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BRIEF REPORT

Performance of α-Defensins in Diagnosing Nosocomial Ventriculitis

Max W. Jacobs[1,2] **and James B. Doub**[1,2]

[1]The Doub Laboratory of Translational Bacterial Research, University of Maryland School of Medicine, Baltimore, Maryland, USA, and [2]Division of Clinical Care and Research, Institute of Human Virology, University of Maryland School of Medicine, Baltimore, Maryland, USA

Nosocomial ventriculitis can be an extremely difficult infectious disease process to diagnose, thereby exposing patients to increased morbidity and unwarranted aggressive antibiotics. Thus, novel ventriculitis diagnostics are drastically needed. In this study, we demonstrate excellent sensitivity and specificity of cerebral spinal fluid α-defensins to aid in diagnosing ventriculitis.

Keywords. α-defensin; cerebral spinal fluid; diagnostics; external ventricular drain; ventriculitis.

Nosocomial ventriculitis is a life-threatening infection that is notoriously difficult to diagnose [1, 2]. This occurs because underlying neurologic conditions such as intracerebral hemorrhage alter cerebrospinal fluid (CSF) leading to deranged glucose, protein, and white blood cell counts [1, 2]. To reduce increased intracranial pressure in patients with intracerebral hemorrhage, external ventricular drains (EVDs) are habitually used, but these can become colonized with microbial biofilms [1, 2]. This adds complexity to diagnosing nosocomial ventriculitis given that biofilm bacteria are difficult to culture with standard techniques. As a result, there is no gold standard diagnostic for nosocomial ventriculitis, and novel diagnostics are drastically needed [1].

One such novel diagnostic is α-defensins. These antimicrobial peptides are secreted by innate immune cells in response to microbes to thereby disrupt microbial membranes [3]. In homeostasis, low levels are found in the serum of healthy individuals, but serum concentrations increase in response to infectious processes [4]. Importantly, in prosthetic joint infections, local concentrations increase significantly in response to microbial biofilms, and the levels of α-defensins are not affected by antibiotic treatments as long as microbial biofilms remain present [4–6]. Therefore, extrapolating to nosocomial ventriculitis, where biofilms on EVDs are similar to biofilms on prosthetic joints, preliminary data suggest that the use of α-defensins may help diagnose nosocomial ventriculitis [7]. Consequently, the aim of this study was to determine the sensitivity and specificity of α-defensins in the diagnosis of nosocomial ventriculitis.

METHODS AND MATERIALS

This study was approved by the University of Maryland internal review board (HP-00103555). From January 2019 through 30 June 2024, patients who were >18 years old, had intracerebral hemorrhages requiring EVDs, and underwent CSF analysis for normal clinical care had their leftover CSF obtained for investigation. CSF was obtained by pipetting at least 200 μL of leftover CSF into 2-mL cryovials and freezing these at −80 °C. For the majority (n = 75, 91%), CSF was obtained from the EVDs, with the other 7 samples being obtained through lumbar punctures.

Patients were deemed to have ventriculitis (n = 41) if they had deranged CSF parameters, had CSF cultures that grew microbes, and were treated with antibiotic durations based on ventriculitis guidelines [1]. CSF parameters suggesting ventriculitis were pleocytosis with hypoglycorrhachia. Patients who had CSF obtained but had negative CSF culture results and were not treated with antibiotics were deemed to have true negative ventriculitis (n = 41). Ten patients from the true ventriculitis cohort who had repeat evaluation of CSF 7 days after starting antibiotics also had their repeat CSF preserved at −80 °C. Furthermore, 10 patients with ample residual CSF volumes had their CSF preserved at −80 °C, and a portion of the same CSF was left at room temperature for 4 hours and then preserved at −80 °C.

The preserved CSF samples were then evaluated for levels of α-defensins by use of a 2-antibody enzyme-linked immunosorbent assay with biotin-streptavidin-peroxidase detection (R&D Systems) as previously discussed [7]. Statistical evaluation was conducted with Prism (GraphPad), with $P < .05$ being considered significant. The number of patient samples needed to produce a 2-sided 95% CI with a width of 20% when the area the under curve is 80% was 41 with culture-proven ventriculitis and 41 with true negative ventriculitis.

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Correspondence: James B. Doub, MD, Division of Clinical Care and Research, Institute of Human Virology, University of Maryland School of Medicine, 725 W Lombard St, Baltimore, MD (Jdoub@ihv.umaryland.edu).

