Routine Broad-Range Fungal Polymerase Chain Reaction With DNA Sequencing in Patients With Suspected Mycoses Does Not Add Value and Is Not Cost-Effective

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Context.—New molecular diagnostic tests regularly become available, and they may be assumed to be superior to traditional diagnostic studies. The added cost of these studies should be considered in conjunction with the value provided for patient care.

Objective.—To assess the cost and diagnostic value of broad-range polymerase chain reaction (PCR) and DNA sequencing for the diagnosis of fungal infections compared with traditional studies.

Design.—We reviewed the cost and clinical impact of broad-range fungal PCR/DNA sequencing for 65 specimens for which this test, a direct fungal examination, fungal culture, and a histopathologic assessment were performed.

Results.—The sensitivity, specificity, and positive and negative predictive values for each of the assays studied were, respectively: histopathology (83.3%, 100%, 100%, and 98.3%); direct examination (66.7%, 100%, 100%, and 96.7%); fungal culture (83.3%, 100%, 100%, and 98.3%); and broad-range fungal PCR/DNA sequencing (83.3%, 95.0%, 62.5%, and 98.3%). The cost for broad-range fungal PCR/DNA sequencing was $32,500, compared with $8,591.70 for all traditional tests combined, for the 65 specimens included in this review.

Conclusions.—Broad-range fungal PCR/DNA sequencing did not detect any infecting fungal pathogen that was not detected by at least 1 of the traditional methods, but 3 false-positives occurred. Broad-range fungal PCR/DNA sequencing is not a substitute for traditional laboratory studies and should be used judiciously to promote care affordability.

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The recent and substantial expansion of molecular diagnostic testing in clinical microbiology and infectious diseases has had both beneficial and deleterious effects. The beneficial effects include the often more sensitive, rapid detection and characterization of pathogens, which in turn affords the tailoring and optimization of therapy, which influence outcomes.3–4 Unfortunately, molecular assays are usually more expensive than traditional methods, and some of the multiplex assays contain microbial targets that are not actionable with current therapeutic regimens.5,6 Additionally, there is commonly a misconception that molecularly derived test results are superior to those from traditional methods of diagnosis.7 It is clear that the use of molecular diagnostic methods may be preferable for the diagnosis of certain syndromic infections, particularly those with overlapping symptomatology that may be caused by a variety of different agents (eg, respiratory viral infections).8–10 However, in other clinical circumstances expensive molecular diagnostics may add cost without appreciable benefit.7,11

Some of these tests have added to the often derided “pan-culture,” the more expensive and equally problematic “pan-polymerase chain reaction (PCR),” when broad-range PCR and DNA sequencing applications for bacteria, mycobacteria, and fungi are used nonjudiciously or as part of the primary means of diagnosis. Furthermore, when quality processes are not in place to ensure that excised, potentially infected tissues are submitted for traditional microbiologic studies, then these more expensive molecular assays may be necessary to secure a diagnosis. Importantly, this delays the identification of the etiologic agent of disease, which could have an untoward effect on patient care. An all-too-common example is when surgically excised, infected tissue is submitted entirely for surgical pathology and a portion is not submitted for culture. When microorganisms are discovered upon histologic examination, then molecular methods must be used in an attempt to identify the pathogen; in most of these instances, a culture would have identified the pathogen in a more timely and cost-effective manner (see cost analysis below) and would have provided a viable isolate should susceptibility testing be necessary.

The use of broad-range PCR followed by DNA sequencing for the detection/identification of fungi was unrestricted at our institute for a certain time period following the
addition of this assay to the test menu. This retrospective study evaluated the incremental cost incurred by the health care system, as well as the analytic performance of this molecular test, which we compared with traditional diagnostic methods (ie, direct examination, anatomic pathology review, and culture) during this time.

MATERIALS AND METHODS

We retrospectively reviewed the results of all of the broad-range fungal PCR/DNA sequencing tests ordered during an approximately 17-month time period (ie, March 22, 2013–August 11, 2014). All the specimens studied were derived from the same surgery and were from normally sterile body sites. Specimens were separated by the clinical team, with 1 portion submitted for each of the 4 tests reviewed; all tissues studied were fresh (ie, none were formalin-fixed, paraffin-embedded). Tests were ordered at the discretion of the clinical provider at the time of specimen submission (ie, molecular studies were not ordered after the presence of a fungus was detected by another method to assist with identification).

