Evaluating the Effects of CSF Protein Content on the Performance of Differential Pressure Valves and Anti-Siphon Devices using a Novel Benchtop Shunting Model

Short Title: Effect of Protein on Differential Pressure Valves

Keywords: Cerebrospinal Fluid, Hydrocephalus, Hydrocephalus Shunt, In Vitro Model, Overdrainage.

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Abstract

2 Background: Hydrocephalus is managed by surgically implanting flow-diversion technologies such as differential pressure valves and anti-siphoning devices, however, such hardware is prone to failure. Extensive research has tested them in flow-controlled settings using saline or deaerated water, yet little has been done to validate their performance in a setting recreating physiologically-relevant parameters including intracranial pressures, CSF protein content, and body-position.

Objective: In order to more accurately chart the episodic drainage characteristics of flow9 diversion technology, a gravity-driven benchtop model of flow was designed and tested continuously during weeks-long trials.

Methods: Using a hydrostatic pressure gradient as the sole driving force, interval flow-rates of six valves were examined in parallel with various fluids. Daily trials in the upright and supine positions were run with fluid output collected from distal catheters placed at alternating heights for extended intervals.

Results: Significant variability in flow-rates was observed, both within specific individual valves across different trials and among multiple valves of the same type. This inter-valve and intravalve variability was greatest during supine trials and with increased protein. None of the valves showed evidence of overt obstruction during 30-days of exposure to cerebrospinal fluid containing 5g/L protein.

Conclusions: Day-to-day variability of ball-in-cone differential pressure shunt valves may increase overdrainage risk. Narrow-lumen high-resistance flow control devices as tested here under similar conditions appear to achieve more consistent flow-rates suggesting their use may be advantageous, and did not demonstrate any blockage or trend of decreasing flow over the three weeks of chronic use.

Introduction

Ventriculoperitoneal shunts (VPSs) are used widely in the surgical management of hydrocephalus[1-14]. While these interventions can be lifesaving, possible complications include infection, obstruction, and overdrainage[5-8,13-19]. Overdrainage in particular can be a major contributor to patient morbidity, and understanding how vulnerable or resilient commonly-used devices may be to overdrainage is critically important to the neurosurgeon. While previous studies characterized the effect of proteins on CSF flow dynamics in fixed differential-pressure valves (DPVs), the effects of protein on the behavior of more complex shunt systems remain largely unknown[16,20]. Shunt technology has evolved over the past years with the advent of programmable valves. Many of these are DPVs gated by mechanisms such as ball-in-cone systems, the opening pressure of which is modulated by increasing or decreasing tension exerted upon it by an adjustable spring. These features enable clinicians to

calibrate a valve's opening pressure to meet their patients' drainage needs. However, such adjustments cannot independently compensate for the wide fluctuations of relative pressures that may occur daily, secondary to simple changes in body position and activity. Despite the advent of anti-siphon devices (ASDs), overdrainage remains a common concern for neurosurgeons, and the effects of protein content on CSF flow through such dynamic devices remains underinvestigated.

To more realistically evaluate flow characteristics through a variety of CSF-diversion devices a gravity-driven benchtop model was developed for the functional assessment of flow-rates over consecutive trials conducted for several weeks. Previous testing systems have utilized steady flow pumps to achieve and maintain target flow-rates, and in doing so may generate unrealistically high pressures unlikely to be seen in the living system[15,20-28]. In contrast, our testbed was developed to maximize similarity to in vivo shunting, operating within the range of physiologic pressure parameters by utilizing gravity as the main driving force for drainage. This system also accommodates episodic alterations in distal catheter position to mimic position changes and consequent pressure differentials seen in ambulatory patients. Furthermore, we used this system to test CSF flow using pathological fluids, evaluating post-hemorrhagic, high-protein CSF (with cellular material removed) to better understand flow dynamics of shunting under these conditions. Here, we demonstrate trends in the flow of normal and pathological fluid through a unique in vitro shunt evaluation system, providing some insight into the inner workings of these variable systems.

Materials and Methods

System

The testing system consisted of a common "ventricle" situated within a tissue-culture incubator (Thermo Fisher Scientific) set to 37°C, 100% humidity, and atmospheric conditions, atop an orbital shaker (Troemner) to prevent sedimentation (Figure 1). On the ventricle's base were ports that allowed for continuous refilling, attachment of a manometer, and entry for six separate proximal catheters (Supplemental Methods). For all trials, three Codman Hakim® ball valves (HBVs) and three Certas® Plus ball valves (CPBVs) (Codman by Integra) were tested in parallel.

