Point-of-Care Cerebrospinal Fluid Detection

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Abstract

Objective. A cerebrospinal fluid leak is one of the most serious complications in otolaryngology. It may occur as a result of injury to the skull base, typically traumatic or iatrogenic. While the presence of a leak is often discerned in the emergent setting, distinguishing normal secretions from those containing cerebrospinal fluid can be difficult during postoperative visits in the clinic. As most current laboratory-based assays are labor intensive and require several days to result, we aim to develop a more user-friendly and rapid point-of-care cerebrospinal fluid detection device.

Study Design. Our laboratory developed a barcode-style lateral-flow immunoassay utilizing antibodies for beta-trace protein, a protein abundant in and specific for cerebrospinal fluid, with a concentration of 1.3 mg/L delineating a positive result.

Setting. Tertiary medical center.

Subjects and Methods. Tests with known concentrations of resuspended beta-trace protein and the contents of discarded lumbar drains (presumed to contain cerebrospinal fluid) were performed to validate our novel device.

Results. Our results demonstrate the ability of our device to semiquantitatively identify concentrations of beta-trace protein from 0.3-90 mg/L, which is within the required range to diagnose a leak, thus making beta-trace protein an excellent target for rapid clinical detection.

Conclusion. Herein we detail the creation and initial validation of the first point-of-care cerebrospinal fluid detection device. This device is a feasible method to more efficiently and cost-effectively identify cerebrospinal fluid leaks, minimize costs, and improve patient outcomes.

Keywords

- cerebrospinal fluid leak
- semiquantitative
- lateral-flow immunoassay
- diagnostic
- point of care
- otolaryngology
- beta-trace protein

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A communication between the fluid surrounding the brain and the outside world, also known as a cerebrospinal fluid (CSF) leak, is a known complication of numerous procedures in otolaryngology. The risk in endoscopic skull base surgery was estimated at 13.8%; endoscopic sinus surgery, 0.17%; and cochlear implantation, 0.4%.1-3 Almost all procedures involving the sinuses, skull base, or ear have some risk of a leak. Individuals who have not undergone procedures are at risk as well. Patients may have leaks resulting from trauma involving facial and skull base fractures, while some individuals are unfortunate enough to present with rare spontaneous conditions, such as middle cranial fossa CSF otorrhea.4

In the acute setting, imaging modalities such as magnetic resonance imaging and computed tomography are used for assessment. If there is high-enough clinical suspicion (obvious facial or skeletal deformities or copious clear rhinorrhea or otorrhea), patients may be taken directly to the operating room for treatment. This involves identifying the site of the leak and using either native tissue or biocompatible materials to patch the affected site.

Identifying a CSF leak in the outpatient or postoperative hospital setting is often more difficult. It is not unusual for patients to have postoperative secretions; therefore, distinguishing normal secretions from those containing CSF may
be a challenging task. When physicians are concerned about a CSF leak, no proven diagnostic modalities exist that allow them to rapidly and noninvasively rule out the presence of a leak. Physicians must then turn to the same imaging techniques used in more acute settings, the cost of which may be difficult to justify for a patient who seems otherwise well.

Alternative methods of identifying CSF have been developed. The current laboratory gold standard for CSF detection involves identification of beta-2 transferrin through either electrophoresis or the enzyme-linked immunosorbent assay. These tests are 94% to 100% sensitive and 98% to 100% specific in detecting CSF; however, they typically require samples to be sent off to a central laboratory where days to weeks pass before results return. Despite the promising role of beta-2 transferrin testing in published guidelines, this test is often not useful to guide clinical decision making, due to the time that it takes for results to return. Other methods of CSF detection have been explored. These include glucose testing, cisternography, and spotting the CSF to look for a “halo” or “ring” sign. Unfortunately, the literature has not found these methods to be sensitive or specific. As an alternative, researchers have looked at beta-trace protein (βTP), also known as lipocalin-type prostaglandin-D2 synthase. βTP is a ubiquitous protein present at various concentrations in different body compartments but notably 1 to 2 orders of magnitude higher in CSF than in nasal secretions and serum. As a result, studies demonstrated that nasal secretions with βTP concentrations above a certain threshold are suggestive of a CSF leak and the need for immediate operative intervention. In a recent study based on the nephelometric assay, Bernasconi and coworkers determined that concentrations of βTP ≥1.3 mg/L indicate the presence of a CSF leak, whereas concentrations of βTP <0.7 mg/L indicate the absence of a CSF leak. βTP concentrations between 0.7 and 1.29 mg/L (denoted the “gray zone”) required further analysis and comparison with the patient’s βTP serum level to confirm the presence or absence of a CSF leak. This approach showed sensitivity and specificity of 98.3% and 96%, respectively.