Broad-range fungal PCR and DNA sequencing was referred to a Clinical Laboratory Improvement Act (CLIA)–certified commercial provider that used broad-range PCR assays that targeted both the 18S rDNA genetic regions using proprietary methods. In our laboratory, fungal elements were sought in clinical specimens via direct microscopic examination using a KOH/calcofluor white preparation (ie, direct examination; Fungi-Fluor Kit, Polysciences Inc, Warminster, Pennsylvania); if present, the fungal elements were subsequently characterized (eg, pseudohyphae, etc). Fungal culture was performed through the inoculation and 28-day, 30°C incubation of potato dextrose (Becton Dickinson Baltimore Biological Laboratories, Franklin Lakes, New Jersey) and Mycosel agar (Remel Inc, San Diego, California). Fungi that grew in culture were identified by standard methods. The histologic examination of slides was performed by staff surgical pathologists at the Cleveland Clinic (Cleveland, Ohio) in a routine manner. If an inflammatory response was present suggestive of an infectious process, then additional histochemical stains for microorganisms were performed at the discretion of the attending pathologist.

To assess the performance of these diagnostic tests, we limited our analysis to those patients for whom specimens were submitted for all 4 tests (ie, broad-range fungal PCR/DNA sequencing, histopathology, direct examination, and culture). The specimen was considered to truly contain a pathogenic fungus (ie, the specimen was truly positive) whenever results from 2 or more tests were positive. Individual test results were considered true positives whenever that result was corroborated by another method (ie, a test result, from any test, was categorized as a true positive when confirmed by any other method). Single positive test results were characterized as false positives. Discrepancies were resolved through chart review to determine the assessment by the clinical team regarding the validity of a result. The sensitivity, specificity, and positive and negative predictive values were calculated for each diagnostic method by standard methods. The PCR/DNA sequencing result for 1 specimen was falsely negative for the pathogenic fungus that the specimen contained, but it was falsely positive for another fungus that was not the pathogen. Biostatistician consultation recommended characterizing this test result as a false negative when calculating sensitivity and negative predictive value, and to characterize it as a false positive when calculating specificity and positive predictive value. The send-out test cost was used for the broad-range fungal PCR/DNA sequencing cost, whereas internal cost information was used for tests performed on site. This review was a quality improvement project and therefore exempt from Institutional Review Board review.

RESULTS

A total of 65 of the 100 specimens reviewed met the inclusion criteria for this study (ie, all 4 tests were performed). Of these 65 specimens, 6 (9.2%) were deemed to truly contain pathogenic fungi by the defined criteria (Table 1). Fungi were demonstrated by broad-range fungal PCR/DNA sequencing in 8 of the 65 specimens (12.3%) in this cohort. A total of 5 of the 8 PCR/DNA sequencing positive results (62.5%) were characterized as true positives, whereas 3 (37.8%) were false positives. The infecting fungus was detected in 5 of 6 truly positive specimens (83.3%) by histopathology, 4 of 6 (66.7%) by direct examination, and 5 of 6 (83.3%) by fungal culture.

The sensitivity, specificity, and positive and negative predictive values for each of the assays studied were, respectively, as follows: histopathology (83.3%, 100%, 100%, and 98.3%); direct examination (66.7%, 100%, 100%, and 96.7%); fungal culture (83.3%, 100%, 100%, and 98.3%); and broad-range fungal PCR/DNA sequencing (83.3%, 95.0%, 62.5%, and 98.3%; Table 2). The cost for broad-range fungal PCR/DNA sequencing for the 100 specimens sent during the time of this study was $50,000, and therefore $32,500 for the 65 specimens that met the inclusion criteria for this study. In contrast, all specimens had been submitted for histopathology, direct examination, and culture the cost incurred would have been $13,218 for the assessment of 100 specimens; the actual cost incurred for the 65 specimens that met the inclusion criteria was $8,591.70.

DISCUSSION

Health care delivery is in the midst of changing from a volume-based to a value-based reimbursement system.12,13 This change is necessitating a thorough assessment of the value of each component of care delivered in every service line. These reviews should eliminate wasteful practices, promote the judicious use of resources, and emphasize evidence-based best practices, while making care affordable.

Molecular diagnostics have revolutionized medicine in many ways. Brain biopsies for the diagnosis of central nervous system infections caused by herpes simplex viruses, toxoplasmosis, and the JC polyoma virus have become a rarity given the widespread use of highly sensitive PCR-based methods.14–18 We have previously called for the enhanced development and application of molecular diagnostics for improvements in the diagnosis and treatment of patients at risk for fungal infections.19,20 There have been several advances in the use of molecular diagnostics for the diagnosis of some fungal infections. Doyle et al,21 for example, have demonstrated the improved sensitivity of PCR for the diagnosis of Pneumocystis pneumonia compared with traditional, morphologic methods. Similarly, amplification-based methods, alone or in conjunction with antigen detection methods, hold promise for the diagnosis of deep-seated fungal infections.22–24 Our study does not challenge the use of these molecular methods for the diagnosis of fungal infections but rather explores the refinement and judicious use of broad-range fungal PCR and DNA sequence-based identification methods when tissue is available for assessment.

