PCR and Electrospray Ionization Mass Spectrometry for Detection of Persistent *Enterococcus faecalis* in Cerebrospinal Fluid following Treatment of Postoperative Ventriculitis

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We describe the use of PCR and electrospray ionization followed by mass spectrometry (PCR/ESI-MS) to evaluate “culture-negative” cerebrospinal fluid (CSF) from a 67-year-old man who developed postoperative bacterial ventriculitis following a suboccipital craniotomy for resection of an ependymoma in the 4th ventricle. CSF samples were obtained on seven occasions, beginning in the operating room at the time of insertion of a right ventriculoperitoneal shunt (VPS) and continuing until his death, 6 weeks later. During the course of the illness, two initial CSF specimens taken before the initiation of antimicrobial treatment were notable for growth of *Enterococcus faecalis*. Once antimicrobial treatment was initiated, all CSF cultures were negative. PCR/ESI-MS detected genetic evidence of *E. faecalis* in all CSF samples, but the level of detection (LOD) decreased once antimicrobial treatment was initiated. When our patient returned with symptoms of meningitis 3 days after the completion of antibiotic treatment, CSF cultures remained negative, but PCR/ESI-MS again found genetic evidence for *E. faecalis* at levels comparable to the pretreatment levels seen initially. This unique case and these findings suggest that determination of CSF LOD by PCR/ESI-MS may be a very sensitive indicator of persistent infection in patients on antibiotic therapy for complex CNS infections and may have relevance for treatment duration and assessment of persistent or recurrent infection at the completion of therapy.

CASE REPORT

A 67-year-old non-insulin-dependent diabetic man with a history of hypertension and coronary artery disease presented for evaluation of ataxia. He complained of approximately 3 months of “listing to the right upon walking”; this symptom was associated with gradually worsening headaches, nausea and vomiting, vertigo, and tremors. Other than tremor and a positive Romberg sign, results of the patient’s examination were unremarkable. The examination showed that motor, sensory, and cranial nerves were intact. A mass consistent with an ependymoma was discovered in the 4th ventricle on magnetic resonance imaging (MRI) of the brain. Due to the significant mass effect and suspicion of malignancy, resection was indicated, and he was scheduled for resection of the 4th ventricle mass.

A suboccipital craniotomy was performed. Preoperative cefazolin antibiotic prophylaxis was administered. After tumor resection, the dura was closed. Pathological examination of the fourth ventricular tumor was consistent with a mixed ependymoma/subependymoma, World Health Organization grade II. The morning following surgery, diplopia developed. Postoperative MRI of the brain demonstrated complete resection without complication. A pseudomeningocele was noted in the soft tissues, although no CSF leak was noted at the wound; a computed tomography (CT) scan of the head 24 h postoperation demonstrated hydrocephalus, prompting insertion of a right frontal external ventricular drain (EVD). The CSF appeared normal. At this time, CSF was not submitted for culture or cell count.

Despite the administration of dexamethasone (10 mg intrave-

nously [i.v.]) every 6 h, the patient remained confused. The white blood cell (WBC) count increased to $23.1 \times 1,000$ cells/ml following EVD insertion but returned to the normal range the following day. The EVD was able to be weaned in 9 days. During this time, CSF profiles and cultures were not drawn due to lack of clinical or laboratory findings suggestive of infection. After a CT scan of the brain confirmed resolution of hydrocephalus, the EVD was removed. Our patient was discharged to the neurological rehabilitation unit 1 day following EVD removal.

On the first day in the rehabilitation unit, he was diagnosed with an *Escherichia coli* urinary tract infection and oral levofloxacin treatment (750 mg a day for 3 days) was initiated. On the fourth day in the rehabilitation unit, he developed headache, somnolence, and confusion. Dexamethasone treatment was resumed. A repeat head CT scan showed recurrence of hydrocephalus and an increase in the size of the preexisting pseudomeningocele. The following morning, a right ventriculoperitoneal shunt (VPS) placement was performed without complication. A grossly bloody CSF specimen that was obtained during the procedure (red blood cell [RBC] count = 40,000 cells/mm³; WBC = 740 cells/mm³) was submitted for culture and Gram stain analysis (Table 1). The

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TABLE 1 PCR/ESI-MS level of detection versus CSF culture results^a

