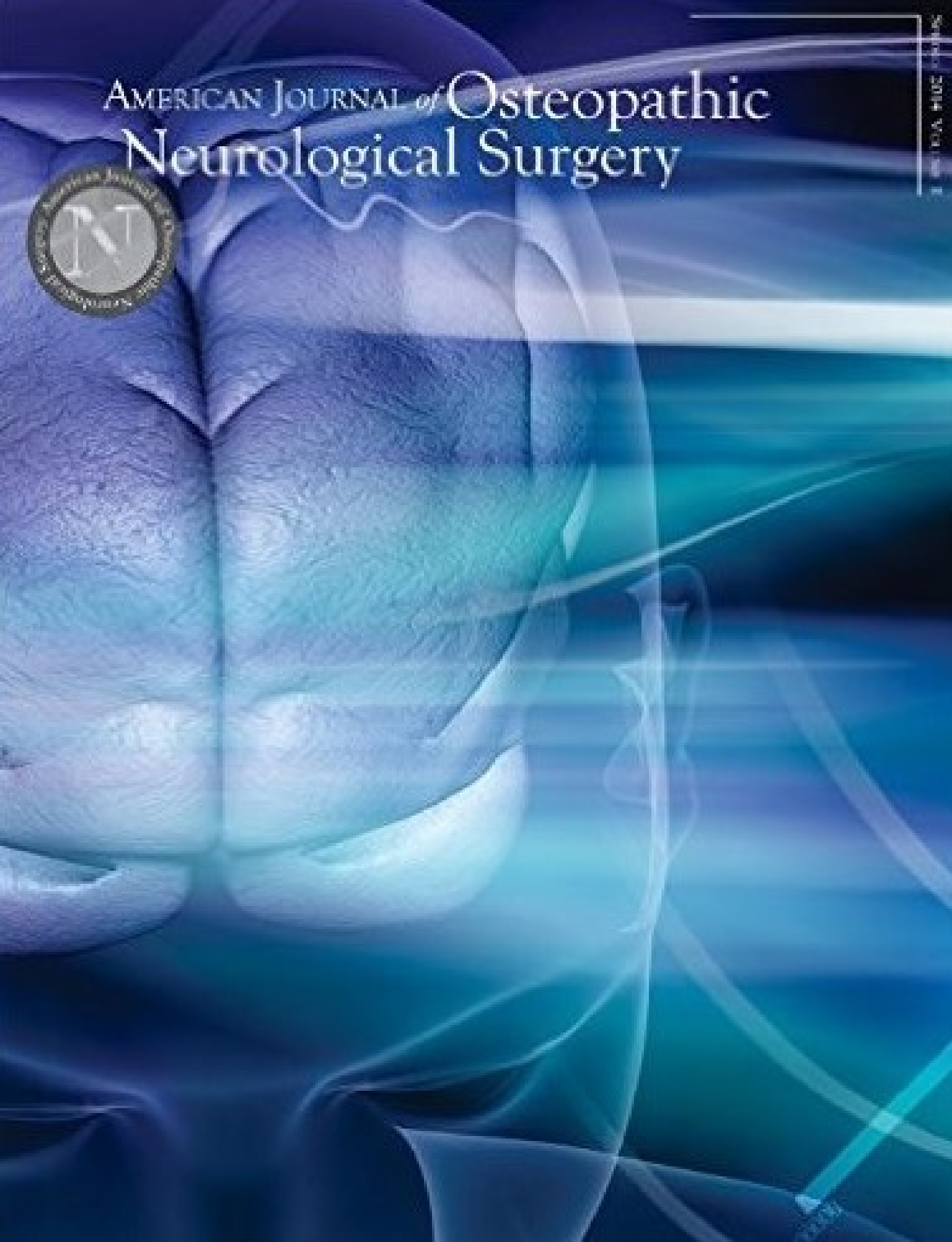

American Journal of Osteopathic
Neurological Surgery

INSTRUCTIONS FOR AUTHORS

Papers submitted should be original documentation, including photographs. The papers should be single column, double-spaced in WORD format. The title should be in title case and bold, followed by Authors, degree, organization and city, state.

The papers should contain an abstract and be separated into sections with bold typing of the section title. The page set-up should be 0-6.5 inches. Paragraphs should be indented 0.5 inches. All tables should be submitted separate from the paper. If possible make the tables up to 3 inches wide so that they could fit into a column. This will allow quicker scanning and preparation.

