-control studies, 11 cohort studies): Summary sensitivity was 93% (95% CI 82 to 98) and summary specificity was 95% (95% CI 87 to 98). The positive likelihood ratio was 18.2 (95% CI 7 to 47.2) and the negative likelihood ratio 0.07 (95% CI 0.03 to 0.2). A higher positive likelihood ratio was obtained when the analysis was restricted to studies performed after 2000 (positive likelihood ratio 24.3, 95% CI 11.3 to 52.4). Higher sensitivity was associated with the use of whole blood samples.

True Positive III individuals (proven/probable/possible invasive candidiasis) versus True Negative at-risk individuals
Summary sensitivity was 73% (95% CI 58 to 83) and summary specificity was 91% (95% CI 82 to 96). The positive likelihood ratio was 8.1 (95% CI 4 to 16.5) and the negative likelihood ratio 0.3 (95% CI 0.9 to 0.48). A higher positive likelihood ratio was obtained when the analysis was restricted to studies performed after 2000 (positive likelihood ratio 14.4, 95% CI 8 to 25.2). Studies that performed more than one PCR test per patient, and studies that used commercial QIAamp (Qiagen) kits were associated with higher specificity.

**Authors' conclusions**
Direct PCR in blood samples may have higher sensitivity for the diagnosis of invasive candidiasis than conventional blood cultures, with a specificity of 90% (acceptable for clinical practice). Future studies should assess the clinical effects of this test.

**CRD commentary**
The review addressed a clear research question and was supported by detailed inclusion criteria. The search strategy included relevant sources and had no language or publication status restrictions, which minimised the risk of publication and language bias. Review processes were performed in duplicate, which minimised the risk of reviewer error or bias.

Study quality was assessed using an appropriate tool. Appropriate synthesis method were used. Whilst the authors’ conclusion that PCR has a high specificity was supported by the evidence presented, the review contained no comparative accuracy data for PCR versus conventional blood cultures.

The authors’ conclusion that PCR had a higher sensitivity compared with conventional blood culture is purely speculative.

**Implications of the review for practice and research**
**Practice:** The authors stated that testing of patients with suspected invasive candidiasis by PCR should accompany, but not replace, blood cultures. They also stated that serial sampling might be considered for patients at high risk for invasive candidiasis. PCR targeting panfungal DNA elements, with subsequent species identification, should be applied to whole blood samples. The assay should have an in vitro sensitivity of at least 10CFU/ml.

**Research:** The authors stated that the assessment of PCR on blood for the diagnosis of invasive candidiasis will have to rely on an interventional study, preferably a RCT, asking whether management directed by PCR testing improves patients’ outcomes. The population should consist of patients at high risk of invasive candidiasis. Serial monitoring and diagnostic PCR samples should be analysed in real time to guide treatment while control patients should be managed using conventional culture-based diagnosis. This should be a multicentred effort.

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