POD	Specimen source (specimen appearance)	Gram stain result/culture result	Glucose level; total protein level; RBC count; WBC count (granulocyte and lymphocyte differentials)	Organism detected by PCR/ESI-MS	Level (no. of GE/well)	Antimicrobial treatment (DOT)
14	O.R. (grossly bloody, slightly cloudy)	No organisms seen/5–10 colonies of <i>E. faecalis</i>	67 mg/dl; 189 mg/dl; 40,000 cells/mm ³ ; 740 cells/mm ³ (83% and 14%)	<i>E. faecalis</i> (<i>vanA</i> negative)	99	Levofloxacin (3), cefazolin (one preoperative dose)
20	Aspiration of VPS (slightly bloody, cloudy)	No organisms seen/<5 colonies of <i>E. faecalis</i>	105 mg/dl; 91 mg/dl; 4,350 cells/mm ³ ; 157 cells/mm ³ (17% and 29%)	<i>E. faecalis</i> (<i>vanA</i> negative)	100	None
23	EVD (xanthochromic, clear)	No organisms seen/no growth (5 days)	78 mg/dl; 101 mg/dl; 68 cells/mm ³ ; 6 cells/mm ³ (7% and 92%)	<i>E. faecalis</i> (<i>vanA</i> negative)	6	Ampicillin (4), cefazolin (one preoperative dose)
26	EVD (clear, slightly cloudy)	No organisms seen/no growth (5 days)	78 mg/dl; 59 mg/dl; 254 cells/mm ³ ; 4 cells/mm ³ (0% and 88%)	<i>E. faecalis</i> (<i>vanA</i> negative)	9	Ampicillin (7)
29	EVD (slightly xanthochromic, slightly cloudy)	No organisms seen/no growth (5 days)	86 mg/dl; 67 mg/dl; 1 cell/mm ³ ; 0 cells/mm ³	<i>E. faecalis</i> (<i>vanA</i> negative)	10	Ampicillin (10)
50	Lumbar puncture (slightly bloody, cloudy) ^b	No organisms seen/no growth (10 days) ^c	41 mg/dl; 240 mg/dl; 41 cells/mm ³ ; 6,317 cells/mm ³ (97% and 3%)	<i>E. faecalis</i> (<i>vanA</i> negative)	91	None
55	EVD (slightly bloody, cloudy) ^b	No organisms seen/no growth (2 days) ^c	—; 10,000 cells/mm ³ ; 52 cells/mm ³ (64% and 30%)	<i>E. faecalis</i> (<i>vanA</i> negative)	19	Ampicillin (6), cefepime (4), vancomycin (6)

^a O.R., operating room; POD, number of days from initial craniotomy for tumor resection; DOT, number of days of therapy; GE/well, number of genome equivalents per PCR; —, not measured.

^b Result from sample collected from patient at discharge.

^c Result from sample collected from patient at home.

Gram stain was negative for organisms, but the culture grew *Enterococcus faecalis*, which was initially considered a contaminant. Antimicrobial treatment was not initiated, and the patient remained drowsy and lethargic. Six days following the VPS insertion, CSF was aspirated from the VPS. The Gram stain was again negative, and *E. faecalis* again grew in culture. The isolate was susceptible to ampicillin (MIC ≤ 2) and vancomycin (MIC = 1). Intravenous ampicillin was initiated on postoperative day (POD) 6 following VPS placement; intrathecal vancomycin was not administered.

Three days later, the patient returned to the operating room for VPS removal. A new ventriculostomy catheter was placed, and CSF obtained from the new EVD was submitted for analysis. Both Gram stain and culture of the CSF were negative.

The patient's sensorium improved remarkably following VPS removal. *Propionibacterium acnes* (ampicillin susceptible) was cultured from the extracted VPS material but did not grow in the accompanying CSF sample, suggesting that *P. acnes* had colonized the surface of the VPS. The EVD remained in place for 8 days. While our patient was receiving antibiotic treatment, three CSF specimens were obtained via the EVD at 3-day intervals. The patient was successfully weaned from the EVD with resolution of hydrocephalus, and the EVD was removed 9 days following insertion. Our patient was discharged with a peripherally inserted central catheter (PICC) to permit continuation of ampicillin (2 g i.v. every 6 h) at home. After 21 days of outpatient i.v. antibiotic treatment, the PICC was removed.