References should be numbered, tab, name of authors, title of paper, journal, year volume:pages.

All papers, correspondence can be submitted to the:

Dan Miulli, DO, FACOS American Organization of Neurological Surgeons

The AJONS is produced and published jointly by the AONS & ACOS-NSD.

EDITOR'S PAGE

Physicians in training, learn and practice research "To formulate, ingrain, and measure, a method of thought, investigation, and evaluation necessary for physicians to have multi-lateral information exchange and communication with experts in areas of scientific and medical discovery, knowledge, and analysis, in order to continuously and efficiently improve human health and patient care." Understanding and performing quality research provides students and residents the tools to propel quality medical care into the community and into the future.

Welcome to the American Journal of Osteopathic Neurological Surgery and the American College of Osteopathic Surgeons Neurosurgical Discipline. This volume is composed of the Residents' annual papers that were submitted but not published elsewhere. It is therefore dedicated to the future Neurosurgeons and their education. All papers were reviewed by the peer review committee and selected for awards. The papers submitted are excellent, representing some of our talented colleagues. Issues will be published quarterly. I hope that this issue will spread the knowledge of our residents and our section. We will continue to solicit annual papers and all papers submitted at the annual meeting. This is your Journal paid for by your annual dues. This issue is available on our website AOANeurosurgery.org. This is your organization; please support it as you can.

Thank you,

Dan Miulli, D.O, F.A.C.O.S
Co-Editor In Chief

2015 Annual Resident Papers Awards

Congratulations on your submission for the 2015 Annual Resident Paper Contest. The winning papers will prepare a 20 minute presentation for the ACOS ACA in Chicago on Sunday October 4, 2015 at 2:00 AM in Chicago Ballroom A/B/C

1st Place Joseph Georges, DO, Use of a Conformational Switching Aptamer for Rapid and Specific Ex Vivo Identification of Central Nervous System Lymphoma in a Xenograft Model

2nd Place Mark Krel, DO, Intraparenchymal Hemorrhagic Stroke Size is Minimal Within an Optimal Range of Total Cholesterol

3rd Place Omid Hariri, DO, Anti-epileptic prophylaxis in traumatic brain injury: A retrospective analysis of patients undergoing craniotomy versus decompressive craniectomy

First place receives $1500.00; Second Place $1000.00; and Third Place $500.00.

ACOS NSD Annual Paper Contest Judging Criteria
The papers judged the best will be original prospective studies in neurosurgery. Case studies and review of the literature are significant contributions and should be combined with retrospective or prospective procedures to qualify as best papers. Papers will receive up to 100 points in each category for a total of 500 points. The categories judged are: 1. Type of Research Paper (basic science, original clinical research, prospective study, chart review, review of literature, case study), 2. Grammar, 3. Addition to Science, 4. Research Conducted, and 5. Change to Neuroscience Practice.

Will Clinical Parameters Reliably Predict External Ventricular Drain Associated Ventriculitis: is Frequent Routine Cerebrospinal Fluid Surveillance Necessary?

Omid R. Hariri, D.O., MSc

Abstract

Introduction: The placement of an external ventricular drain (EVD) for monitoring and treatment of increased intracranial pressure is not without risk, particularly for the development of an associated ventriculitis. The goal of this study was to investigate the presence of any CSF, serum or clinical parameters that would help to predict ventriculitis before it occurs; thus allowing for the determination of optimal timing of CSF collection for patients with in-situ EVDs.

Methods: An observational retrospective study was conducted on patients from the Neurosurgery census between January 2006 and May 2012. A total of 466 patients were identified as having an in-situ EVD placed. Inclusion criteria were age >18 years, GCS 4-15, and placement of EVD for any indication. Our exclusion criteria included any recent history of meningitis, cerebral abscess, cranial surgery or open skull fracture within the previous 30 days. A broad definition of ventriculitis was used to separate patients into two categories: Suspected Ventriculitis (meeting all 3 CSF criteria while having no organism on CSF culture); and Confirmed Ventriculitis (presence of any organism on CSF culture). CSF sampling was conducted every other day of the week (Monday, Wednesday, Friday).

Results: A total of 466 patients were identified as having an EVD placed during the study and 123 patients were included in the final analysis after exclusion criteria were applied. The incidence of ventriculitis was found to be 8.8%. Only one of variables being studied, the ratio of glucose CSF:serum < 0.5 was found to be of statistical significance, though not predictive of developing a ventriculitis.