New valves and catheters were provided from the manufacturer with integrated SiphonGuard® (SG) ASDs (Codman by Integra). The ventricle was continuously refilled by reservoir flasks (Corning) situated above the ventricle. This height differential determined the hydrostatic potential driving fluid through the shunt systems.

Valves were connected to Bactiseal® catheters (Codman by Integra), reinforced with ligatures, and submerged underwater to prevent air entry. The distal catheters emptied into collecting flasks which were placed 2cm- or 28.5cm-below the height of the valves to recreate different position-dependent flow-rates seen in the horizontal (supine) and vertical (upright) states, respectively. Every 24-hour period consisted of a 6-8 hour supine trial and a 12-15 hour upright trial. At the conclusion of each trial, the total fluid output was weighed and the average flow rates were calculated for each valve. Groups of contiguous sets of trials extended over at least three weeks with different fluids.

Fluids

The system was designed to run a variety of fluids mimicking different physiological and pathological states. It was calibrated using both normal saline (Hospira) and saline containing 1g/L of Bovine Serum Albumin (BSA, Sigma Aldrich). 1g/l of BSA was chosen as it represents a pathologically elevated level of the specific protein found to be the major component of protein deposition detected in recovered catheters[29]. To further challenge the hardware under more extreme conditions, human CSF with protein elevated beyond the normal pathological range was used in additional trials. CSF was collected from 87 patients at our institution with intraventricular hemorrhage following IRB approval and informed consent. CSF samples were combined, forming an aggregate with a protein concentration of 1.63g/L, and BSA was added to a final concentration of 5g/L. CSF was centrifuged to remove blood and cellular material. The supernatant was filtered, and amphotericin B (Sigma Aldrich), penicillin/streptomycin (Gibco), and chlorhexidine dihydrochloride (Sigma Aldrich) were added to minimize risk of contamination. Protein was measured throughout the trial to ensure that concentration remained stable.

Results

Continuous trials of six programmable ball-in-cone valves exposed to physiologic pressures were carried out over a minimum two-week duration for system validation and calibration of resistance settings. The goal of validation was to determine if the valve flow-rates varied independently or uniformly between trials. Low-resistance saline trials were run with three CPBVs maintained at a low-resistance setting of 2 and three HBVs at an equivalent setting of 50. Valves were subsequently tested under medium-resistance settings (CPBV:4, HBV:100) for 27 days (Figure 2). Regular-interval measurements over the course of each daytime trial confirmed that flow-rates remained constant and independent for each valve. As the system ran, ICP level depreciated linearly with time, however this trend was minimal and did not appear to influence interval flow rates in either state.

Saline, Low-resistance

The mean flow-rates of saline through CPBVs and HBVs with valves at low-resistance settings in the supine position were 31.0ml/h (SD 5.2) and 35.2ml/h (SD 6.5), respectively. During upright-position trials in otherwise identical conditions, mean flow-rates were 27.4ml/h (SD 0.76) and 22.5ml/h (SD 1.23) through the same respective valves (Table 1).

Saline, Medium resistance

When valve opening pressures were readjusted to medium-resistance settings, mean flow-rates in the upright position decreased only slightly compared to those with low-resistance settings for both types of valves (CPBV: 21.29ml/h, SD 1.56; HBV: 20.23ml/h, SD 1.84). In contrast, the mean flow-rates in the supine position slowed dramatically to 0.05ml/h and 0.06ml/h (SD 0.08 and 0.09) for CPBVs and HBVs, respectively. For all subsequent trials testing saline with protein, medium-resistance settings were maintained throughout.

Saline + BSA (1g/L)

In the supine position, mean flow-rate with saline containing 1g/L of BSA through CPBVs was 3.23ml/h (SD 4.54). Their mean flow-rate in the upright position was 22.1ml/h (SD 1.72) over alternating trials within the same time period.

Similarly, HBVs drained an average of 0.22ml/h (SD 0.76) in the supine position and 20.12ml/h (SD 2.75) when upright. An increase in variability was noted, as evidenced by the greater SD values compared to normal saline trials.