Three days later, symptoms of headache and fatigue developed. The patient returned to the medical center for evaluation. He was afebrile but difficult to arouse. Physical examination results were notable for nuchal rigidity. The WBC count was 16.3×10^3 cells/μl. A head CT scan did not reveal evidence of an increase in ventricle size, or of a change in contour, but did disclose a new right thalamic infarct; this finding was confirmed by MRI. A lumbar puncture (LP) was performed, revealing cloudy CSF which was submitted for analysis (WBC = 6,317 cells/mm³; RBC = 41 cells/mm³; glucose = 41 mg/dl; protein = 240 mg/dl). Gram stain of the fluid was negative for organisms. Cultures of blood and CSF were negative for growth. Despite resumption of i.v. vancomycin and ampicillin treatment, the patient remained confused. On day

3, cefepime was added to the empirical antimicrobial treatment. A follow-up head CT scan on day 4 demonstrated that there was stable ventricle size and morphology. An EVD was placed to monitor intracranial pressure. CSF obtained from the EVD was submitted for analysis. Gram stain of the fluid was negative for organisms, and the culture was negative for growth.

On the morning of hospital day 8, the patient suffered cardiopulmonary arrest, and efforts to resuscitate him were unsuccessful. Meningitis was not thought to be the cause of death, but an autopsy was not performed, and the cause of death was uncertain.

PCR/ESI-MS is a diagnostic tool that has demonstrated utility in detection of disseminated CNS infection (1, 2). To date, the role of PCR/ESI-MS in monitoring responses to antimicrobial treatment of bacterial meningitis has not been examined. When our patient returned for evaluation, the clinical and laboratory examination was consistent with recurrent bacterial meningitis. Although CSF Gram stain results were negative and cultures of CSF obtained following readmission were unrevealing, our patient was lethargic, nuchal rigidity was observed, and the CSF WBC count had increased from 0 to 6,317 cells/mm³ (97% granulocytes).

PCR/ESI-MS was performed on all stored samples of CSF. The protocol previously developed and validated by Kaleta et al. (3) was followed. Compared to clinical samples, the assay performs with 98.7% and 96.6% concordance at the genus and species levels, respectively (3). PCR/ESI-MS detected *E. faecalis* in every CSF specimen tested (Table 1). A decline in the level of detection (LOD) of the pathogen, *E. faecalis*, was noted once antimicrobial treatment was initiated, suggesting that the organism was being eradicated by antibiotics. Following initiation of intravenous ampicillin, PCR/ESI-MS continued to detect *E. faecalis*, but the LOD was 1 log lower than pretreatment levels. Culture of CSF obtained by LP 4 days following the last dose of antibiotics was negative, but *E. faecalis* was detected by PCR/ESI-MS in this sample (LOD = 91 genomes/well). The deterioration in neurologic status demonstrated a striking correlation with the observed return in the LOD of *E. faecalis* to >90 genome equivalents/well. Clinical evidence of

relapse of infection in our patient correlated with the results of PCR/ESI-MS testing, suggesting that the LOD from serial CSF samples could have an impact on the duration of therapy in patients with CNS infection.

When CSF cultures are negative because of administration of antimicrobial treatment, surrogate markers of infection are typically used to judge if there is clearance of infection from the CNS (4–6). In our case, CSF cultures obtained during antibiotic administration were negative, but PCR/ESI-MS continued to detect low levels of *E. faecalis*. The challenging aspect of this technology is the inability to distinguish live organisms from extracellular DNA released by dead organisms. In retrospect, given the unfortunate recurrence of infection in our patient, the results of both culture and PCR/ESI-MS were potentially concerning.

Future investigations conducted prospectively to analyze the diagnostic accuracy and utility of PCR/ESI-MS for the more common causes of bacterial meningitis in the setting of neurosurgical procedures must be conducted before antimicrobial treatment decisions should be made on the basis of PCR/ESI-MS results. The correct threshold for detection must be determined. A prospective investigation of the diagnostic accuracy and clinical utility of PCR/ESI-MS using serial CSF samples from neurosurgical patients following EVD placement is being conducted.

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