While the nephelometric assay is more rapid than electrophoresis, it still requires centralized laboratory equipment that may not be available in every clinical setting. To address this issue, we describe the development of a semiquantitative barcode-style lateral-flow immunoassay (LFA) for the detection of βTP. By classifying a sample as having a concentration of <0.7, between 0.7 and 1.29, or ≥1.3 mg/L of βTP, we predict that our equipment-free and disposable device will allow clinicians to more rapidly and affordably identify the presence of a CSF leak.

### Methods

**Preparation of the Barcode-Style LFA**

All reagents and materials were purchased from Sigma-Aldrich (St Louis, Missouri) unless noted otherwise. First, to form antibody functionalized gold nanoparticles (GNPs), 35 μL of a 0.1M sodium borate (pH 9) solution was added to 1 mL of a 40-nm citrate-capped gold nanoparticle suspension (Nanocomposix, San Diego, California). Subsequently, 8 μg of anti-βTP antibodies were added, and the mixture was allowed to react for 30 minutes. To prevent nonspecific binding of other proteins to the gold nanoparticles, 100 μL of a 10% w/v bovine serum albumin (BSA) solution was added to the mixture and allowed to react for 10 min. To purify unbound antibodies from the nanoparticles, the mixture was centrifuged and the pellet resuspended in 100 μL of a 0.1M sodium borate (pH 9) solution.

The LFA test strip is composed of overlapping pads secured to an adhesive backing (Figure 1). These pads include a sample pad, a conjugate pad, a nitrocellulose membrane, and an absorbent pad. The sample pad consists of a 3 × 10-mm fiberglass paper treated with a 0.1M Tris (pH 9) solution containing 1% BSA. GNPs were dehydrated onto a 3 × 10-mm fiberglass paper with 1% BSA in diH2O to form the conjugate pad. Both the sample and the conjugate pads were dehydrated under very low pressure with a Labconco FreeZone 4.5 lyophilizer (Fisher Scientific, Hampton, New Hampshire) for 2 hours.

Anti-βTP antibodies were printed and immobilized on a nitrocellulose membrane at 3 test line locations (T1, T2, and T3) to form the test zone. The antibodies at the T1, T2, and T3 locations were immobilized at 2, 0.5, and 0.35 mg/mL, respectively. These concentrations were experimentally determined to give the desired detection cutoffs. Protein A was immobilized at a concentration of 0.2 mg/mL on the nitrocellulose membrane downstream of the test zone to form the control line. Test and control line printing was performed with an Automated Lateral Flow Reagent Dispenser (Claremont BioSolutions, Upland, California) and a Fusion
200 syringe pump (Chemyx, Stafford, Texas) with a flow rate of 250 µL/min. The printed membrane was left in a vacuum-sealed desiccation chamber overnight. After protein immobilization, membranes were immersed in a 0.1M Tris (pH 9) solution containing 1% BSA for 1 hour and dehydrated under very low pressure overnight with a lyophilizer.

To assemble the test strip, the absorbent pad was first adhered at the far end of the test strip, downstream of the control line, overlapping the nitrocellulose membrane. The conjugate pad was placed at the opposite end of the strip, upstream of the test zone, overlapping the nitrocellulose membrane. Last, the sample pad was placed on the test strip overlapping the conjugate pad. After assembly, the LFA strips were placed in bags containing drierite desiccant (Fisher Scientific), which were then placed inside an autodesiccant chamber (Fisher Scientific) for storage.

Detection of βTP with the Barcode-Style LFA

To demonstrate the ability of the barcode-style LFA to detect and quantify βTP, we tested it with a variety of samples, including recombinant βTP (Mybiosource, San Diego, California), human serum, and human CSF from lumbar drains mixed with human serum (University of California Los Angeles [UCLA] Institutional Review Board approval 16-000045). These samples were first diluted in various amounts in phosphate-buffered saline (PBS) to adjust the βTP concentration to find the optimal detection range of the barcode-style LFA. Briefly, samples containing known concentrations of recombinant βTP in PBS (0.3-90 mg/L) were diluted 150-fold in PBS. Pooled human serum (Sigma-Aldrich), which served as human βTP-containing samples that were negative for CSF, was diluted 50-, 150-, and 500-fold in PBS. Last, varying dilutions of CSF in human serum (2-, 5-, 10-fold), which served to simulate nasal drip samples containing varying amounts of CSF, were diluted 150-fold in PBS.

Prior to running the assay, the PBS-diluted samples were further diluted 2-fold by mixing 25 µL of the samples in a test tube with 25 µL of running buffer (0.4% BSA, 0.6% Tween 20, 0.2% polyethylene glycol, 0.1M Tris buffer, pH 9). The LFA test strip was dipped vertically into the tube with the sample pad submerged. After 20 minutes, the test strips were imaged by a Canon EOS 1000D camera (Canon USA, Inc, Lake Success, New York) in a controlled lighting environment. Three CSF samples were tested with our assay. Representative images from 1 of these samples are shown.