CSF + Protein (5g/L)

A total of 35 trials with 5g/L BSA CSF were run over 18-days throughout a 30-day period in which the valves were continuously exposed to the fluid. For the final 22 trials, all valves were maintained in their respective medium-resistance settings. At these settings, CPBVs recorded a mean flow-rate of 11.85ml/h (SD 7.67) in the supine position and 21.78ml/h (SD 3.94) in the upright position among 12 and 10 trials, respectively. HBV performance during the same trials achieved mean flow-rates of 10.74ml/h (SD 6.88) and 18.97ml/h (SD 1.68) in their respective supine and upright positions.

Device performance over time

None of the CPBVs, HBVs, or their integrated SGs showed any signs of overt failure or downward trend in flow-rates related to progressive occlusion over the duration of these trials (Supplementary Table 1).

Discussion

In this study, two types of differential pressure ball-in-cone valves were tested over prolonged intervals in a novel gravity-driven testing apparatus. Increased flow and variability were found after the addition of protein, across all valve types and positional states. These changes were observed using a testbed specifically designed for assessing performance of devices within physiologically relevant conditions. 149 Although both valve types are manufactured by the same company, their different ball-in-cone mechanisms, in conjunction with and without an additional high-resistance pathway represent a broad range of devices used today.

In contrast to models utilizing steady-flow pressure pumps, the use of a hydrostatic pressure gradient calibrated to physiologic parameters provided a platform to accurately sense changes in flow secondary to variations in head position and protein concentration[16-18,20,22,23]. Normal

diurnal positional changes were mimicked by raising and lowering the distal catheter height relative to the valves. Various ASDs have been designed that respond to a surge or siphoning of fluid by dampening the immediate CSF outflow. Combining programmable valves with ASDs allows the primary device to further respond to sudden positional changes. Nevertheless, concerns over the long-term performance of DPVs and ASDs both alone and in series are still debated. SG, a secondary mechanism designed to divert a surge of fluid along a narrower, higher-resistance pathway, provided flow regulation in response to these positional changes[25,28,30].

To provide insight into the real-world behavior and interactions between DPVs and ASDs, specific valves with integrated SG were chosen. When disengaged, the SG's suspended ruby ball allows unobstructed flow from the DPV through the primary outflow path. When a greater hydrostatic differential across the system forces the SG's ruby ball to engage, this primary path is blocked and flow is diverted through a narrower lumen pathway which by increased resistance consequently reduces flow. These hardware selections allowed the study of fluid output across alternating flow states within the same device.

Having all six of the tested valves connected to the same fluid chamber (ventricular representation) allowed uniform control of ICP changes, temperature, and fluid composition. Other variables such as ICP, ventricle volume, and CSF cellularity were held constant or eliminated from this model to reduce confounding factors.

A range of biologically relevant fluids were used in order to better simulate the intraventricular environment and theoretically increase the system's sensitivity to hardware obstruction due to protein compared to water-filled rigs[15,21-23]. To challenge these devices, they were tested using post-hemorrhagic CSF with added protein to observe how the hardware might handle exposure to high protein concentration over time. This realistic, independent testing of VP shunt hardware is critical for assuring the safety and reliability 179 of CSF flow-diversion technologies widely used to manage adult and pediatric hydrocephalus[15,20-23,25,27].

Effects of Protein on Mean Flow-rate

Previous studies by Brydon et al. on PS Medical Flow Control and Cordis-Hakim valves

demonstrated that the addition of protein decreases opening and closing pressures[16,20]. We further investigated these variables on the performance of two commonly used, newer generation valves in a long-term setting and within the context of positional changes using both saline and protein-augmented CSF. Our findings are consistent with Brydon's studies, demonstrating a trend towards increased flow rates with the addition of protein. Specifically, when tested over 30-days, trials of valves in the supine position showed higher flow-rates with 1g/L BSA in normal saline, compared to normal saline (Figure 3). While data reflected a statistically significant trend in all CPBVs ($p<0.0001$), significance was not reached in any of the HBVs (Figure 3).