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Table 1. Demographics of the Cohorts

	Mean (Range) or No. (%)	
	Ventriculitis (n = 41)	No Ventriculitis (n = 41)
Age, y	56.5 (41–77)	57.3 (44–82)
Sex: female, %	54	62
Hypertension	36 (88)	35 (85)
Diabetes	9 (22)	12 (29)
Immunosuppressed	4 (10)	3 (7)
Cerebrospinal fluid		
Red blood cells	41 (100)	41 (100)
Pleocytosis[a]	41 (100)	39 (95)
Hypoglycorrhachia[b]	41 (100)	21 (56)
α-Defensin, mg/L	366.9 (153.8–790.9)	57.6 (0.4–417)

[a]Pleocytosis is defined as >5 cells/mm^3 after correcting for red blood cells.

[b]Hypoglycorrhachia is defined as a cerebrospinal fluid to serum glucose ratio ≤0.5.

RESULTS

Table 1 shows the demographics of the cohorts. Of note, 39 (95%) in the true negative cohort had pleocytosis, defined as >5 white cells/mm^3. When the level of CSF α-defensins in the ventriculitis cohort (mean, 366.9 mg/L; SD, 148.6) was compared with the no-ventriculitis cohort (mean, 57.6 mg/L; SD, 86.1), there was a statistically significant difference ($P < .01$; Figure 1*A*). Most patients with no ventriculitis had low levels of α-defensins (<220 mg/L) in their CSF. There were 2 patients in the no-ventriculitis cohort who had an α-defensin level >220 mg/L, but these patients were also experiencing persistent bacteremia despite having CSF parameters that did not suggest that they had ventriculitis. Moreover, the receiver operating characteristic (Figure 1*B*) shows an area under the curve of 0.9714 ($P < .0001$; 95% CI, .9357–1.000). When the cutoff value of 220 mg/L was used, there was a correlated sensitivity of 85.37% (95% CI, 71.56%–93.12%) and specificity of 95.12% (95% CI, 83.86%–99.13%). No significant differences ($P = .9229$) in α-defensin levels were observed between gram-negative (n = 24; mean, 368.8 mg/L; SD, 139.5) and gram-positive (n = 17; mean, 364.2 mg/L; SD, 164.9) ventriculitis (Supplementary Figure 1).

When patients who experienced ventriculitis had CSF α-defensin concentrations evaluated before 7 days of antibiotics (mean, 452 mg/L; SD, 170.9) vs after (mean, 20.67 mg/L; SD, 34.1), there was a statistically significant decrease ($P < .01$) in this biomarker in their CSF (Figure 1*C*). Yet, when this biomarker was evaluated for stability at room temperature, there was no statistically significant change in the amount of α-defensins in the CSF at time 0 (mean, 39.65 mg/L; SD, 50.67) as compared with 4 hours later (mean, 42.42 mg/L; SD, 54.73; Figure 1*D*).

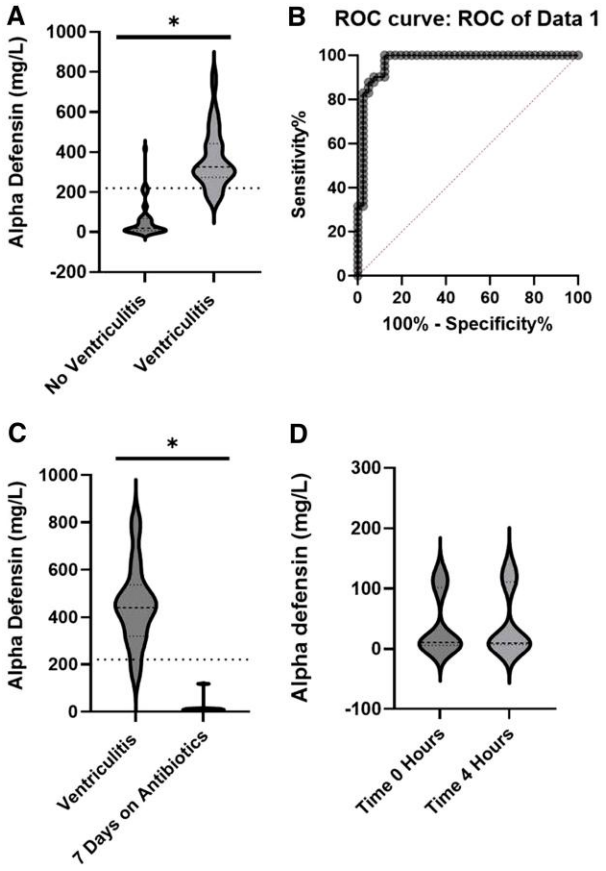

Figure 1. Evaluation of CSF α-defensin as a ventriculitis diagnostic. *A*, Violin graph shows a statistically significant difference ($P < .01$) in concentrations of α-defensins in CSF between the cohorts. Dashed line represents a cutoff of 220 mg/L. *B*, ROC graph indicates an area under the curve of 0.9714 ($P < .0001$; 95% CI, .9357–1.000). *C*, Violin graph illustrates concentrations of α-defensins in CSF after 7 days of antibiotics in which there was a significant difference ($P < .01$) vs the time of initial diagnosis. Dashed line represents a cutoff of 220 mg/L. *D*, Violin graph shows stability of CSF α-defensin concentrations after 4 hours at room temperature in which there was no significant change. CSF, cerebrospinal fluid; ROC, receiver operating characteristic.