Conclusions: The authors feel confident that this study demonstrates no reliable CSF, serum, or clinical parameters that can effectively predict the development of ventriculitis in an EVD patient. Thus, we recommend and will continue to draw CSF samples on patients with in-situ EVDs on our previously established schedule of Monday/Wednesday/Friday for as long as the EVD remains in place.

Introduction

The use of the external ventricular drain (EVD) serves a dual function in the neurosurgical patients. It not only serves as a diagnostic monitor to detect elevated intracranial pressure (ICP), but also serves a therapeutic role in its ability to drain cerebrospinal fluid (CSF) in the setting of elevated ICP. While the ICP device is used in many neurosurgical patients, including but not limited to, those with subarachnoid hemorrhage (SAH), intracranial hemorrhage (ICH), or acute hydrocephalus; it is not without complications. The most commonly noted complication is ventriculitis. Incidence rates vary depending on the institution, and range

from 0-22%, but more commonly tend to lie within the 10- 17% range[1-6,8,10]. The rate of ventriculitis tends to be associated with a higher morbidity and mortality for the neurosurgical patient[5,6]. Recent improvements in sterility techniques, antibiotic-impregnated catheters, and appropriate antibiotic administration have helped to decrease the rate of ventriculitis[5,6,].

In a prospective epidemiologic study, Mayhall et al[7] analyzed the risk factors for ventriculitis in 172 consecutive neurosurgical patients. Their proposal stated that infection rates may be lowered by prophylactic exchange of catheters at 5-day intervals. However, a decade later, Holloway et al[1] showed that replacement of catheters prior to 5-days did not show a lower infection rate as compared to catheters exchanged at intervals of 5-days or greater. Over time, several approaches have been suggested to prevent ventriculitis, such as use of prophylactic antibiotics at time of insertion, during the first 24 hours post-insertion, and in a prolonged systemic fashion while the EVD is in place[11]. However, there is no consistent practice regarding the use of prolonged systemic antibiotics with EVD and the preference is largely related to tradition and training[11, 12].

Early detection of ventriculitis is imperative for successful treatment and for minimizing the possibility of future infections[3]. However, the clinical detection of ventriculitis can be difficult. For example, a study by Muttaiyah et al[4] determined that there was no associated temperature spikes with ventriculitis. However, CSF glucose levels were notably lower in these patients.

An important consideration in the control of ventriculitis is the surveillance of CSF and monitoring for clinical symptoms of ventriculitis[9]. Currently, the issues of when and how often CSF sampling should be performed remains controversial[9]. Routine CSF sampling has been reported as daily[2,9,16,17], every 3-5 days[6,18-20], only at EVD insertion, or only as clinically indicated[1,21,22]. In a study by Williams et al[3], investigators sought to examine how the incidence of ventriculitis was affected by CSF sampling frequency, by decreasing surveillance from daily to every three days. Their results demonstrated that the incidence of ventriculitis decreased from 17% to 10.8% after changing CSF surveillance to every three days[3].

Naturally, one would assume that a breach of the EVD drainage system for sampling the CSF would increase the risk of contamination and subsequent infection. However, this risk must be balanced by the morbidity and mortality of ventriculitis if it remains undetected. This is especially important in light of the established literature showing the difficulty of clinical detection of ventriculitis. Current standard practice at our institution is to sample CSF upon insertion of EVD, followed by sampling every Monday, Wednesday, and Friday for as long as the EVD remains in place. As a result of these challenges, we decided to examine whether changes in any CSF, serum, or clinical parameters can help predict ventriculitis before it occurs, and if the current collection schedule of CSF is necessary.

Materials and Methods

The patients included in this study were identified during the course of the study using their medical record numbers. We performed a retrospective observational study of the

Neurosurgical Census, for patients with in situ EVD from January 2006 to May 2012. A total of 466 patients were identified. Medical records were then used to classify patients by age and gender, indication for EVD, the duration of EVD, CSF and serum laboratory data, daily temperatures, changes in GCS, CSF culture, and the presence of other infections. To be included in the study, patients had to be 18 years or older, have an EVD (regardless of indication), and GCS 4-15. Patients with any recent (defined as within the last 30 days) history of meningitis, cerebral abscess, craniotomy or open skull fracture were excluded from the study.