Results

In our barcode-style LFA, the presence of the target biomarker would produce 1 to 3 visible test lines, as the GNPs would first bind to the βTP in the sample and then be captured at the test lines (Figure 1). Each test line has a cutoff, which is the minimum concentration of βTP in the sample that is necessary for that test line to become visible, and this cutoff can be adjusted by varying the density of the capture antibody immobilized. Regardless of the presence of βTP in the sample, protein A at the control line would bind to the antibodies on the GNPs resulting in the formation of a visible red control line, indicating successful sample flow through the strip and thus valid test results.

We aimed to design our barcode-style LFA to have test line cutoff values that correspond to the threshold values established by Bernasconi and coworkers.13 At βTP concentrations <0.7 mg/L, 0 or 1 test line should appear indicating no CSF leak. βTP concentrations between 0.7 and 1.3 mg/L, which served to simulate nasal drip samples containing varying amounts of CSF, were diluted 150-fold in PBS.

Prior to running the assay, the PBS-diluted samples were further diluted 2-fold by mixing 25 µL of the samples in a test tube with 25 µL of running buffer (0.4% BSA, 0.6% Tween 20, 0.2% polyethylene glycol, 0.1M Tris buffer, pH 9). The LFA test strip was dipped vertically into the tube with the sample pad submerged. After 20 minutes, the test strips were imaged by a Canon EOS 1000D camera (Canon USA, Inc, Lake Success, New York) in a controlled lighting environment. Three CSF samples were tested with our assay. Representative images from 1 of these samples are shown.

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L, which are in the “gray zone,” should result in 2 test lines appearing. Samples classified within the gray zone cannot confidently be diagnosed and should undergo further testing before a clinical decision is made. Samples containing concentrations >1.3 mg/L should result in the formation of 3 test lines, which would indicate the presence of a CSF leak.

Initial range of detection and cutoff values of the LFA were determined with known concentrations of recombinant βTP in PBS. In all cases, pink control lines were visible, indicating valid test results. βTP was accurately detected at all concentrations tested, as indicated by the formation of visible pink test lines on all strips. Specifically, when the original sample contained 0.3 mg/L of βTP, 1 visible test line at the T1 position was present; at 0.9 mg/L, 2 visible test lines at the T1 and T2 positions were present; and at 3 mg/L, 3 visible test lines at the T1, T2, and T3 positions were present (Figure 2). These results correctly correspond with the desired cutoff values.

Next, the LFA was tested with samples of pooled human serum. It was able to detect native βTP for all dilutions tested, as indicated by the appearance of a visible line at the T1 location on all strips (Figure 3). For the 300- and 1000-fold preanalytic sample dilutions of pooled human serum, T1 was the only visible line. These samples were thus classified as negative for the presence of CSF with our assay. In contrast, when the serum was diluted only 100-fold, there were visible test lines at the T1 and T2 locations, corresponding to the gray zone.

Finally, multiple samples of CSF obtained from de-identified lumbar drains were mixed with human serum to simulate patient nasal drip samples containing varying amounts of CSF. These samples were then diluted 300-fold prior to application to the LFA (Figure 4). When tested on the LFA, CSF that was not mixed in serum (CSF only) produced visible lines at all 3 test line locations. Three visible test lines were also visible down to the 5-fold dilution of CSF in human serum, correctly indicating that these samples were positive for CSF. The 10-fold dilution of CSF in human serum produced 2 visible test lines, classifying this sample as being in the gray zone, where further testing is recommended. Last, human serum without any CSF produced only 1 test line, correctly indicating the sample as negative for CSF.

Discussion

There is a need for a point-of-care test that would allow for rapid detection of skull base violation at the bedside and in clinics. The implications of a device capable of detecting CSF extend beyond the field of otolaryngology. Such a device could be used to identify injuries to the spinal cord or globe and may have a role in ruling out CSF leaks after neurosurgery for patients with a low pretest probability of having a leak. One biomarker for detecting CSF leaks is βTP, which has shown excellent sensitivity and specificity in a research setting. Unfortunately, it has not been widely accepted as a target in clinical detection, partly due to the current assays requiring expensive and centralized equipment that is not readily available in many clinical settings. This makes it an excellent candidate for novel translational advances. Therefore, we have created a barcode-style LFA that quantifies the βTP concentration in a sample for the rapid detection of CSF leaks.