During upright trials over the same time interval, flow-rates in the CPBVs were consistently higher with the addition of 1g/L BSA compared to normal saline ($p<0.001$). In the upright position, two HBVs showed significant increases in flow-rates with BSA compared to saline ($p<0.02$, $p<0.0001$), however a slower mean flow-rate was recorded in one valve ($p<0.0001$). When patient-derived CSF was introduced, markedly increased rates were recorded in the supine position ($p<0.0001$ for all six valves) compared to saline both with and without BSA. Interestingly, in the upright position, no pattern of flow-rate changes were found compared with other fluids (Figure 3). These observations suggest that the effect of protein on increased flow is dependent upon the ball-in-cone mechanism, as these flow-rate increases are lost when the ASD engages and diverts fluid through the narrow high-resistance pathway.

Variability Among and Within Valves at Baseline

During the saline calibration trials it became clear that each of the valves tested had its own set point. These values all fell within the parameters described in the product safety literature. Longitudinal flow-rates for each valve varied slightly but showed no directional trend (Supplemental Figures 1-9).

Effects of protein on differential pressure and high-resistance valves.

Previous experiments have examined CSF shunt hardware using a narrow scope of fluids, which limits the generalizability of these results regarding the performance of such devices. In particular, ISO standards for medical devices only require testing with distilled water,

which may misrepresent flow in the biological setting. Our studies sought to incorporate factors that more realistically represent and challenge the working environment of flow diversion hardware in normal and pathological states. With the introduction of these variables, a number of observations were noted. For example, in supine position trials, addition of protein resulted in increased range and variation of flow-rates (Supplemental Figure 10). These findings were amplified in 5g/L protein-CSF compared to 1g/L BSA saline, suggesting a dose responsiveness of protein content with shunt flow-rate variance. In concordance with previous studies[16], we hypothesize that this increase in variability could be the result of a slower activation of the ball-in-cone mechanism due to increased adhesive properties of protein in solution. Possible explanations for these observations may be related to rheological factors affected by the increased protein concentration, namely a potential influence on viscosity, surface tension, and binding to the DPV's internal components. The fact that these changes in SD and range were not seen in upright trials may indicate that SG's high-resistance pathway might be less prone to protein-induced effects. Clinical concerns have been raised regarding the reliability and risk of obstruction of shunting devices with narrow pathways. In order to explore the limits of these shunting devices, we tested supraphysiologic protein levels flowing continuously over prolonged intervals in a virtually sterile environment. This setup was designed to allow greater sensitivity to gradual decreases in flow and acute valve failures, excluding blockages secondary to air bubble formation, fluid contamination, and protein sedimentation. Furthermore, the use of filtered CSF avoided shunt blockages due to blood product accumulation, a well-known and previously investigated etiology of shunt failure[16,20]. To the best of our knowledge, these studies represent the longest continuously running model of shunt flow using a non-pump-driven system.
Although no obstructions or flow decreases were detected, it must be noted that even trials lasting several weeks represent only a snapshot of these systems' intended lifespans in patients.

Clinical Significance. We designed our valve testing apparatus to operate within physiologic parameters. Our findings are consistent with previous evidence showing that shunt valves with ASDs perform reliably in the upright position over long periods of time with

nonparticulate fluids[28,30]. The use of high protein CSF resulted in significantly increased supine flow-rates compared to normal saline. An 8-hour supine period combined with 14 hours in the upright position with 1g/l of BSA resulted in average increases of 36.8ml, translating to a total daily fluid output of 335.3ml, which may lead to overdrainage in certain situations. These results suggest that protein content can have an important influence on shunt flow-rates, and might be of particular consideration when managing valve settings in patients predisposed to increased CSF protein. However, the effects of protein on fluid output across a prolonged series of trials indicates that these effects are somewhat variable. In the supine position, for example, the range of flow-rates in saline (low-resistance settings), saline with 1g/l BSA, and 5g/l protein-CSF varied dramatically when compared to similar upright trials. One particular CPBV demonstrated supine outputs ranging from 8.0ml (flow-rate 0.96ml/h) to 123.84ml (flow-rate 15.48ml/h) with low protein. This pattern was seen in all valves (CPBV and HBV) and was independent from external variables such as temperature, ICP, valve setting/position. This variability in supine flow-rates might be clinically relevant, leading to higher uncertainty with regards to the actual volume drained by their shunts, especially among those who are bedridden.

These findings, although preliminary, suggest that the engagement of SG's narrow path confers less flow-rate variation in response to the challenges of elevated protein. These results may reinvigorate arguments favoring the use of a long, high-resistance pathway as means of tighter regulation of CSF-diverting flow, though further studies are warranted.

