Not Over Yet: Fungal Infections following Methyl Prednisolone Injections Smoulder On

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The multistate outbreak of fungal infections in the United States following contaminated methyl prednisolone injection has been well reported (1). Meningitis was the most frequent consequence, but other infections included arachnoiditis, with local spinal infection and peripheral joint infections seen in 12% of cases. The black mold Exserohilum rostratum was implicated as the main causative agent, but a wide variety of other fungi have also been identified. More than 13,500 individuals received potentially contaminated injections, and at the latest count there have been 751 infections across 20 states of the United States and 64 deaths. As follow-up of exposed and infected patients continues, it is becoming apparent that cohorts of exposed patients are emerging with chronic symptoms (2).

The paper presented by Zhao and colleagues (3) describes the investigation of blood and synovial fluid specimens from 20 patients with symptoms more than a year after receiving contaminated injections. Not all of the patients met the case definition, and no cause had been identified. E. rostratum-specific and panfungal PCRs were performed, and 10 patients gave positive results, of which 7 were considered significant. Only one of these was confirmed to be E. rostratum, but a wide range of other environmental fungi were identified by sequencing of the panfungal PCR product. Aspergillus sp. was the commonest mold implicate. It is worth remembering that the index case in the outbreak suffered an aspergillus infection (4).

The work highlights a number of aspects deserving of further recognition. The speed and efficiency with which the CDC investigated and contained the outbreak are impressive. From the first notification of potential cases in September 2012 to the recall of the contaminated products, contact and communication with 99% of all potentially exposed individuals, and identification of E. rostratum as the major causative organism, a mere 4 weeks elapsed.

Fundamental to this achievement was the ability of the CDC and Public Health Research Institute Centre to respond to this crisis and develop diagnostic assays (5, 6). From an initial panfungal approach, they were able to develop a specific PCR for E. rostratum which proved clinically useful during the outbreak (6). Although this was not without problems, a molecular test was the only type of nonculture diagnostic test that could be developed in such a short time. The assays were developed “on the hoof,” such that the specimens of cerebrospinal fluid (CSF), synovial fluids, abscess aspirates, and tissues from the outbreak cases were used to validate the assay. In the initial study, PCR sensitivity was relatively poor, at 20 to 38%. However, in a subset of 139 cases, PCR sensitivity was double that of culture, although some cases were positive only by one of the assays, highlighting the difficulty in diagnosing invasive fungal disease (IFD) and the need for a multifaceted diagnostic approach (6). Specificity was high (100%) and could be used to confidently diagnose disease.

Since these novel assays lack FDA or CE approval, it was stressed that it should not be used for diagnostic or patient management purposes but as a research tool only. However, this also highlights the limitations of relying on FDA- or CE-approved tests to diagnose novel or emerging pathogens in an emergency situation, where the time required to attain approval impairs diagnostic utility. The same is true for changes within the nucleic acid sequence of pathogens that then render current assays redundant. Diagnostic tests can be applied in two ways, either employing high sensitivity to screen specimens and reliably exclude disease (albeit accepting a significant false positivity rate) or utilizing high specificity to confidently rule in a diagnosis while accepting that a negative will not exclude disease. In this study, the aim was toward the latter.

The study of 20 patients and 6 controls described here is the first to use predominately blood samples to investigate this outbreak, permitting samples to be taken at multiple time points and enhancing the chance of diagnosis (3). It confirmed the benefit of molecular testing in culture/magnetic resonance imaging (MRI)-negative patients with persistent or progressive symptoms and highlighted the possibility of mixed and multiple causative agents and the consequent effects on diagnostic requirements. It also provides some interesting technical insights. Efficient DNA extraction from whole blood requires additional steps to remove blood cells and disrupt fungal cell walls (7). This was not performed and, if used, may have improved sensitivity. Apart from this, the methodology is sound, with use of appropriate controls, and the real-time E. rostratum PCR appears to follow MIQE (minimum information for publication of quantitative real-time PCR experiments) guidelines (8). The cutoff chosen for the panfungal PCR is rather early given the low fungal burden in blood but is driven by false positivity, and a single control sample still exceeded this threshold. Investigation into the false positivity would be beneficial to determine whether this was a result of low-grade contamination, as suggested, or a result of oligonucleotide interactions/cycling conditions that could be resolved via further assay optimization. Unfortunately, no information concerning false positivity in the no-template controls is provided. It would also have been beneficial to sequence all positives, since this may have identified a common contaminant that possibly could be used for differentiation of true/false positives. Nevertheless, by using an...
early positivity threshold assay, specificity is increased, improving diagnostic accuracy.

The paper suffers from a lack of clinical information. Specifically, there are no data on disease manifestations (important considering that blood was tested) and concurrent antifungal usage, which could affect PCR positivity.

Nonetheless, it is becoming apparent that only a minority of cases from the outbreak have been microbiologically confirmed, and there remains a large pool of exposed individuals who may be at risk of indolent and chronic infection from a broad range of fungal pathogens. Given that 50% of undiagnosed but symptomatic patients were positive in this study and the limitations of classical mycological investigations, these assays are essential in the follow-up of this outbreak. More multicenter evaluation and standardization are required before wide-scale rollout.

REFERENCES


