Has *Aspergillus* PCR Come to the Age of Maturity?

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The diagnosis of invasive aspergillosis (IA) has always been a challenge in immunocompromised patients. Until recently, classical mycological tests were the only diagnostic tools available in this highly lethal condition. However, fungal culture and antibody detection are known for having a low diagnostic sensitivity. Histopathological examination is difficult, because biopsies in such immunocompromised hosts are risky and commonly not obtained, due to low platelet counts. Galactomannan (GM) testing has completely modified this scenario, allowing for an early, sensitive (78 %) and specific (81 %) diagnosis of IA, particularly in the neutropenic population [1]. In conjunction with computed tomography of the thorax, GM has become the backbone of preemptive antifungal therapy. Nowadays, most patients with IA enroll clinical trials or receive antifungal therapy based on GM testing. Nevertheless, there are a number of

caveats that sometimes cloud the interpretation of GM results, including a high rate of false-positive results, cross-reaction with fungi other than the *Aspergilli*, and the reduced sensitivity of serum testing in non-neutropenic patients [2]. Therefore, the scientific community has been at a constant search for alternative non-culture-based and noninvasive diagnostic tests. Polymerase chain reaction (PCR) tests seem to fit quite well these requirements.

The diagnostic performance of PCR in IA has been evaluated in several studies. A previous meta-analysis included 1618 at-risk patients from 16 studies to reveal that PCR had a sensitivity of 88 % in the diagnosis of IA [3]. Specificity was 75 % (increased to 87 % if two positive results are obtained). Most studies included in the meta-analysis used whole blood, and sample volume and DNA extraction protocols varied considerably, compromising reproducibility. More recently, a meta-analysis published at the Cochrane Library revealed that the mean sensitivity and specificity of *Aspergillus* PCR were 80 and 78 % for single positive test results, and 58 % and 96 %, respectively, for two consecutive positive results [4]—a performance very similar to what is known for GM. The elevated negative predictive value of *Aspergillus* PCR allows for IA to be virtually excluded in the presence of a negative test result. The performance of GM and PCR has been compared in a recent publication. The study showed that the highest diagnostic odds ratio (DOR) was obtained when GM was combined with PCR

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(DOR 135), in comparison with two positive GM tests (DOR 30) or two positive PCR results (DOR 18), separately. Both GM and PCR showed a high (>90 %) negative predictive value in the diagnosis of IA. Authors proposed that patients at high risk for IA should be screened regularly with both GM and PCR: IA will be highly unlikely if both tests are negative and highly likely if these are both positive. If only one of the tests is positive, IA cannot be confirmed or excluded [5].

Zhang et al. [8] recently published in *Mycopathologia* a study comparing PCR and GM for the diagnosis of IA, using serum and bronchoalveolar lavage (BAL) samples from predominantly non-neutropenic individuals. In BAL samples, sensitivity was elevated for both PCR and GM (90 %). However, specificity was higher in the BAL for PCR, in comparison with GM (92.5 % vs. 68.8 %, respectively). In serum samples, as expected for non-neutropenic patients, sensitivity was limited for both methods. The study concluded that PCR could be a valuable tool for diagnosis of IA in non-neutropenic patients, offering a higher specificity in comparison with GM.

Nevertheless, the study by Zhang et al. [8] has some limitations. In addition to its retrospective design, the authors studied a heterogeneous and small population (the number of IA cases in the study was only 10). Moreover, the manuscript discussed very little on the potential utility of performing a quantitative real-time PCR test, in contrast to conventional PCR, in patients at risk for IA. This brings some interesting questions to the role of PCR in the diagnosis of IA: Would DNA quantification allow for a better discrimination of *Aspergillus* colonization and disease? And what would be the ideal cutoff, for different patient populations? That deserves further investigation. Also, if PCR is going to be used in association with GM, we still need to know about the best strategy for the execution of these diagnostic tests (i.e., concomitantly or sequentially).

PCR critics say the method cannot be a reference diagnostic tool for IA, due to lack of standardization in methods. In fact, PCR was excluded from the current EORTC/MSG criteria for the definition of invasive fungal diseases [6]. However, the international community—particularly in Europe—has joined efforts

toward standardization in the PCR [7]. As a result, the newest EORTC/MSG consensus—which should be published soon—promises to include *Aspergillus* PCR (blood and bronchoalveolar lavage) as a mycologic criterion for probable IA.

The field of diagnostic mycology is expanding fast. We celebrate the arrival of PCR to the podium, not as a definitive solution, but as a good companion to its existing buddy, GM.

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