Limitations

Our attempt to recreate physiologic parameters of CSF flow is not without limitations. While this in vitro model of shunting using gravity-driven flow controlled for many variables, there is no doubt that it oversimplifies in vivo shunting by not taking into account multiple daily positional changes and features such as CSF pulse pressure, cardiorespiratory variations, abdominal backpressure, and CSF absorption. Importantly, we recognize that even the extended duration of these benchtop trials is nevertheless eclipsed by the intended lifespan of these devices in vivo. Lastly, while the valves studied were chosen due to their mechanistic

variety, further studies with an even broader range of hardware should be pursued in the future.

Conclusions

Here, we created a novel benchtop model of VP shunting with gravity-driven flow, allowing for parallel testing of multiple shunts at near-physiologic experimental conditions. Using this model, we examined the effects of both diurnal positional change and protein concentration on shunt flow in two commonly used DPVs with high-resistance ASDs. These studies showed no protein dependent flow obstruction on any valve system, including the narrow high-resistance pathway.

While these studies provide some of the longest continuously collected data from a realistically flowing system with high-protein CSF, the test durations do not approach the intended lifespan of these devices, limiting one's ability to conclusively comment on their long-term resistance to protein-related occlusion in long-term chronic use. Significant variability was demonstrated in ball-in-cone DPVs. The addition of protein resulted in higher flow in the supine trials, whereas engagement of the high-resistance pathway in upright trials provided more consistent performance in that position. Furthermore, attenuation of protein-related increases in day-to-day variability by narrow-lumen devices suggests they may play an important role in preventing overdrainage.

These findings suggest that clinically significant changes in flow-rates in DPVs may increase with highly elevated protein levels, and that this variability is potentially attenuated by activating a narrow-lumen high-resistance pathway as used with SG. While questions remain about possible risks of overdrainage using unvalved pathways alone, and about possible longer-term blockage of narrow pathways, this study suggests that further exploration of alternatives to ball-in-cone differential pressure valves is warranted.

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Figure Captions

Figure 1: Illustration of the Bench-top Shunt Model with a Comparative Schematic. Reservoir units (A) provide a hydrostatic driving force that keeps the central ventricle unit (B) filled with fluid as it drains through proximal catheters (C) that penetrate the bottom of the unit. This driving force can be adjusted to mimic intracranial pressure (ICP) by controlling the height differential between the reservoir units on the top shelf and the valves (D) below. Fluid that flows through the valves passes through distal catheters (E) which empty into their respective measuring flasks which can be positioned 28.5 or 2 cm below the height of the valves in order to recreate the patient's upright (F) or supine (G) position states.

Table 1: Average flow-rates and standard deviations of each individual CPBV (A, C) and HBV (B, D) for all trials in the supine (A, B) and upright (C, D) states.

Figure 2: Daily average flow-rates grouped by valve type in the supine (solid) and upright 384 (dashed) positions for saline without (A, B) and with protein (C), and CSF with protein (D). No downward trends in flow-rates were noticed over time. For supine trials with saline,

flow-rates decreased when resistance settings were increased from low to medium. Supine flow-rates subsequently rose with the addition of BSA and furthermore CSF with BSA.

Figure 3: Scatter plot of every individual flow-rate measured in the (A) upright and (B) supine positions for all three CPBVs and HBVs over the duration of all trials, separated by study fluid. Each dot represents a single trial, with each of the three distinct CPBVs represented by shades of blue and each of the three HBVs shown as shades of red. The narrow clustering of each distinct valve's upright flow-rates in saline (with and without protein) is lost in the upright CSF+Protein trials.

Supplemental Digital Content. Table 1. Statistical analysis of variance (ANOVA) among sets of valves according to different positional states and fluids. Repeated measures ANOVA showed no significant downward or upward trend in CPBVs and HBVs valves during trials with 400 normal saline, normal saline + BSA 1 g/l and CSF + 5 g/l.

Supplemental Digital Content. Figure 1-2. Consecutive Trial Flow Rates of all CPBVs and HBVs in Saline. A combined total of 55 supine and upright trials of three CPBVs (1) and three HBVs (2) valves in saline were alternatingly tested for 6- and 14-hour periods, respectively, over a span of 28 days. Average flow rates are given in ml/h for each individual trial. Upright trials
are represented by lines connecting open circles (white areas), and supine trials by lines connecting filled circles (shaded areas). Horizontal axis depicts both cumulative hours (grey labels) and day numbers (black labels). All CPBVs and HBVs were maintained at settings of 4 and 50, respectively.

