In encephalitis case, next-gen sequencing is the star

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April 2014—In what may be a first for the burgeoning field of next-generation sequencing, this powerful new technology was used to identify the cause of encephalitis in a teenage boy who had been critically ill in the intensive care unit for several weeks. Diagnosis suggested a specific treatment. Within two weeks of initiating therapy, the boy had recovered and was discharged.

It is becoming common practice to use NGS to detect mutations that can help select drug therapy in cancer cases and to find genetic variations responsible for inherited diseases. However, NGS has not previously been considered a useful tool in critical care situations, where a short turnaround time is crucial. "Next-generation sequencing can have great utility in these types of cases," Joe DeRisi, PhD, professor of biochemistry and biophysics and Howard Hughes investigator at the University of California, San Francisco, School of Medicine, said in a presentation at the Advances in Genome Biology and Technology (AGBT) conference in February. In the case Dr. DeRisi described, the 48-hour turnaround time from extraction of nucleic acid to identification of the cause of disease provides a new perspective on the utility of this rapidly expanding molecular technology.

Encephalitis could be a particularly fruitful field in which to exploit NGS' newly demonstrated ability. In a seven-year study, California Encephalitis Project investigators found that, after extensive workup with existing methods, 63 percent of cases went undiagnosed (Glaser CA, et al. Clin Infect Dis. 2006;43:1565–1577). The problem, says Charles Chiu, MD, PhD, professor of laboratory medicine and infectious diseases at UCSF and a senior investigator in the study, is that "the causes of encephalitis are broad in scope," ranging from infectious agents, especially in immunosuppressed individuals, to non-infectious autoimmune disease and vasculitis.

Michael Wilson, MD, professor of neurology at UCSF, was working in Dr. DeRisi's laboratory to learn NGS technology at the time of this case. "From the neurologist's standpoint," he tells CAP TODAY, "when a patient with encephalitis comes into the hospital, you expect that more often than not you won't come up with a diagnosis, no matter how many tests you run. Infectious disease doctors share that frustration. This kind of case is exactly why I wanted to learn next-generation sequencing.

"What still kind of amazes me is that not only do we fail to identify a pathogen many times, but often we fail to differentiate between an autoimmune or an infectious cause. That's what happened in this case, too," he says. "Any technology that might increase that otherwise very poor yield is exciting."

Dr. DeRisi and his colleagues are seeking to exploit this newly discovered potential of NGS by setting up a protocol for investigating cases of encephalitis and meningitis. Prospectively obtained samples will be divided between brain slices to search for evidence of autoimmune disease and next-gen sequencing to find infectious agents.

In his AGBT talk, Dr. DeRisi presented the case in classic fashion, describing the diagnostic process without revealing the solution to the puzzle until the end. The patient was a 14-year-old Wisconsin boy who had severe combined immunodeficiency disease due to adenosine deaminase (ADA) deficiency partially corrected by a bone marrow transplant. He was receiving monthly intravenous immunoglobulin.

In September 2012 the boy presented to the ophthalmology clinic at a hospital in Madison, Wis., with bilateral conjunctivitis, along with six days of fever and headache. Symptoms resolved over the next 10 days.

In October the boy went to the ophthalmology clinic again, this time with photophobia. He was given steroid eyedrops and ciprofloxacin. His symptoms resolved by December.

He also had thrombocytopenia which, Dr. DeRisi said, was thought at the time to be of autoimmune origin from a

transplant effect. His platelet count returned to normal with rituximab treatment.

At this point in his talk, Dr. DeRisi made known the boy's prior exposures, which included a trip to Puerto Rico in August, after which he had four days of fever and hematuria. Later, his family had vacationed in Florida. During these vacations the boy swam in the ocean and a resort pool.

A few months later the boy's condition became more severe. In April he presented to the emergency department with six days of headache and fever to 103 degrees. A workup for infectious agents was done, including PCR assays for enterovirus, influenza, Epstein-Barr virus, and cytomegalovirus and bacterial culture. All results were negative. In the same month he developed photophobia again, with fever, fatigue, and weight loss. Blood work results were normal. An MRI was unrevealing.

Workup of the cerebrospinal fluid showed unusual findings, including high protein, 97 mg/dL, and low glucose, 24 mg/100 mL. Leukocytes were also high, at 125 cells/mL.

In July the boy's condition began to deteriorate, with fever, headache, vomiting, and weakness. Cerebrospinal fluid levels of glucose and protein became worse. This time rituximab produced no improvement. Abnormalities were seen on MRI—irregular hyperintensities in the basal ganglia and inflammation in the brainstem suggesting meningitis. An even more extensive workup for infectious agents on multiple specimens came back negative. Histology on a biopsy from the right frontal lobe showed nonspecific inflammation but was otherwise unrevealing.

Empiric treatment with cefuroxime, an antibiotic, had no impact on the patient's status. Physicians started intravenous steroids and pegylated-ADA. He subsequently developed altered mental status and onset of seizures, for which he was put into an induced coma.

At this point the patient had been in the hospital for 28 days. Doctors still did not know whether the encephalitis was due to autoimmune disease or infection, which was the crucial distinction because the two would be treated differently. For the former, the immune system would be suppressed, whereas for infection, immunosuppressive therapy would be withdrawn and targeted antibiotics started depending on the identity of the causative pathogen.

