

Polymerase Chain Reaction for Diagnosing Invasive Aspergillosis: Getting Closer but Still a Ways to Go

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(See the article by White et al. on pages 479–86)

The technique of PCR for detection of *Aspergillus* in human specimens has been around in one form or another for almost 2 decades [1], but it is still regarded as too experimental to use for defining invasive fungal disease [2]. The reasons for not including PCR in the European Organisation for Research on Treatment of Cancer and the Mycoses Study Group criteria are as frustrating as they are manifold but include the lack of standardization and the absence of a commercially available system [3]. Notwithstanding the slow development, there have been advances, as attested by the study by White and colleagues in this issue of the journal [4]. This group is based in Cardiff, United Kingdom, and has employed a modified PCR method from a previous coworker [5] for use in a clinical setting. Their primary aim was to assess the performance of PCR in terms of diagnosing invasive aspergillosis, and they succeeded in supporting the value of a negative PCR result for ruling

out IA, thereby helping to limit empirical therapy to those patients who are most likely to have the disease.

The authors went to a great deal of trouble to undertake the study. They took the British Society for Medical Mycology's proposed standards of care for patients with invasive fungal infections as their starting point [6] but did not seem to manage to enlist the full cooperation of their hematology colleagues. High-resolution CT scans were recommended for all patients with suspected IA, but the compliance of the clinicians was only 37%. Although no one expects 100% compliance for this patient population, others have shown that this type of imaging is attainable in the majority of cases [7, 8]. Compliance in submitting blood samples for the galactomannan ELISA test twice weekly was also low, ~50%. This makes estimating the true incidence of IA in this population unreliable and may have led to underdiagnosis of IA.

The authors also made every effort to minimize variation in the performance of PCR by choosing whole blood samples, rather than plasma or serum samples, and employing a modified version of the extraction method used by a Tübingen group [9] and the standardized Light Cycler (Roche) method for real-time PCR. In this way, they had an assay capable of reliably detecting the equivalent of 5 cfu

of *Aspergillus*, which is in the same range as found in experimentally infected mice [10]. It is true that they used *Aspergillus* primers that were different from those employed by others [11–19], choosing instead ones that performed well in a previous study [5]. Nonetheless, their assay was sensitive by any standards, so that a negative result effectively meant the absence of fungal DNA, even though the converse was not the case.

The authors succeeded in showing a clear relationship between positivity rates for PCR and those for patients but not those for samples. Of the 13 patients with proven or probable IA, 12 (92%) had serial positive PCR results, whereas this was the case for only 6 (15%) of the 40 patients with possible IA and 8 (5%) of the 149 patients at risk who had no evidence of IA. The negative predictive value was 99%, with corresponding sensitivity of 92% and specificity of 95%. The performance of their PCR also compares favorably with that of previous studies, yielding high likelihood ratios of probable/proven IA for a positive test result (table 1).

Therefore, it is fair to conclude, as the authors did, that failure to detect *Aspergillus* by PCR in blood specimens obtained twice weekly should allow a wait-and-see approach to be taken, thereby reducing the need for empirical antifungal therapy by ~50%. This is in line with the recent re-

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Table 1. Comparison of different studies of PCR for invasive aspergillosis (IA).

Reference	No. of patients (episodes) in study	No. (%) of patients with probable/proven IA	PCR sensitivity, %	PCR specificity, %	Likelihood ratio of probable/proven IA	
					Positive PCR test result	Negative PCR test result
[13]	86	21 (24)	100	98	50.00	<0.01
[20]	93	22 (24)	100	89	9.09	<0.01
[14]	84	7 (8)	100	65	2.86	<0.01
[15]	92	14 (15)	100	73	3.70	<0.01
[5]	37	13 (35)	81	100	>100	0.19
[19]	121	3 (2)	75	96	18.75	0.26
[11]	218	33 (15)	2	81.3	4.90	0.10
[17]	122	33 (27)	79	92	9.88	0.23
[12]	165 (205)	11 (7)	64	63.5	1.74	0.57
[18]	96 (149)	11 (11)	64	87	4.92	0.41
[16]	78 (175)	8 (10)	75	70	2.50	0.36
[4]	202	13 (6)	92	95	17.09	0.08

sults of a study from Leuven, Belgium, by Maertens et al. [21], who found that detection of galactomannan 3 times weekly in serum drawn on a daily basis could reduce the need for empirical therapy by at least 78%.

What other conclusions can be drawn? It is striking that, as with the development of galactomannan, the progress toward using PCR seems, with the notable exception of a Tokyo group [17, 18], to be a primarily European affair involving a few centers. The researchers who opt to study PCR for screening high-risk patients seem to be not particularly enamored of galactomannan testing, and vice versa. This is a pity because combining these 2 tests, perhaps also with β -D-glucan testing, might, in fact, offer a better means of including or excluding the diagnosis of IA, because each measures different components of fungi. However, it may not be that simple, because Kami et al. [17] showed that PCR detected the presence of *Aspergillus* earlier than did either galactomannan ELISA or the β -D-glucan test, and, in a later study, the same group found that combining the tests reduced sensitivity [18]. A smaller study [22] indicated why this might be, showing (using a variant of the method of Einsele et al. [13]) that PCR was more sensitive than the galactomannan ELISA, whereas the latter test

was more specific. The apparent discrepancies are likely to be partly the result of widely differing frequencies of probable and proven IA in the different populations, because the actual performance of any test is dependent on the a priori probability—that is, the prevalence. As shown in table 1, the frequency of probable/proven IA varied markedly for tests with a high specificity, for which a positive result suggests a diagnosis of IA, which was also reflected in the likelihood ratios. Similar variation was seen for tests with a high sensitivity, for which a negative result rules out the diagnosis of IA. This makes it difficult for others to adopt PCR for screening.

Clearly, only a prospective, multicenter study of sufficient size can provide an answer to the question of whether screening would help direct therapy to patients that need it while exempting those that do not. The study of White et al. [4] gives grounds for optimism while clearly highlighting the need for minimum standards of diagnosis in any undertaking. Their expertise together with that of others in the field provides enough critical mass to help generate a common PCR protocol. Centers, including my own, that have opted for galactomannan ELISA will be more keen to join forces to form a multicenter base that would be sufficiently large to allow re-

cruitment, within a reasonable time, of the necessarily large number of patients at risk. The costs incurred could be defrayed by the savings on expensive antifungal agents, some of which cost several hundred euros a day.

It would be ideal if funding from grant agencies could be found, but this is unlikely in the current climate of budget restrictions, because IA fails the test of high societal impact. On the other hand, it would be wrong to expect commercial enterprises to entirely foot the bill for this undertaking. The problem is clearly the province of health care providers. The impetus provided by White et al. [4] should encourage all parties to combine their efforts and establish a sort of consortium to support a study that is clearly needed to obtain evidence on which clinicians can base their decisions now and in the future. Let us hope we will not have to wait another decade.

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