Is Real-Time Polymerase Chain Reaction Ready for Real Use in Detecting Candidemia?

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(See the article by McMullan et al. on pages 890–6)

In this issue of *Clinical Infectious Diseases*, McMullan et al. [1] present a carefully crafted and cautiously interpreted study of real-time PCR for detection of *Candida* species in serum samples obtained from nonneutropenic patients with candidemia. The test result was positive for 8 of 9 patients with *Candida albicans* in their routine blood culture and for both patients with *Candida glabrata* infection. The test detected only the *C. albicans* in a patient with mixed fungemia due to both *C. albicans* and *C. glabrata*. The test was not designed to detect *Candida famata* and missed the single case of fungemia due to that rare species. Remarkably, there were no false-positive results in 491 serum samples from 128 patients judged unlikely to have invasive candidiasis. Of the 13 serum samples from 6 patients who were considered to be at high risk of invasive candidiasis, the only positive results were for samples obtained from a patient who later developed candidemia.

This provocative study raises several issues that are currently of high interest. McMullan et al. [1], like many others, believe that more rapid identification of candidemia could improve outcome. As logical as that seems, it remains controversial whether early therapy decreases overall mortality associated with this very morbid infection [2, 3]. Nevertheless, a rapid test for candidemia might decrease hospital stay or use of inappropriate treatments. In most hospitals, candidemia is now detected by an automated broth blood culture system. Culture medium is inoculated at the bedside and placed in the automated incubator soon after the specimen reaches the laboratory. Depending on inoculum, volume of blood cultured, *Candida* species, medium, and detection system, growth is detected in ∼24–48 h [4]. At that time, the clinician is notified that a yeast is growing. There are some clues as to species, such as the more rapid growth of *C. glabrata* in anaerobic than aerobic culture in at least one system [5]. *C. glabrata* and *C. albicans* can be identified within 3 h using a commercial peptide nucleic acid fluorescent in situ hybridization (PNA FISH) method [6]. For a firm identification of other yeast species, subculture and overnight incubation is needed for isolation of individual colonies. The laboratory can then select from a variety of techniques for identifying the yeast colony on subculture, with *C. albicans* being identified the same day and other species up to 3 days later. As with most PCR and phenotypic tests, the identification is as good as the database. Designation of the isolate as “non-*albicans*” is not predictive of fluconazole resistance, because most of these species are as azole-susceptible as *C. albicans*.

Whether real-time PCR is faster than routine culture depends on how often the real-time PCR batch is run. The need for specially trained technicians, a dedicated space for PCR and another for real-time PCR, and the costs saved by running the test in batches would limit this to 1 or 2 weekdays each week in most laboratories. The real-time PCR used by McMullan et al. [1] began with manual extraction of DNA from serum, followed by a PCR, followed by a nested real-time PCR. Technician hands-on time is considerable and expensive. Another concern is the extraordinary precautions required of this procedure. Routine laboratories have avoided using nested PCR techniques because the opportunities for contamination are so great that, even with dedicated work areas and skilled employees, false-positive results are difficult to prevent. It is often unclear whether such false-positive results are truly false or are detecting levels of candidiasis below the limit of blood culture. A similar nested real-time PCR found positive results for *Candida* in 27 of 113...
immunosuppressed febrile patients, only 3 of whom had a positive blood culture result [7]. It was impossible in that study for the authors to tell from the case histories whether these were false-positive results or reflected a more sensitive test for candidiasis than blood culture. Use of prophylactic and empirical antifungal therapy in high-risk patients further compounds difficulties in interpretation. There is no useful definition of “probable” deeply invasive candidiasis; there are only factors that place patients at varying degrees of risk. Absence of a reference standard beyond blood culture has impeded and confused work in this diagnostic area.

Routine blood culture has the advantage of detecting bacteria and yeasts in the same system, including mixed cultures, whereas real-time PCR is designed to detect a defined number of species. McMullan et al. [1] designed their real-time PCR to distinguish 4 fluconazole-susceptible species (C. albicans, Candida tropicalis, Candida parapsilosis, and Candida dubliniensis) from each of 2 relatively fluconazole resistant species (C. glabrata and Candida krusei). The latter species occurs in no more than 1% of Candida isolates from the bloodstream, but C. glabrata causes roughly 15%–20% of candidemia cases reported from developed countries. The experimental design used [1], explicitly targeting C. glabrata, raises the question of whether it is necessary to identify this species rapidly to prevent inappropriate usage of fluconazole. Although some institutions use an echinocandin to treat candidemia until the species is identified, the added cost and question about higher echinocandin MICs for C. parapsilosis makes this choice less obvious for every patient. In the 3 randomized trials of fluconazole to treat candidemia in non-neutropenic patients, the rate of global response to fluconazole in C. glabrata was 56% (31 of 55 patients) versus 62% (165 of 265 patients) with C. albicans, C. parapsilosis, and C. tropicalis bloodstream infections [8–10]. Whether one should amalgamate results across different study designs and populations is open to debate, but the 6% difference in outcome is not large. One explanation of the similar response in C. glabrata bloodstream infections is that other factors, such as removal of intravascular catheters, the patient’s host defense status, and intensive care support, are more pivotal in survival and ultimate response than is the fluconazole MIC. It does not seem obvious that a patient administered fluconazole to treat candidemia and whose condition is clearly improving should have the treatment switched to an echinocandin or an amphotericin B formulation when the species is determined to be C. glabrata. One recommendation is to avoid administration of fluconazole when the patient has been exposed to azoles in the recent past, is neutropenic, or is in septic shock [11]. This is a decision made at the bedside, not in the microbiology laboratory.

At present, the report by McMullan et al. [1] of nested real-time PCR for diagnosis of candidemia is best viewed as a proof of principle, a project definitely worth further study.

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References


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