"This is where a diagnostic can have an actionable outcome," Dr. DeRisi told AGBT attendees. "It can help make a decision about treatment."

At 38 days of hospitalization, one of the boy's treating physicians spoke on the phone with Dr. DeRisi, with whom he was doing a research collaboration on asthma. Dr. DeRisi agreed to do NGS on a sample of the boy's CSF. An emergency institutional review board was convened to allow the boy to be enrolled for NGS for diagnostic purposes, since NGS is a research-only technology in this context.

On day 42, CSF and blood from the patient were transferred to Dr. Chiu's laboratory at UCSF for analysis. The Chiu lab had previously optimized a rapid protocol for processing clinical samples as part of the ongoing study using NGS to investigate causes of acute illness. In addition, the Chiu laboratory had developed and validated a rapid cloud-compatible bioinformatics pipeline, named SURPI, for pathogen detection and discovery from mining of NGS data. By use of SURPI, only 96 minutes of the 48-hour total processing time were taken up with analysis of the sequence reads.

A clear result emerged from the data. "SURPI quickly identified Leptospira in the bacterial portion of the sequence output," a bacterium never seen before in the laboratory, Dr. Chiu says. Bacterial DNA made up just 475 out of more than 8 million reads. Identification was confirmed with Sanger sequencing. Additional evidence that this finding was real, Dr. Chiu says, was that "the reads were distributed all over the Leptospira genome. They weren't just repeats of one segment."

While rare in the U.S., Leptospira is "one of the most widespread zoonoses in the world," Dr. DeRisi said. It is contracted from swimming in fresh water in which infected animals have urinated. It also typically presents with conjunctivitis and uveitis, congruent with the boy's disease course. Subsequently it was found to be the Leptospira santarosai strain, which is seen in Puerto Rico.

With a probable diagnosis and a specific etiologic agent in hand, the next step was to determine whether to institute therapy on the basis of a result from a research-only test. Several multidisciplinary specialists, including a bioethicist, discussed this question in a series of conference calls. Dr. DeRisi said the consultants were concerned with whether the result could be due to contamination and the evidence that it was the actual pathogenic cause. "We had confidence in the result," he tells CAP TODAY. "And it was not such a hard decision in this case because the treatment was relatively harmless."

"These were intense discussions," says Dr. Wilson, who took part in the calls. "Charles and I initially discussed whether we should recommend treating the patient in the absence of a confirmatory test. It was a Friday afternoon, and they wouldn't be able to send out a specimen to a public health laboratory for confirmatory testing until the following week. Even then it would be two weeks at least before they would get an answer. They said he didn't have two weeks. When the clinicians in Wisconsin heard we had a possible diagnosis that was very treatable, to them it wasn't too hard of a call to use the information and treat."

Therapy was started—13 million units of penicillin and tapering steroids. Fourteen days later the boy was discharged with baseline CSF levels. Dr. DeRisi called his rapid recovery "unreal."

Could this be called a lifesaving diagnosis? "I never evaluated the patient personally," Dr. Wilson says, "so it is hard for me to say. The treating physicians in Wisconsin believe it was lifesaving. They were worried he wouldn't last through the weekend."

A 17-year-old boy who went on the same trip to Puerto Rico also became ill upon return, with fever and kidney problems. However, he spontaneously recovered. "The CDC is doing the testing now, but we think that probably he was infected with Leptospira as well," Dr. Wilson says. Perhaps the difference in disease course was due to a difference in the two boys' immunocompetence.

An alternative to initiating treatment immediately would have been to send a sample to a public health laboratory for the approved confirmatory assay—PCR for a specific Leptospira gene. At the same time penicillin therapy was started, a sample was sent to the Centers for Disease Control and Prevention for PCR testing. Two weeks later, the result came back negative. "This is not surprising," Dr. Chiu says, "since the assay had not been validated with CSF or with clinical samples infected by the Leptospira santarosai strain." He tells CAP TODAY that the CDC subsequently obtained positive results by tweaking the PCR amplification mix, a modification that was not part of an approved clinical assay. "So if we had waited for a confirmatory test, it would have taken extra time and would have been negative," Dr. DeRisi said.

Dr. Wilson points out another aspect of this situation, one that supports early diagnosis and rapid treatment. "Leptospira made this boy very sick," he says, "but it was not destructive to his brain. When the infection cleared, his mental function rapidly recovered. However, with many brain infections, even if you make the diagnosis and treat, if you wait too long, the damage can be irreversible." Herpes encephalitis is a notable example.

According to this line of thinking, introducing NGS earlier might be warranted in cases of undiagnosed encephalitis. "The bulk of diagnoses with conventional techniques are made early on," Dr. Wilson says. "There is definitely a role for something like this pretty early. If you come up empty after the first week, you need to think hard about other ways to come up with the answer." Perhaps the encephalitis/meningitis protocol Dr. DeRisi and colleagues are developing will provide guidance on this question of timing.

From a financial point of view, performing NGS on an individual case calls up a value judgment, Dr. DeRisi said. "As an individual test, next-generation sequencing might seem expensive. However, when you compare the extensive workup and a hospital stay potentially costing millions of dollars to the cost of next-generation sequencing, it was relatively cheap.

"Next-generation sequencing has great promise," he said, "as an unbiased, relatively inexpensive, sensitive, and accurate assay for infectious disease agents."

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