In the Literature

PCR for Invasive Aspergillosis


The diagnosis of invasive Aspergillus infection is often problematic and seldom definitively proven either before or after the start of antifungal therapy. The serum galactomannan assay, which is widely available, has been disappointing. A number of investigators have developed polymerase chain reaction (PCR) assays for detection of Aspergillus nucleic acid and have reported variable results. The AmBiLoad trial, which found no difference in outcome in patients with proven or probable invasive filamentous fungal infection (97% with aspergillosis) treated with 2 different doses of liposomal amphotericin B [1], provided an excellent opportunity to evaluate the performance characteristics of PCR for detection of Aspergillus DNA in plasma.

Hummel and colleagues tested serially collected samples from 91 patients (median of 4 samples per patient) treated for invasive aspergillosis in the AmBiLoad trial at 6 German centers. Seventy percent of samples were obtained while the patient was receiving antifungal chemotherapy. Based on the modified criteria of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG), 7 of the patients had proven and 52 had probable invasive fungal infection (IFI). The nested PCR used by the investigators had a sensitivity of 1–5 colony-forming units per milliliter of blood.

Of the total of 459 samples, Aspergillus DNA was detected in 43 with a positive result in samples from 3 of the 7 patients with proven IFI (43%), 18 of 52 with probable IFI (35%), and 8 of 31 with possible IFI (26%). Among those patients with a positive PCR, only 8 of 21 with proven or probable IFI had more than 1 positive sample, as did only 2 of the 8 with possible IFI. PCR was more likely to be positive in samples from patients who had an unfavorable response to therapy than in those with a favorable response (48% vs 21%, respectively). Of those with an unfavorable response and a positive PCR, one-half had more than 1 positive test. Of the 7 patients with proven infection, only 1 had a positive result with the serum Platelia galactomannan assay (a positive result required more than 1 sample with an optical density index result ≥1.0).

Whereas the PCR used by these investigators was more sensitive than the commercially available Platelia galactomannan assay, its clinical usefulness is questionable. The authors point out a number of potential reasons for the insensitivity of their assay, including the fact that it was specific for Aspergillus fumigatus and that infections with other fungi may have been etiologic in some of these cases. Although broadening the range of fungi detected by the assay may prove helpful, it is not likely to raise the sensitivity of the assay to a suitably high level. The world of invasive filamentous fungal infections in immunocompromised patients remains one with more questions than answers and, often, more art than a science.

References


A New Central Nervous System Syndrome in HIV-Infected Patients?


Retrospective evaluation of the records of 30,954 human immunodeficiency virus (HIV)–infected patients in the UK between 1996 and 2007 identified 613 patients (2.0%) who had developed intercurrent central nervous system (CNS) diseases [1]. These were, in decreasing order of frequency, HIV encephalopathy, progressive multifocal leukoencephalopathy, toxoplasmosis, and cryptococcal meningitis. Other diagnoses such as lymphoma and cytomegalovirus encephalitis were excluded from the analysis because of their rarity. The incidence of these CNS complications decreased from 13.1 to 1.0 per 1000 patient-year over the period of observation, a time when progressively more patients received effective combination antiretroviral therapy (cART).

Given this marked reduction in CNS complications of HIV infection, it would be reasonable to guess that there would “nothing new under the sun.” Newsome
and colleagues in Baltimore, however, now describe 10 patients among 2754 (0.4%) patients seen from 1993 through 2008 with what they believe is a unique CNS disorder in HIV-infected users of illicit drugs. The 7 men and 3 women were included in the series on the basis of HIV infection and active drug use and the presence of noninfectious bilateral basal ganglia lesions revealed by magnetic resonance imaging, which showed diffuse hyperintense FLAIR or T2-weighted basal ganglia abnormalities in each patient. Thalamic lesions were also detected in 5 patients, and 6 had lesions outside their deep gray matter. No evidence of ischemia was observed, and the lesions all failed to enhance with gadolinium infusion.