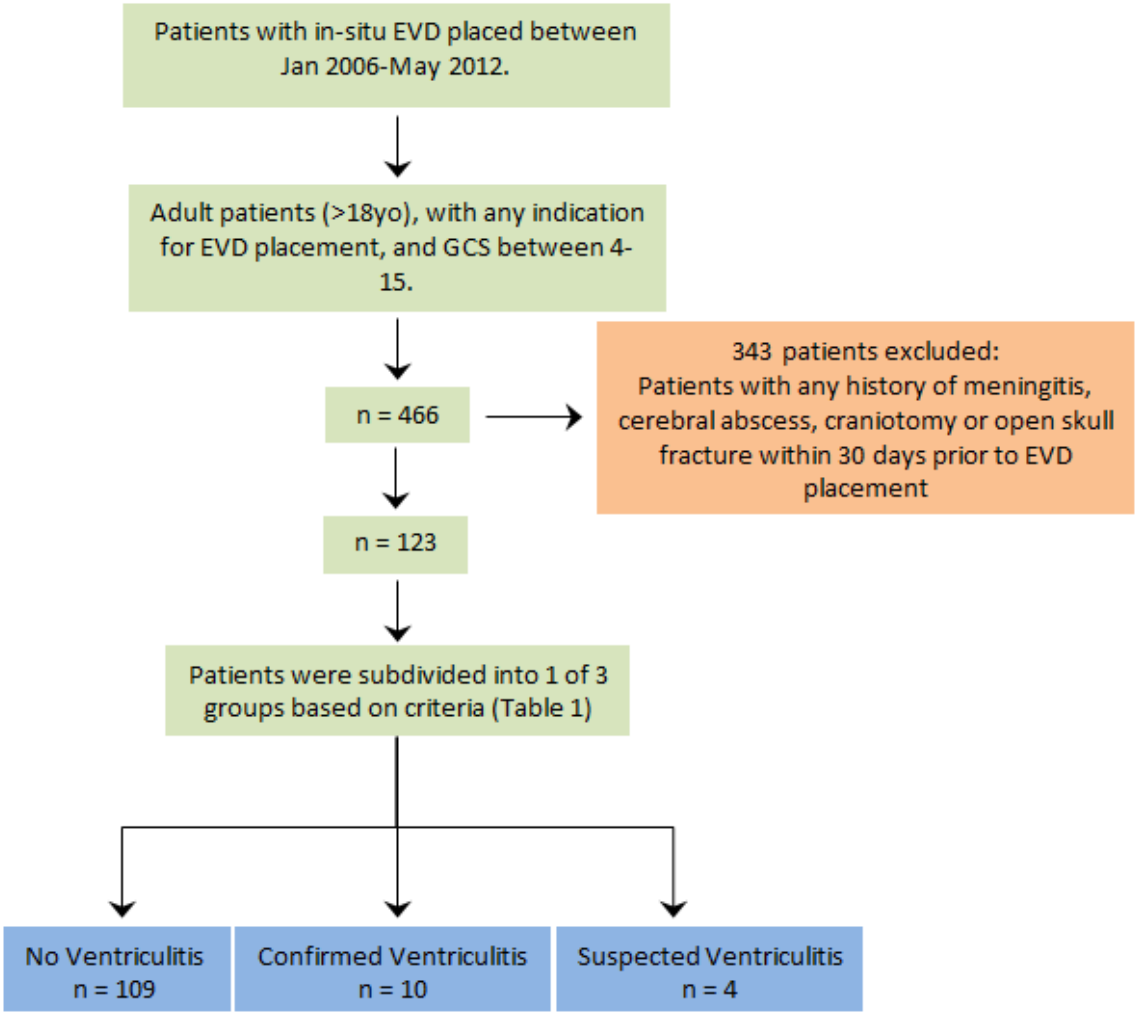


Figure 1: The criteria for patient selection, as well as the number of patients that were included in the study.

At our institution, EVDs are placed by standard neurosurgical sterile technique in either the operating room, the emergency department, or in the intensive care unit, and are then connected to a sterile closed circuit system. All ventriculostomy catheters were non-antibiotic impregnated Integra trauma catheters. After the EVD has been sutured into place, a BioPatch is placed where the catheter exits the skin and a Tegaderm is placed to cover the BioPatch and catheter. As per standard Neurosurgical protocol, a non-contrast Computerized Tomography

(CT) scan was obtained after placement to rule out hemorrhage caused by placement and to confirm satisfactory placement of the catheter.