Supplemental Digital Content. Figure 3. Saline - Consecutive average flow rates by trial alternating supine and upright flow states in CPBVs (red line) and HBVs (blue line).

Supplemental Digital Content. Figure 4-5. Consecutive Trial Flow Rates of all BPBVs and

HBVs in Saline + 1 g/l BSA. Thirty three trials testing three CPBVs (4) and three HBVs (5) in saline with 1g/l BSA were alternatingly tested in the supine and upright positions for 6- and 14-hour periods, respectively, over a span of 23 days. Average flow rates are given in ml/h for each individual trial. Upright trials are represented by lines connecting open circles (white areas), and supine trials by lines connecting filled circles (shaded areas). Horizontal axis depicts both cumulative hours (grey labels) and day numbers (black labels). All CPBVs and HBVs were maintained at settings of 4 and 50, respectively.

Supplemental Digital Content. Figure 6. Saline + 1 g/l B - Consecutive average flow rates by trial alternating supine and upright flow states in CPBVs (red line) and HBVs (blue line).

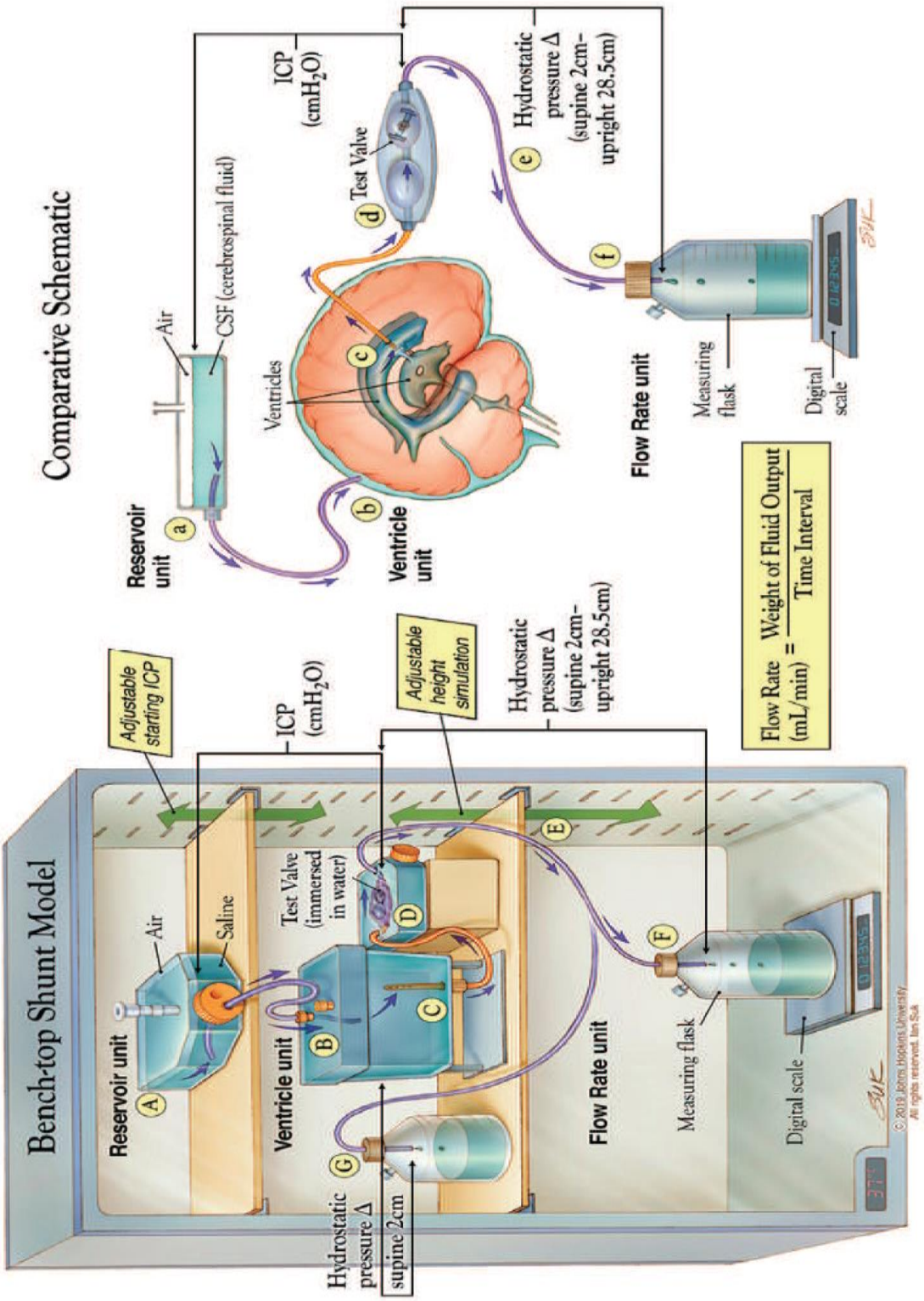
Supplemental Digital Content. Figure 7-8. Consecutive Trial Flow Rates of all CPBVs and HBVs in CSF + 5g/l Protein. Thirty five trials of three CPBVs (7) and three HBVs (8) in CSF with 5g/l of protein were alternatingly tested in the supine and upright positions for 6- and 14-hour periods, respectively, over a span of 30 days. Average flow rates are given in ml/h for each individual trial. Upright trials are represented by lines connecting open circles (white areas), and supine trials by lines connecting filled circles (shaded areas). Horizontal axis depicts both cumulative hours (grey labels) and day numbers (black labels). Different valve settings were used throughout the trial, as indicated by the blue arrow bars under the horizontal axis.