The patients were initially seen because of 1 or more seizures or altered mental status, with symptom onset at a mean of only 2.4 ± 4.2 days before presentation. Of the 10 patients, 9 used cocaine, often with heroin, whereas 1 used heroin alone; 8 had renal dysfunction (acute in 6); and 6 had chronic hepatitis C virus infection, but this was active in only 1 patient. Their mean CD4 T-cell count was 19 ± 21 cells/mm³ (range, 1–68 cells/mm³) and their HIV plasma viral loads were ≥193,392 copies/mL. Cerebrospinal fluid pleocytosis was generally absent (range, 0–12 WBC/mm³), but the protein concentration was significantly elevated (range, 63–838 mg/dL). No infectious etiology was detected in any patient.

Of the 10 patients, 8 died after a median of 21 days. The 2 survivors, only 1 of whom had renal dysfunction, were the only patients in whom cART was started during their hospitalization. Neuropathological examination was performed and this revealed diffuse microglial activation within the basal ganglia with perivascular microglial nodules, but no evidence of HIV.

These HIV-infected, cocaine-using or heroin-using patients with advanced immunodeficiency appear to have suffered from a previously unreported syndrome marked by acute onset of seizures or altered mental status with nonenhancing basal ganglia lesions on magnetic resonance imaging and elevated cerebrospinal fluid protein concentrations with minimal or no pleocytosis. They had rapidly progressive courses, with the only survivors being the 2 patients in whom cART was started during their hospitalization. The etiology of this process is unknown. A relationship with illicit drug use is suggested, but the HIV population at Johns Hopkins, where these patients were seen, is heavily weighted with such patients, making a definitive association potentially fraught with uncertainty resulting from a selection bias.

References

KSHV—A Master Manipulator


Herpesviruses have complex interactions with the innate immune system [1]. Kaposi sarcoma–associated herpesvirus (KSHV, or human herpesvirus 8 [HHV-8]), a β-herpesvirus, devotes one-fourth of its genomic coding capacity to production of immunomodulatory molecules, a number of which allow it to evade the host immune response during both latent and lytic infection [2]. An etiologic agent of Kaposi sarcoma, primary effusion lymphoma, and multicentric Castleman disease, KSHV has previously been demonstrated to interact at different points in its life cycle with the innate immune system. Thus, during primary infection, KSHV upregulates Toll-like receptor (TLR) 3 expression in monocytes and induces TLR3-specific cytokines and chemokines [3], while TLR7/8 agonists reactivate latent KSHV and induce viral lytic gene transcription and replication [4]. TLRs, and other pattern recognition receptors, signal immune cell activation on interaction with pathogen-associated molecular patterns. Activation of a subset of the pattern recognition receptors—nucleotide-binding and oligomerization, leucine-rich repeat (NLR)—leads to the formation of inflammasomes, key regulators of the innate immune system. The inflammasome consists of a complex of an NLR, procaspase-1, and an adaptor protein. This complex activates caspase 1, which leads to the proteolytic processing of interleukin 1β and interleukin 18, and is associated with pyroptosis, a form of cell death with, in contrast to apoptosis, a concomitant inflammatory response.

Gregory and colleagues identified a known KSHV tegument protein, Orf63, as a viral homolog of human NLRP1, a member of the NLR family that does not, however, contain the caspase activation or pyrin domains, 2 components necessary for its activation. This suggested to the investigators that Orf63 functioned as an inhibitor of NLRP1, a hypothesis that was confirmed by demonstrating that it blocked NLRP1-dependent innate immune responses, interfering with processing of IL-1β and IL-18, as well as with caspase-1 activation. Thus Orf63, acting as a decoy inhibitory protein, resulted in protection of KSHV-infected cells from NLRP1-dependent cell death and, furthermore, was necessary for efficient reactivation of latent virus and production of new virions.

The fact that the ORF63 protein is also found in other herpesviruses suggests that this strategy evolved long ago and that it may well have similar functions in other viruses of this group.
References


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