In the Literature

Rapid Distinction between Candida albicans and Non-albicans Yeast by Direct Gram Stain of Candida-Positive Blood Cultures


A commonly used strategy for patients who are found to have candidemia is to initiate therapy with high-dose fluconazole if the bloodstream isolate is Candida albicans but to use alternative therapy, such as an echinocandin, if the isolate is identified as a non-albicans species of Candida. The rationale for this approach takes account of the relatively inexpensive acquisition cost of generic fluconazole relative to the costs of most agents and the fact that, although it is unusual for C. albicans to exhibit high-level resistance to fluconazole, some non-albicans species, such as Candida glabrata, frequently exhibit resistance. This strategy only applies, of course, to the preferable circumstance in which the laboratory has identified Candida species in blood culture and antifungal therapy has not already been initiated. The initial distinction between C. albicans and non-albicans species is often made in the laboratory by means of the germ tube test. As this test is usually performed, a colony is picked from subculture on solid media, incubated for 2.5–3 h, and then microscopically examined. The test results are positive for ~95% of C. albicans isolates, but false-positive results are occasionally reported, usually as a result of the presence of Candida tropicalis, an organism that is usually susceptible to fluconazole. A test that avoids the need for subculture, which generally requires 24 h, and that avoids the ~3-h incubation period would make this process more efficient and would possibly improve patient treatment.

One alternative procedure, which uses fluorescent in situ hybridization, shortens the required time by allowing for direct application on a Gram stain–positive blood culture, thus avoiding the need for subculture [1]. This procedure is, however, significantly more expensive than the germ tube test. Recently, it has been reported that the germ tube test can be performed directly on an aliquot from Gram stain–positive blood culture media [2]. Now, an even simpler method, which is performed directly on positive blood culture media, has been described.

Harrington and colleagues at the University of Washington (Seattle) microscopically examined Gram-stained aliquots of the contents of blood culture bottles for 60 consecutive patients with candidemia for the presence of pseudohyphae clusters. A single blood culture typically involved the use of an aerobic and an anaerobic bottle, as well as a fungal bottle (Myco/F). When a bottle’s result was indicated as positive by the Bactec 9240 continuous monitoring system, a sample was stained, and the isolate was then identified by use of germ tube testing after subculture and standard biochemical methods. A specimen was considered to have findings consistent with the presence of C. albicans (i.e., a positive test result) if “one or more clusters of budding yeast with elongated, overlapping, and branching pseudohyphae were present at 10× magnification” (p. 326). Examiners were masked to the final identification of the organism, and interobserver agreement was 100%.

The most frequently isolated yeasts were C. albicans (which accounted for 43% of the total), followed by C. glabrata (28%), Candida parapsilosis (8%), Candida krusei (7%), and C. tropicalis (5%). Examination of the first blood culture bottle to trigger the growth indicator found that 22 of 26 that contained C. albicans tested positive for pseudohyphal clusters, and only 1 of 34 non-albicans isolates (C. tropicalis) yielded positive results. The results were improved if only the aerobic blood culture bottle was considered, regardless of which bottle first yielded positive results, with only 1 false-negative result; however, the C. tropicalis isolate continued to yield a false-positive result. Of note is that the aerobic bottle, in fact, was the first to yield a positive result in two-thirds of cases. The sensitivity, specificity, positive predictive value, and negative predictive value of examination of the first positive bottle were 85%, 97%, 96%, and 89%, respectively, whereas all values were 95%–96% if only the examination of the aerobic bottle was considered. The sensitivity of examination of the Myco/F fungal blood culture bottle was only 25%.

If confirmed by others, this simple technique could prove to provide a low-cost means of reducing, by a few hours, the time to optimal initial selection of antifungal therapy for patients with candidemia. One important caveat mentioned by the authors is the potential for misleading results in patients with fungemia that is simultaneously due to >1 species—an occurrence identified in 2 patients (3%) in this study (who were not considered in the evaluation of test performance). One of the 2 patients with mixed fungemia was infected with both C. albicans and C. glabrata, which would result in a positive result for clustered pseudohyphae, potentially leading to therapy with fluconazole, which could be ineffective against C. gla-
brata. It should be mentioned, however, that this drawback also applies to the other methods discussed above.