Supplemental Digital Content. Figure 9. CSF + protein (5g/L) - Consecutive average flow rates by trial alternating supine and upright flow states in CPBVs (red line) and HBVs (blue line).

Supplemental Digital Content. Figure 10. Individual flow rates by trial and fluid (saline – black dots, saline + protein 1g/l - red dots, CSF + protein 5 g/l - blue dots) in supine and upright states in CPBVs and HBVs.

Supplemental Digital Content. Materials and Methods.

Supplemental Digital Content SupFig10 Click here to access/download;Supplemental Digital Content (for online-only posting);Supplemental Digital Content Figure 10



Supplemental Digital Content. Materials and Methods

System

The shunt flow testing system consisted of a common central water-tight "ventricle" placed upon the middle shelf of a tissue culture incubator (Thermo Fischer Scientific, Waltham, MA) maintained at 37˚C, 100% humidity, and atmospheric CO_2 and pressure (Figure 1). The ventricle was housed on the middle shelf of the incubator atop an orbital shaker to prevent sedimentation (Troemner, Thorofare, NJ). Affixed to the ventricle's base were twelve luer-style adapter ports (Qosina, Ronkonkoma, NY). Six of these ports housed separate proximal catheters, to allow for simultaneous flow measurement in six complete shunts (proximal catheter, valve, distal catheter) in parallel. For all flow trials, three Codman Hakim® ball valves (HBVs) and three Codman Certas® Plus ball valves (CPBVs) were tested in parallel. All valves and catheters were new, provided directly from the manufacturer (Codman by Integra, Plainsboro, NJ) and contained integrated SiphonGuard® (SG) anti-siphoning devices. The ventricle was filled with fluid of varying composition and constantly refilled by five reservoir flasks (Corning Inc., Corning, NY) situated upon the incubator's top shelf, connected to the ventricle by tubing attached to ports at the base of the ventricle. The height differential between the incubator's top and middle shelves, which housed the filling flasks and the ventricle, respectively, determined the hydrostatic potential driving fluid through the attached shunt systems. The ventricle's twelfth and final bottom port was