In regards to patients with an EVD, per current institutional protocol for the Department of Neurosurgery, CSF samples are drawn every other day during the week (ie: Monday, Wednesday, Friday) for as long as the EVD remains in place. CSF samples are drawn solely by the neurosurgery residents or attending physicians. The stopcock just distal to the catheter insertion site is turned off from the drainage system and the proximal port is cleaned with chlorahexidine twice. A sterile syringe is connected to the port and 3-5 ml of CSF is slowly withdrawn and then discarded. The port is then cleaned a second time and another 3-5ml of CSF is withdrawn, and sent for culture and analysis using standard methods.

For patients in our study, the aforementioned laboratory and clinical parameters were collected on the day of EVD placement ("day 0"). Additionally, the following data was collected on the day of EVD placement ("day 0"), as well the day prior ("day -1") and two days prior ("day -2): serum WBC, temperature, GCS, and cultures from other bodily sources (including sputum, nasal, urine, blood, neck, or tracheostomy). The schema for the time-based collection of all laboratory and clinical data can be found in Table 1.

Timeline of data collection		
Day -2 (2 days prior to CSF collection)	**Day -1 (1 day prior to CSF collection)**	**Day 0 (day of CSF collection)**
WBC_{Serum}	WBC_{Serum}	WBC_{Serum}
Temperature	Temperature	Temperature
GCS	GCS	GCS
Culture of other bodily sources	Culture of other bodily sources	Culture of other bodily sources
		CSF culture
		CSF protein level
		Ratio $Glucose_{Serum} : Glucose_{CSF}$
		Ratio $WBC_{CSF} : RBC_{CSF}$

Table 1: schema showing the timeline of data collection on the day of CSF draw (Day 0), and previous two days.

To define ventriculitis, we used the criteria proposed by Lozier et al[13], including fever (≥ 38.5°C, with/without clinical signs of meningitis), associated with at least one positive CSF culture and at least one element of CSF abnormality, including low CSF glucose levels (<40mg/dl, or <50% of serum glucose in patients with hyperglycemia), high CSF protein (> 100mg/dl), or CSF pleocytosis (> $100/mm^3$). In addition, we also looked at the ratio of CSF glucose to serum glucose (<0.5) and the ratio of white blood cells (WBC) in the CSF to red blood cells (RBC) in the CSF (>1:250).

Patients were broadly divided into 3 categories: No Ventriculitis, Suspected Ventriculitis, and Confirmed Ventriculitis. A CSF culture with the growth of any organisms denoted confirmed ventriculitis. In the absence of any organisms, the previously mentioned criteria from Lozier et al were used to denote suspected ventriculitis: CSF glucose to serum glucose ratio < 0.5, CSF white blood cell (WBC) to red blood cell (RBC) ratio > 1:250, and CSF protein > 100 mg/dL [Figure 2]. Patients who did not meet all of these criteria, or did not have any organisms present on CSF culture were placed into the No Ventriculitis group.