This review has demonstrated that the unrestricted use of broad-range fungal PCR/DNA sequencing is not only costly, but it does not increase the diagnostic yield compared with traditional morphologic methods. Miller et al2 demonstrated similar findings when they reviewed the unrestricted use of broad-range mycobacterial PCR/ DNA sequence testing for mycobacteria. As described by Miller et al,2 we have shown...
Table 1. Comparison of Tests With Any Positive Results

| Pt | Anatomic Location | Fungal Infection | Inflammatory Response | Histologic Findings | Fungi Seen With Special Stains | Direct Exam | Fungal Culture | Fungal PCR/Sequencing |
|----|-------------------|------------------|-----------------------|---------------------|-------------------------------|-------------|---------------|----------------------|
| 1  | Sinus tissue      | Present (TP)     | Present               | Present             | No                            | FN          | Positive       | TP                   |
|    |                   |                  |                       |                     |                               |             |               | Malassezia sp      |
| 2  | Aortic valve      | Present (TP)     | Present               | Present             | Yes                           | Yes         | Positive       | TP                   |
|    |                   |                  |                       |                     |                               |             |               | C albicans         |
| 3  | Right knee        | Absent (TN)      | Absent                | No                  | N/P                           | TN          | Negative       | TN                   |
|    |                   |                  |                       |                     |                               |             |               | Cladosporium sp    |
| 4  | Basal ganglia     | Present (TP)     | Present               | Present             | Yes                           | Yes         | Positive       | TP                   |
|    |                   |                  |                       |                     |                               |             |               | Negative            |
| 5  | Epidural tissue   | Absent (TN)      | Present               | No                  | N/P                           | TN          | Negative       | TN                   |
|    |                   |                  |                       |                     |                               |             |               | Cryptococcus spp   |
| 6  | Prosthetic mitral valve | Present | Present             | Present             | Yes                           | Yes         | Negative       | FN                   |
|    |                   |                  |                       |                     |                               |             |               | Candida parapsilosis |
| 7  | Prosthetic mitral valve | Present | Present             | Present             | Yes                           | Yes         | Positive       | TP                   |
|    |                   |                  |                       |                     |                               |             |               | Histoplasma capsulatum |
| 8  | Aortic valve      | Present (TP)     | Present               | Present             | Yes                           | Yes         | Negative       | FN                   |
|    |                   |                  |                       |                     |                               |             |               | H capsulatum       |

Abbreviations: FN, false negative; FP, false positive; H&E, hematoxylin-eosin; N/P, not performed at the discretion of the pathologist of record; PCR, polymerase chain reaction; Pt, patient; TN, true negative; TP, true positive.

a The presence or absence of a fungal infection in the specimen, based on the criteria described (ie, ≥2 positive test results for the etiologic agent).
that in all instances of true mycoses, one or more of the traditional diagnostic methods (ie, histopathology, direct examination, and fungal culture) established the diagnosis, eliminating, in almost all instances, the need for molecular studies.

Gomez and colleagues undertook an interesting study evaluating culture-independent methods for diagnosing fungal infections on specimens from 233 patients who had negative culture results but who had microscopic and ancillary study results indicative of an invasive fungal infection. For patients with a known fungal infection, they reported a high sensitivity (96.6%) and specificity (98.2%). However, the diagnostic yield dropped to 62.9% overall when patients who were tested were merely suspected of having an invasive fungal infection. They concluded that the diagnostic yield was dependent on the amount of tissue available; we would add to this “and evidence that some type of fungus may be present, based on traditional studies.”

A recognized and appropriate use of molecular diagnostic testing is for the characterization of an infecting fungus when other means are not available to establish the etiologic agent. The fungus from 1 specimen (ie, patient 4) that failed to grow in culture was identified by broad-range fungal PCR and DNA sequencing as Rhizopus oryzae. Although this application was arguably the most appropriate use of molecular diagnostics in this study, both the histopathologic evaluation and the direct examination demonstrated mucoraceous hyphae (ie, broad, pauciseptate hyphae), and these results were present long before the results from the molecular studies (ie, 1–2 days after surgery versus approximately 1 week). The findings from the traditional methods informed the clinical team that voriconazole was not a therapeutic option because of innate resistance in this group of fungi. Although the molecular findings were confirmatory of the histopathologic and direct examination findings, medical record review disclosed that there was no change in the therapy of this patient based on the molecular test result.