connected to tubing that served as a manometer, continuously monitored to ensure homogeneous pressure in the physiologic ICP range, measured in cmH_2O.
This system was designed for compatibility with a range of VPS hardware. In all studies, valves were attached to silicone Bactiseal® proximal and distal shunt catheters (Codman by Integra, Plainsboro, NJ). Each proximal and distal catheter-valve interface was reinforced with a silk suture and the unit was submerged underwater to prevent air entry and provide surrounding pressure similar to a subcutaneously-implanted valve. Proximal catheters were passed into the ventricle to expose the hardware directly to the test fluid. The distal ends of the valves were connected to a distal Bactiseal® catheter and ultimately emptied into a collecting flask which was measured by weight with a digital scale. Placement of collecting flasks alternated between the incubator's middle and bottom shelves to recreate the different position-dependent flow rates seen with a patient in the horizontal (supine) and vertical (upright) states, respectively. Every 24-hour period consisted of a 6-8 hour supine trial and a 12-14 hour upright trial, with distal catheter tips 2 cm and 28.5 cm below the height of the valves, respectively. All fluid containing flasks were opened to atmospheric pressure through 0.22 uM air filters (Corning Inc., Corning, NY) to provide a barrier against contamination while allowing pressure equilibration across the system.
At the conclusion of each trial, the total fluid output collected from each of the six distal catheters was weighed and the trial's overall average flow rate was calculated for each shunt
system.

Fluids
The system was designed to run a variety of fluids mimicking different physiological and pathological states. It was first calibrated for a month using normal saline (Hospira, Lake Forest, IL), followed by another month-long protein trial using saline containing 1g/L of Bovine Serum Albumin (BSA, Sigma Aldrich, St. Louis, MO). To further test the hardware under more challenging pathological conditions, human CSF with elevated protein was also used in several sets of trials that lasted for 30-days using new valves. CSF was collected from external ventricular drainage catheters in adult and pediatric patients at our institution with intraventricular hemorrhage after obtaining IRB approval and informed consent (IRB NA_00029413). All CSF samples were deidentified and combined, forming an aggregate with a baseline protein concentration of 1.63 g/L. BSA was added to reach a final protein concentration of 5 g/L. CSF was centrifuged at 2500 rpm for 20 minutes to remove blood and other cellular material, and the supernatant was collected and filtered with 0.22 μm low-protein binding sterilizing filters (Corning Inc., Corning, NY, USA). Amphotericin B (final concentration of 2.2 mg/dL, Sigma Aldrich, St. Louis, MO), Penicillin/Streptomycin (3% v/v, Gibco Laboratories, Gaithersburg, MD), and Chlorhexidine dihydrochloride (Sigma Aldrich, St. Louis, MO) were added to this acellular, protein-enriched human CSF to minimize the risk of contamination.
Samples were frozen and stored at -80° C until use after thawing. Protein concentration was measured at our institution laboratory core before, during, and after the end of the trial, to ensure that concentrations remained stable throughout.

Valve Resistance Settings

Supplemental Digital Content SupFig10 Click here to access/download;Supplemental Digital Content (for online-only posting);Supplemental Digital Content Figure 10

For the initial calibration trials with normal saline and high-protein CSF, CPBVs and HBVs were programmed to low-resistance settings of 2 and 50, respectively. For all other trials, the valves were increased to settings 4 and 100.

Statistical Analysis

Hourly and average flow rates were therefore calculated along with standard deviations, and statistical analysis was performed using two samples t-test for independent samples and paired ttest for same sample measured at different conditions or time points. Repeated measures ANOVA was used to compare valves' performance over time. GraphPad Prism Software® (Version 8.0, GraphPad Software, San Diego, CA) was used to perform statistical tests. Statistical significance was set to a P value of 0.05.

Supplemental Digital Content 3. Table 1 Click here to access/download;Supplemental Digital Content (for online-only posting);Supplementary Digital Content 3. Table 1

Supplemental Table 1: Statistical analysis of variance (ANOVA) among sets of valves according to different positional states and fluids.

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Supplemental Digital Content SupFig2 Click here to access/download;Supplemental Digital Content (for online-only posting);Supplemental Digital Content Figure 2 CMYK.tif

Supplemental Digital Content SupFig3 Click here to access/download;Supplemental Digital Content (for online-onlyposting);Supplemental Digital Content Figure 3 CMYK.tif

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Supplemental Digital Content Figure 6 CMYK.tif Click here to access/download;Supplemental Digital Content (for online-onlyposting);Supplemental Digital Content Figure 7 CMYK.tif

Supplemental Digital Content SupFig8 Click here to access/download;Supplemental Digital Content (for online-only posting);Supplemental Digital Content Figure 8 CMYK.tif

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Supplemental Digital Content SupFig9 Click here to access/download;Supplemental Digital Content (for online-only posting);Supplemental Digital Content Figure 9 CMYK.tif