DISCUSSION

Overall this study suggests that α-defensin can be a reliable biomarker for nosocomial ventriculitis in that there was a statistically significant difference ($P < .01$) in α-defensin levels between patients with and without ventriculitis (Figure 1*A*). In patients who did not have ventriculitis but did have pleocytosis, their innate immune cells did not secrete high levels of α-defensins, which supports that microbes are needed to stimulate innate immune cells to secrete α-defensins in the CSF. Correlated with this, in patients without ventriculitis, the levels of α-defensins were typically < 220 mg/L, suggesting that when no central nervous system infection is present, the levels of α-defensins in the CSF are typically low. Yet, when patients have severe systemic infections, such as persistent bacteremia, elevated levels of

α-defensins in CSF can also occur given that α-defensins can pass through the blood-brain barrier. However, evaluating patients for alternative infectious processes is important and routinely conducted by clinicians when evaluating ventriculitis [1].

Nonetheless, the use of α-defensins for diagnosing nosocomial ventriculitis had an area under the receiver operating characteristic curve of 0.9714, which demonstrates excellent performance of CSF α-defensin as a biomarker for ventriculitis with regard to its specificity and sensitivity (Figure 1*B*). When a cutoff of 220 mg/L was used, there was excellent specificity (95.12%) and sensitivity (85.3%). Consequently, these findings are important because at the present time numerous CSF markers, such as lactate, glucose, and others, lack significant sensitivity and specificity for diagnosing ventriculitis [1]. Thus, the findings here support further development of this biomarker in the diagnosis of nosocomial ventriculitis, especially since it is already part of the diagnostic workup for prosthetic joint infections [6, 8, 9].

While proving excellent performance of this diagnostic is vital, ensuring that this biomarker does not have persistent elevated levels in the CSF for prolonged periods after treatment with antibiotics is also important. Here we show that in a subset of patients with ventriculitis who had repeat CSF α-defensin measured after 7 days of antibiotic therapy, all had a statistically significant ($P < .01$) decrease in α-defensin levels after treatment with antibiotics. This is an important finding demonstrating that after elimination of microbes, innate immune cells stop secreting this biomarker, and elevated levels of this biomarker are thus cleared from the CSF. Yet this study also showed that α-defensins are stable at room temperature for 4 hours. As a result, leftover CSF in patients who are diagnostically challenging could theoretically be used to determine α-defensin concentrations to aid in diagnosing ventriculitis.

While the findings of this study are interesting, this study has several limitations. For one, it was a single-center retrospective study evaluating α-defensin levels in patients with and without ventriculitis. It is plausible that a small proportion of patients in the true ventriculitis cohort may have had bacteria grow on cultures but that the growth was not representative of ventriculitis, as others have shown [1, 10]. To mitigate this, care was taken to include in the true ventriculitis cohort only those patients with positive culture results and symptoms, whom infectious disease and neurocritical care clinicians deemed to have ventriculitis. As well, this biomarker was assessed only in patients whose ventriculitis status was confirmed. Patients who had culture-negative ventriculitis were not included, but this clinical cohort might also benefit from this diagnostic. However, it was not feasible to establish baseline sensitivity and specificity of this diagnostic while including these patients, given the potential to confound the results. Therefore, follow-up studies assessing this biomarker as a point-of-care test to help diagnose ventriculitis in real time are needed.

In conclusion, CSF α-defensin has excellent sensitivity and specificity in diagnosing nosocomial ventriculitis and is stable at room temperature for at least 4 hours. Therefore, further research should be conducted to continue to develop this diagnostic as a point-of-care test to reduce morbidity associated with underdiagnosing ventriculitis or overdiagnosing ventriculitis and therefore exposing patients to unwarranted antibiotics. However, before the broad use of this diagnostic to aid in diagnosing ventriculitis, follow-up studies should be conducted to support the findings seen here.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Author contributions. J. B. D. designed the study and obtained and analyzed the data. M. W. J. wrote a significant portion of the manuscript.

Data availability. The data generated and analyzed during the current study are available upon reasonable request to the corresponding author.

Patient consent. This study does not include factors necessitating patient consent. The study was approved by the University of Maryland Internal Review Board (HP-00103555).

Potential conflicts of interest. J. B. D. has a patent pending for use of α-defensin in the diagnosis of ventriculitis. The other author reports no potential conflicts.

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