We encountered a challenging molecular test result with a sinus specimen, which was categorized as both a false-negative and a false-positive result. The molecular testing of the sinus specimen (patient 1, Table 1) did not detect the pathogens detected by the direct examination and culture (ie, a false-negative test result). This false-negative test result was included in the calculation of sensitivity and negative predictive value for the PCR/sequencing test. The PCR/sequencing result from this sinus specimen detected a fungus that was discordant with the direct examination and culture results, and it was not considered clinically relevant by the infectious disease clinicians caring for this patient (ie, a false-positive test result). This false-positive test result was included in the calculations for the specificity and positive predictive value for the PCR/sequencing test.

The value of the molecular test results for the 4 cardiac valve specimens that were among the true positives should also be questioned. In each instance, these specimens had either a positive histologic study and/or fungus demonstrated on direct examination and/or culture.

Although we have compared the cost per test of new and traditional tests above, the broad-range PCR/DNA sequencing does not represent a replacement for histopathology, direct examination, and culture for a variety of reasons. Histopathology could not be discontinued because tissue analysis may disclose a disease other than a fungal infection (eg, neoplasia, noninfectious etiologies, or another cause of infection). It would be unacceptable to discontinue the direct examination because this inexpensive assay provides a rapid result for the presence of a fungal infection in a time frame that cannot currently be achieved with PCR with DNA sequencing (ie, approximately a week as a send-out test, in our experience). Finally, although both broad-range PCR/DNA sequencing and culture afford the identification of the infecting fungus, only culture provides a viable isolate for susceptibility testing, which may be clinically necessary. Therefore, broad-range PCR/DNA sequencing cannot be considered as a replacement for the current standard of care assays, but rather as a supplemental test in a subset of cases when further identification is necessary to guide clinical decision-making.

The sensitivity of the direct examination (ie, KOH/calcofluor white exam) was the lowest of all the assays, which necessitates further investigation. The sensitivities of histopathology, culture, and broad-range fungal PCR/sequencing were equivalent in this study. Although broad-range fungal PCR/sequencing was found to be highly specific (ie, 95.0%), in this study the specificities of direct exam, histopathology, and culture were all 100%. We also recognize that false-positive results may also occur with the

| Specimen Categorization | Truly Positive (n = 6) | Truly Negative (n = 59) | Measures of Test Performance, % |
|-------------------------|----------------------|------------------------|---------------------------------|
|                         | TPs                  | FNs                    | FPs                             | TNs                | Sensitivity | Specificity | PPV | NPV |
| Histopathology          | 5                    | 1                      | 0                               | 59                 | 83.3        | 100         | 100 | 98.3 |
| Direct examination      | 4                    | 2                      | 0                               | 59                 | 66.7        | 100         | 100 | 96.7 |
| Fungal culture          | 5                    | 1                      | 0                               | 59                 | 83.3        | 100         | 100 | 98.3 |
| PCR and sequencing      | 5                    | c                      | 1c                             | 2                  | 57          | 83.3        | 95.0 | 62.5 | 98.3 |

Abbreviations: FN, false negative; FP, false positive; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value; TN, true negative; TP, true positive.

a Specimen contained pathogenic fungi by defined criteria.
b Specimen did contain pathogenic fungi by defined criteria.
c One specimen (patient 1, Table 1), which contained pathogenic fungi, had a broad-range fungal PCR and sequencing result that was categorized as an FN when calculating sensitivity and NPV, and as an FP when calculating specificity and PPV.
traditional diagnostic methods. These findings reiterate the diagnostic power of traditional methods, which remain, in our opinion, the most cost-effective, equally sensitive (ie, histopathology and culture), and more specific methods for the detection of medically important fungi. Importantly, there were no etiologic fungal pathogens detected by molecular methods that were not also detected by one or more of the traditional methods studied. Otherwise stated, the traditional methods did not fail to identify fungal infections that were detected by molecular methods. Importantly, there were no additional actions taken based on the presence of fungi detected by the molecular tests. Ergo, there were no changes in patient outcomes, treatment decisions, or length of stay, but rather only the addition of cost.

Clinicians and pathologists should work together to improve processes to ensure appropriate diagnostic studies are ordered to optimize patient care and maintain care affordability. This study demonstrates that direct or routine (nonalgorithmic) use of fungal broad-range PCR with DNA sequencing does not add value and is not cost effective. The indiscriminate ordering of this molecular testing in the manner described is a waste of limited resources. This study also supports the incorporation of results from traditional studies to triage specimens for the use of broad-range fungal PCR and DNA sequencing. It also demonstrates the feasibility of using this technology when fungi are present in human tissues or detected in the direct examination, but the culture fails to grow the infecting fungus. Even in these situations, sending the specimen for molecular studies should be critically assessed with the clinical providers to determine whether or not the results of the molecular studies will change or impact therapy. The implementation of an appropriate triage process affords the opportunity to preserve diagnostic quality while reducing health care costs.

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