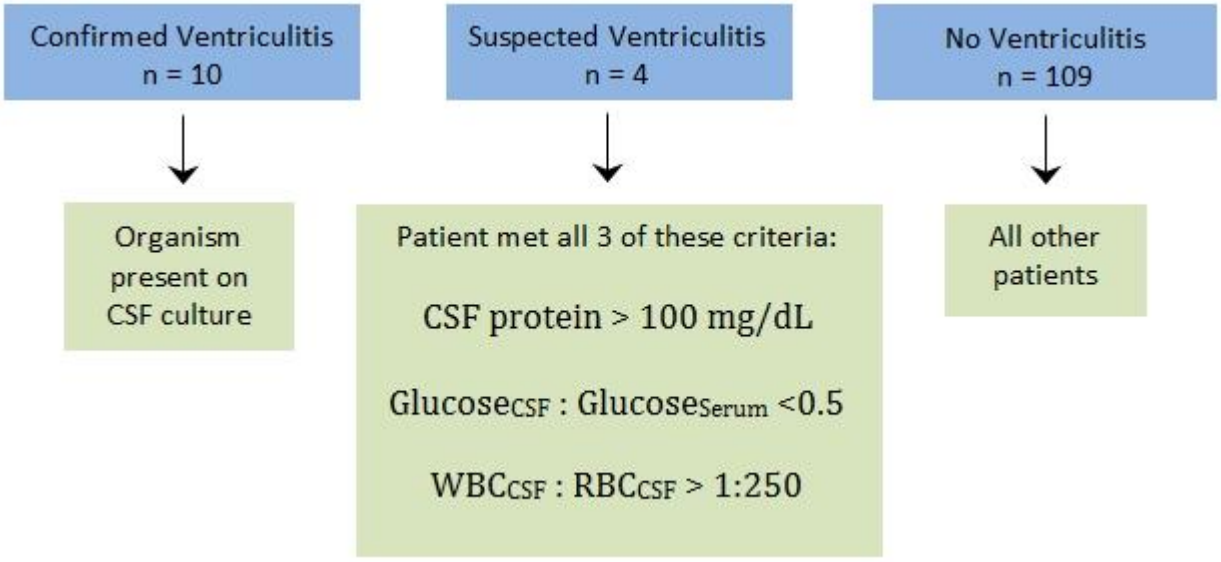


Figure 2: illustrates the criteria that were used to place patients into one of three categories.

Statistical Analysis: All statistical analyses were conducted using the SAS software for Windows version 9.3 (Cary, NC). Descriptive statistics were presented as means and standard deviations for continuous variables (e.g., age), and frequencies and percentages for categorical variables. Crosstab analyses were conducted to identify the association between two categorical variables using the Chi-square test or Fisher's exact test if the expected cell count does not meet the assumption. Given the small count in the suspected ventriculitis (n=4), this category was excluded from the analysis. Independent t-test was adopted to analyze whether there is a statistically significant difference between patients with and without ventriculitis. All statistical tests were two-sided. P-value <0.05 was considered to be statistically significant.

Results

A total of 123 were included in the final analysis. The majority of these patients were males (n=87, 70.7%) with the average age being 48.8 ± 17.2 years. A total of 10 (8.1%) patients diagnosed with ventriculitis, 4 (3.3%) with suspected ventriculitis and 109 (88.6%) without ventriculitis were identified in this sample.

Demographic breakdown with respect to ventriculitis status						
		No ventriculitis n = 109	Confirmed ventriculitis n = 10	Suspected ventriculitis n = 4	All patients combined n = 123	P-value
Age (years) mean ± SD		48.64 ± 17	45.5 ± 16.41	60.75 ± 25	48.78 ± 17.22	0.5759
Sex	M	79 (72.5%)	5 (50%)	3 (75%)	87 (70.7%)	-
	F	30 (27.5%)	5 (50%)	1 (25%)	36 (29.3%)	

Table 2 illustrates the demographic breakdown all patients with respect to their ventriculitis status.

Crosstab analyses were conducted using the Fisher's exact test. The suspected ventriculitis patients were excluded due to the extremely small sample size (n=4). Regardless of the sampling time frame (2 days ago, 1 day ago, or 0 day ago), temperature >100.4F or WBC >

11,000 or decline in GCS by two or more points, were not associated with the ventriculitis status.

Crosstab analysis of temperature with ventriculitis status						
		No ventriculitis n = 109	Confirmed ventriculitis n = 10	Suspected ventriculitis n = 4	All patients combined n = 123	P-value
Temp >100.4°F 2 days prior to CSF collection	Yes	11 (18%)	1 (10%)	1 (25%)	13 (17.3%)	1.000
	No	50 (82%)	9 (90%)	3 (75%)	62 (82.7%)	
	Data Unavailable	48	-	-	48	
Temp >100.4°F 1 day prior to CSF collection	Yes	11 (13.1%)	0 (0%)	1 (25%)	12 (12.2%)	0.6001
	No	73 (86.9%)	10 (100%)	3 (75%)	86 (87.8%)	
	Data Unavailable	25	-	-	25	
Temp >100.4°F on day of CSF collection	Yes	14 (14.1%)	2 (20%)	0 (0%)	16 (14.2%)	0.639
	No	85 (85.9%)	8 (80%)	4 (100%)	97 (85.8%)	
	Data Unavailable	10	-	-	10	
Crosstab analysis of serum white blood cell count with ventriculitis status						
		No ventriculitis n = 109	Confirmed ventriculitis n = 10	Suspected ventriculitis n = 4	All patients combined n = 123	P-value
WBC >11,000 2 days prior to CSF collection	Yes	55 (68.8%)	5 (50%)	2 (50%)	62 (66%)	0.2915
	No	25 (31.3%)	5 (50%)	2 (50%)	32 (34%)	
	Data Unavailable	29	-	-	29	
WBC >11,000 1 day prior to CSF collection	Yes	65 (62.5%)	6 (60%)	3 (75%)	74 (62.7%)	1.000
	No	39 (37.5%)	4 (40%)	1 (25%)	44 (37.3%)	
	Data Unavailable	5	-	-	5	
WBC >11,000 on day of CSF collection	Yes	61 (56%)	6 (60%)	4 (100%)	71 (57.7%)	1.000
	No	48 (44%)	4 (40%)	0 (0%)	52 (42.3%)	
	Data Unavailable	-	-	-	-	
Crosstab analysis of Glasgow Coma Scale with ventriculitis status						
		No ventriculitis n = 109	Confirmed ventriculitis n = 10	Suspected ventriculitis n = 4	All patients combined n = 123	P-value
GCS decline >2 between day of CSF collection & 2 days prior	Yes	43 (41.4%)	5 (50%)	3 (75%)	51 (43.2%)	0.7402
	No	61 (58.7%)	5 (50%)	1 (25%)	67 (56.8%)	
	Data Unavailable	5	-	-	5	

