Use of PCR for diagnosis of invasive aspergillosis: systematic review and meta-analysis

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A systematic review and meta-analysis was done on the use of PCR tests for the diagnosis of invasive aspergillosis. Data from more than 10 000 blood, serum, or plasma samples obtained from 1618 patients at risk for invasive aspergillosis were retrieved from 16 studies. Overall, the mean diagnostic odds ratios (DORs) of PCR for proven and probable cases were similar whether two consecutive positive samples were required to define positivity (DOR 15·97 [95% CI 6·83–37·34]) or a single positive PCR test was required (DOR 16·41 [95% CI 6·43–41·88]). Sensitivity and specificity of PCR for two consecutive positive samples were 0·75 (95% CI 0·54–0·88) and 0·87 (95% CI 0·78–0·93), respectively, and if only a single positive sample was required, these values were 0·88 (95% CI 0·75–0·94) and 0·75 (95% CI 0·63–0·84), respectively. Whereas specificity based on a single positive test was significantly lower (p=0·027) than two positive tests, the sensitivity and DOR did not differ significantly. A single PCR-negative result is thus sufficient to exclude a diagnosis of proven or probable invasive aspergillosis. However, two positive tests are required to confirm the diagnosis because the specificity is higher than that attained from a single positive test. Populations at risk varied and there was a lack of homogeneity of the PCR methods used. Efforts are underway to devise a standard for *Aspergillus* sp PCR for screening, which will help enable formal validation of PCR and estimate its use in patients most likely to benefit.

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Introduction

Invasive aspergillosis is a fairly common opportunistic fungal infection of patients who are profoundly neutropenic, either as a result of intensive cytostatic chemotherapy to treat acute leukaemia or myelodysplastic syndrome or after having received haemopoietic stem-cell or solid-organ transplantation.[1] Prevalence of invasive aspergillosis is 1–15% and mortality can exceed 90%.[2] Prophylaxis is recommended for patients at high risk of developing invasive aspergillosis,[2] but many institutions rely on early treatment strategies instituted either empirically or on the basis of early diagnosis.

Early diagnosis of invasive aspergillosis remains a challenge, and few diagnostic tools are available. The screening of patients for the presence of galactomannan may be useful in establishing an earlier diagnosis and may result in improved outcomes.[3] Other methods, such as PCR, are being investigated to improve the diagnosis of invasive aspergillosis.[4,5]

Several studies have assessed the diagnostic yield of PCR techniques for the diagnosis of invasive aspergillosis. A recent review evaluated the quality of 12 clinical studies of PCR for diagnosing invasive aspergillosis among patients at high risk, and concluded that, although valuable, implementation of PCR was hampered by a lack of standardisation.[6] We undertook a more systematic review of these and other, more recent, clinical studies by use of meta-analysis to assess the PCR techniques for their accuracy with diagnostic odds ratios (DORs) and other performance indicators.

Methods

Search strategy

The search was carried out using Medline (from 1980 to July, 2008) and Embase (from 1980, to July, 2008). We used the search terms "aspergillosis", "aspergillus"; "diagnosis", and "polymerase chain reaction", and the syntax used for Medline searches was as follows: "aspergillosis" OR "aspergillus" AND "diagnosis" AND/OR "polymerase chain reaction". The search was supplemented by consultation of the bibliographies of the articles retrieved. No language restrictions were applied to the search.

Inclusion criteria

Studies that used PCR techniques on blood, serum, or plasma samples were included if (1) they compared the results of PCR with the diagnosis made after the published case definition criteria for invasive fungal disease proposed by the European Organisation for Research and Treatment of Cancer (EORTC) and Mycoses Study Group (MSG)[1] or, for studies published before the publication of these criteria in 2002, similar criteria were used as a reference standard;[1] (2) they reported data separately on false-positive, true-positive, false-negative, and true-negative results of the diagnostic tests under investigation; and (3) the assessments of the tests were done prospectively in a cohort of consecutive patients from a relevant clinical population, defined as a group of individuals at high risk for invasive aspergillosis. These criteria were used to cover the spectrum of diseases likely to be encountered in the current or future use of this diagnostic test. The diagnostic methods were classified according to the criteria used to define a positive result as either (1) positive PCR in at least two consecutive blood samples drawn from the same patient, or (2) a single sample of blood that showed a PCR-positive result. Studies that assessed use of PCR on bronchoalveolar lavage alone were not included in the analysis.

