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 his-
tories whether these were false-positive re-
sults or reflected a more sensitive test for
candidiasis than blood culture. Use of pro-
phyllactic and empirical antifungal therapy
in high-risk patients further compounds
difficulties in interpretation. There is no
useful definition of “probable” deeply in-
vasive candidiasis; there are only factors
that place patients at varying degrees of
risk. Absence of a reference standard be-
yond blood culture has impeded and con-
fused work in this diagnostic area.

Routine blood culture has the advan-
tage of detecting bacteria and yeasts in the
same system, including mixed cultures,
whereas real-time PCR is designed to de-
tect a defined number of species. Mc-
Mullan et al. [1] designed their real-time
PCR to distinguish 4 fluconazole-suscep-
tible species (C. albicans, Candida tropi-
calis, Candida parapsilosis, and Candida dubliniensis) from each of 2 relatively flu-
conazole 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], explic-
antly targeting C. glabrata, raises the ques-
tion of whether it is necessary to identify
this species rapidly to prevent inappro-
priate usage of fluconazole. Although
some institutions use an echinocandin to
treat candidemia until the species is iden-
tified, the added cost and question about
higher echinocandin MICs for C. parap-
silosis 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 re-
sponse to fluconazole in C. glabrata was
56% (31 of 55 patients) versus 62% (165
of 265 patients) with C. albicans, C. par-
apsilosis, and C. tropicalis bloodstream in-
fec tions [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 re-
sponse in C. glabrata bloodstream infec-
tions is that other factors, such as removal
of intravascular catheters, the patient’s
host defense status, and intensive care sup-
port, are more pivotal in survival and ul-
timate response than is the fluconazole
MIC. It does not seem obvious that a pa-
tient administered fluconazole to treat
candidemia and whose condition is clearly
improving should have the treatment
switched to an echinocandin or an am-
photericin B formulation when the species
is determined to be C. glabrata. One re-
commendation 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 diag-
nosis of candidemia is best viewed as a
proof of principle, a project definitely
worth further study.

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