References


The Medicinal Leech Is Not What It Seems


In 2004, the US Food and Drug Administration (FDA) approved the use of the medicinal leech, Hirudo medicinalis, as a medical device as „an adjunct to the healing of graft tissue when problems of venous congestion may delay healing, or to overcome problems of venous congestion by creating prolonged localized bleeding” [1]. It now turns out that the H. medicinalis that the FDA thought it was approving is not H. medicinalis.

Siddall and colleagues evaluated a variety of wild-caught and commercial medicinal leeches, making species assignments on the basis of examination of mitochondrial sequences and nuclear microsatellites. Among the wild leeches, all of which were captured in Europe, they identified 3 distinct species: H. medicinalis, Hirudo verbana, and Hirudo orientalis. Analysis of 10 leeches obtained from 4 major commercial suppliers in the United States and United Kingdom, however, revealed that all were H. verbana; none were the approved species, H. medicinalis.

Does this misidentification matter? The investigators point out that >100 bioactive compounds have been isolated from medicinal leeches, and many are the subject of active investigation. Some of these molecules may have significant interspecies variability that may have implications with regard to both previously reported research. It also raises the likelihood that more-potent molecules may exist in species other than those that were previously examined.

Other implications have some potentially unusual consequences. In Europe, H. medicinalis is listed as an endangered species. In contrast, the species that is the commercially available medicinal leech, H. verbana, is not protected. Perhaps more importantly, in a remarkable bureaucratic snarl, H. verbana, in contrast to H. medicinalis, has been not approved for use as a medical device by the FDA, and mislabeling of a medical device is proscribed by law. Whoops!

Reference


Excluding the Diagnosis of Bacterial Meningitis


The Bacterial Meningitis Score consists of a set of elements, the absence of which has been proposed as a means to identify patients at very low risk of bacterial meningitis. The elements, all of which must be absent, are a positive CSF Gram stain result; CSF absolute neutrophil count, ≥1000 cells/µL; CSF protein concentration, ≥80 mg/dL; peripheral blood absolute neutrophil count, >10,000 cells/µL; and a history of seizure before or at the time of presentation. In a study of 3295 children with CSF pleocytosis, 121 (3.7%) of whom proved to have bacterial meningitis, the prediction rule had a sensitivity of 100%, a specificity of 63.5%, and a negative predictive value of 100%. The risk of bacterial meningitis was only 0.1% when all the elements were absent. Because children who had received an antibiotic ≤72 h before undergoing lumbar puncture were excluded from the study, the results cannot be applied in that circumstance.

Phase II Study of Raltegravir, an Inhibitor of HIV-1 Integrase


HIV-infected patients (n = 179) who had CD4 T cell counts >50 cells/µL and plasma viral loads >5000 copies/mL and who had experienced documented multidrug resistance, including resistance to at least 1 protease inhibitor, were randomized to receive placebo or raltegravir at 1 of 3 dosages (200 mg, 400 mg, or 600 mg twice daily); each patient also received optimized background therapy. At week 24, the mean change from the baseline viral load was -0.35 log10 copies/mL among placebo recipients and -1.80 to -1.87 log10 copies/mL among raltegravir recipients. Raltegravir was well tolerated.

A Novel Method of Methicillin-Resistant Staphylococcus aureus (MRSA) Decolonization


A nurse, having been successfully treated for a urinary tract infection due to MRSA, was found to have gastrointestinal carriage of MRSA, but not nasal or throat carriage. It was recommended that she undergo attempted decolonization, but she refused antibiotic therapy and, instead, was given a preparation of lytic S. aureus bacteriophage. The preparation, which contained 3 different phages (676/F, A3/ R, and A5/80) in a titer of 7 × 106/mL, was administered orally 3 times daily for 4 weeks. MRSA was subsequently undetectable in rectal swabs after the first week of treatment and remained so over the next half year.