Studies were classified on the basis of the sampling method as being consecutive if samples had been

collected prospectively from a cohort of patients, non-consecutive if the samples of some, but not all, patients had been included, or unspecified if it was not clearly stated. Studies that assessed samples from a group of patients known to have aspergillosis and from a separate group of individuals without evidence of disease were regarded as case–control studies and were excluded from analysis because these studies tend to overestimate or underestimate the diagnostic yield of a test.[7]

The quality of the studies included was assessed as recommended in the Standards for Reporting of Diagnostic Accuracy (STARD).[8] For this purpose, we used the QUADAS tool, which contains 14 items specifically developed to assess the quality of systematic reviews of primary studies of diagnostic tests.[9]

We obtained in full any study that potentially met the inclusion criteria on the basis of title, abstract, or both, and critically reviewed these studies to ascertain whether they met our inclusion criteria. Three reviewers (CM, MC, JPD) independently screened the titles and abstracts of references, and doubts or disagreements were resolved by discussion.

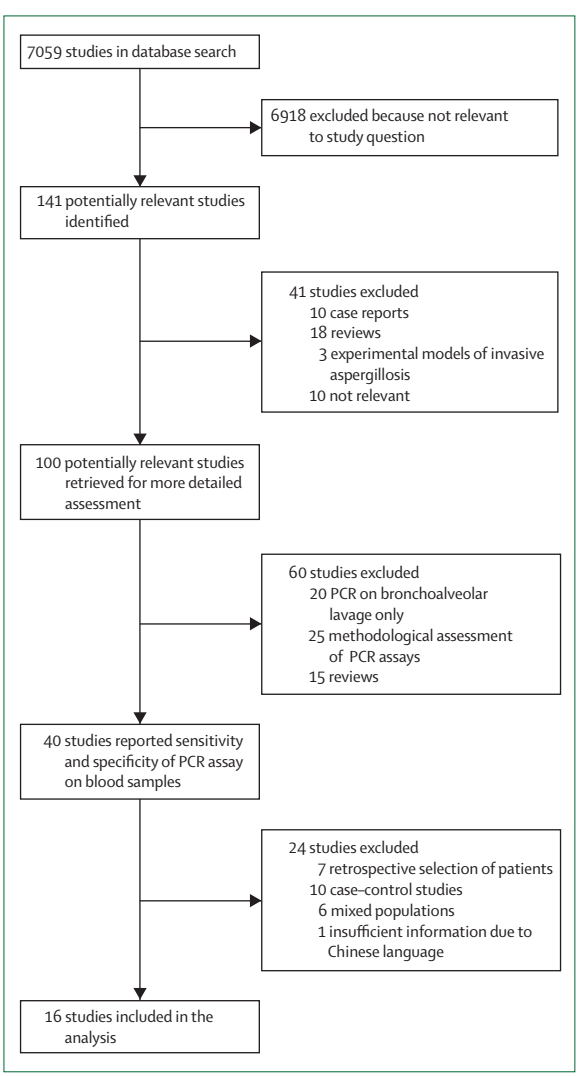

***Figure 1*: Study selection**

Statistical analysis

The DOR is a single indicator measure of the accuracy of a diagnostic test. It describes the odds of positive test results in individuals with the disease compared with the odds of positive results in those without disease, and corresponds to particular pairings of sensitivity and specificity. The mean DOR was adopted as an accuracy index and was estimated by classic meta-analytic pooling, along with an assessment of the heterogeneity of the various studies included.[10] The meta-analytical pooling was based on the inverse variance method for calculating weights.

Heterogeneity was assessed by means of the Cochran Q method and by the test of inconsistency (I^2).[11,12] DerSimonian and Laird random-effects models were used if heterogeneity was present.[13] Funnel plots were generated to allow visual inspection for publication bias, and tests for rank correlation and for regression asymmetry (Egger's test) were used to detect asymmetry.[14,15] The "trim and fill" method was used if there was evidence of an asymmetrical funnel plot to provide an estimate of the publication bias-adjusted effect.[16] The summary receiver operating characteristic (ROC) curve analysis was done to determine the presence of a threshold effect.[10,17] The Q-point was detected by plotting the summary ROC curve in a sensitivity versus 1–specificity space, where sensitivity equals specificity.

The mean sensitivity and specificity of the diagnostic test under scrutiny was assessed by a bivariate random-effects approach.[18,19] In this Review, the original logit/linear, mixed-effects approach was modified by use of a binomial/logistic (exact) procedure to avoid the difficulties arising from small datasets and the necessity for ad-hoc continuity correction.[20] The model adopted for this systematic review assessed the two interpretive criteria for a PCR-positive result: a single positive PCR result and two positive PCR results. We also calculated positive and negative likelihood ratios, which describe the discriminatory properties of positive and negative test results.[10] Sensitivity analyses were done using Stata version 10 to determine whether quantitative results differed if individual studies were excluded.

Results

Of the 7059 references identified, 141 potentially relevant citations were selected. After screening titles and abstracts, 100 articles were selected for full-text review (figure 1). 40 articles reported sensitivity and specificity of PCR tests on blood, serum, or plasma samples for the diagnosis of invasive aspergillosis. Of these, 24 studies were excluded (figure 1). Therefore, 16 studies published between 2000 and 2008 met the inclusion criteria and were included in the meta-analysis.[21–36]