Initial testing demonstrated that our LFA was able to detect concentrations of recombinant βTP much lower than the desired cutoffs, so it was determined that a 300-fold preanalytic sample dilution would be required to adjust the range of detection and reduce the instance of false positives (Figure 2). When moving to patient samples, this preanalytic sample dilution had the added benefit of reducing viscosity to enable better flow. Next, dilutions of human serum were tested with the LFA to confirm its ability to detect native human βTP. The βTP concentration of the serum is...
expected to be close to the previously reported average of 0.59 mg/L.10,11 This is below the 0.7-mg/L cutoff for a negative result, and so we expect 1 test line to be visible on the LFA strip with the appropriate dilution. The results shown in Figure 3 support the use of the previously determined 300-fold preanalytic sample dilution to ensure this true negative result (1 test line). While we acknowledge that the βTP concentration in serum is different (and greater) than that in nasal secretions, this was an important negative control, as serum is a possible contaminant in nasal secretions.

Last, when a sample of CSF from lumbar drains (CSF only) was diluted by 300-fold and tested with the LFA, it was correctly identified as positive (Figure 4). In addition to being able to detect pure CSF, it is important that our assay correctly detect CSF that has been diluted in serum, as it is common for CSF to be diluted in nasal or otologic secretions prior to collection. When the sample to be tested containing CSF was diluted in the human serum, positive detection was observed down to the 5-fold dilution, while the 10-fold dilution was classified as being in the gray zone. Whereas the gray zone is not a confirmatory result and future laboratory testing should be performed in a clinical setting, it does suggest that our device has the ability to identify leaks that have been diluted 10-fold by nasal or otologic secretions. This is comparable to previously reported values for beta-2 transferrin electrophoresis.14,15

Note that the absence of a visible control line on all tests containing human serum (Figures 3 and 4) was not due to inadequate flow of the sample solution through the test, as all of the sample was wicked out of the test tube. We attributed this weak control line to endogenous antibodies in the human serum outcompeting the antibodies on the GNPs for protein A on the control line. In the future version of our test strip, we will adjust this by replacing protein A with a species-specific secondary antibody.

Limitations of this device may include the detection of very dilute (<10%) samples and detection of intermittent leaks. These limitations, however, both similarly apply to samples analyzed electrophoretically for beta-2 transferrin.14,15 Also, while the stratified barcode readout makes interpretation simple, further testing and evaluation may still be required when samples test within the “gray zone,” limiting its use as a stand-alone test.

Other important considerations include contamination of samples with other body fluids known to contain βTP. Individuals with end-stage renal disease, cardiovascular disease, and bacterial meningitis may have serum concentrations of βTP in the range of positive detection by our device.16,17 For cardiovascular disease, this has been cited as 1.33 ± 0.63 mg/L, well within the range of positive detection by our device.16 These findings emphasize the importance of ensuring that samples do not have blood contamination, and they may cause clinicians to more carefully consider patient comorbidities when a positive result is obtained.18,19 Furthermore, concentrations of βTP in nasal secretions are much lower than those in serum and, in the absence of serum contamination, should be 1 to 2 orders of magnitude lower than the βTP concentrations in CSF.11 An uncontaminated nasal secretion sample is thus unlikely to be incorrectly diagnosed as containing CSF. These findings highlight the importance of pretest clinician suspicion for a CSF leak and suggest that this device may have a powerful role in excluding CSF leaks in appropriately selected patient populations.

Future work will involve the testing of healthy human nasal secretions to ensure true negative test results in this population. Additionally, equivocal samples from patients with suspected CSF leaks will be tested to evaluate the sensitivity and specificity of our device.

Conclusion

CSF leaks are an excellent target for point-of-care testing in otolaryngology. The ability of a simple device to accurately detect CSF will help diagnose these devastating leaks more rapidly and rule them out less expensively. Here we describe the creation of a barcode-style LFA that detects βTP and classifies the concentration of detected protein in a semiquantitative fashion into 3 ranges that allow for ease of clinical interpretation. In initial studies, this device was able to detect resuspended βTP as well as CSF samples diluted in serum up to 10%. This device shows that bedside CSF identification is feasible and has the potential to influence decision making without additional expensive diagnostic workup.

Author Contributions

Ashley E. Kita, idea conception, device creation and development, device validation, drafting of manuscript; Daniel W. Bradbury, idea conception, device creation and development, device validation, drafting of manuscript; Zachary D. Taylor, idea conception, device creation and development, device validation, manuscript revision; Daniel T. Kamei, idea conception, device creation and development, device validation, manuscript revision; Maie A. St. John, idea conception, device creation and development, device validation, manuscript revision.
Disclosures

Competing interests: Ashley E. Kita, inventor on provisional patent on this technology filed through UCLA; Daniel W. Bradbury, inventor on provisional patent on this technology filed through UCLA; Zachary D. Taylor, inventor on provisional patent on this technology filed through UCLA; Daniel T. Kamei, cofounder of the company Phase Diagnostics, which has interest in commercializing this technology; inventor on provisional patent on this technology filed through UCLA; Maie A. St. John, inventor on provisional patent on this technology filed through UCLA.

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