Table 3 shows a summary of results with respect to temperature, white blood cell count, and GCS.

However, despite the lack of statistically significance (p=0.0866), patients who were diagnosed with ventriculitis were twice as more likely to have a WBC to RBC ratio>1:250 (40% vs 16.5% for ventriculitis and no ventriculitis cohort, separately). Similarly, patients who were diagnosed with ventriculitis were twice as likely to have CSF Glucose<0.5 serum (50% vs 21.2% for ventriculitis and no ventriculitis cohort, separately). Lastly, using glucose as a continuous variable, patients with ventriculitis had statistically significant lower concentration of glucose

than the counterpart (0.48 and 0.62 for ventriculitis and no ventriculitis cohort, separately, p=0.0298).

Crosstab analysis of CSF parameters with ventriculitis status						
		No ventriculitis n = 109	Confirmed ventriculitis n = 10	Suspected ventriculitis n = 4	All patients combined n = 123	P-value
WBC_{CSF} : RBC_{CSF} > 1:250	Yes	18 (16.5%)	4 (40%)	4 (100%)	26 (21.1%)	0.0866
	No	91 (83.5%)	6 (60%)	0 (0%)	97 (78.9%)	
	mean ± SD	0.0032 (1:310) ± 0.0010	0.0034 (1:290) ± 0.0013	0.0055 (1:180) ± 0.0011	0.0033 (1:300) ± 0.0012	0.5517
CSF protein > 100 mg/dL	Yes	43 (39.5%)	2 (20%)	4 (100%)	49 (39.8%)	0.3156
	No	66 (60.6%)	8 (80%)	0 (0%)	74 (60.2%)	
	mean ± SD	178.99 ± 297.4	141.7 ± 264.09	434.5 ± 283.14	184.27 ± 296.04	0.7027
$Glucose_{CSF}$: $Glucose_{Serum}$ < 1:2 (0.5)	Yes	23 (21.1%)	5 (50%)	4 (100%)	32 (26%)	0.0539
	No	86 (78.9%)	5 (50%)	0 (0%)	91 (74%)	
	mean ± SD	0.62 ± 0.18	0.48 ± 0.25	0.33 ± 0.16	0.59 ± 0.2	0.0298

Table 4 summarizes the crosstab analysis of CSF parameters with respect to ventriculitis status

Discussion

Our study sought to investigate the presence of any CSF, serum or clinical parameters that would help to predict ventriculitis before it occurs, thereby allowing for the determination of an optimal CSF surveillance schedule. Our study population consisted mostly of intubated and sedated ICU patients who presented a challenge for evaluation of clinical symptoms and signs of ventriculitis. In addition, evaluation of any new focal neurological symptoms or change in mental status in our patient population proved difficult, thus necessitating the use of additional parameters for detection of ventriculitis. In our study, the incidence of ventriculitis was determined to be 8.8% overall. This was found to be slightly lower than the established literature values[1-6,8,10]. The rates of ventriculitis have a wide range in the established literature, which is likely due to the variety of definitions used for ventriculitis along with different CSF collection techniques. The importance of being able to diagnose ventriculitis as early as possible is critical for the treatment of the infection as well as mitigating the morbidity and mortality associated with it.

In our patient population, Coagulase-negative staphylococci (including *Staphylococcus epidermidis*) was found to be the most common organism isolated from positive CSF cultures, occurring approximately 53% of the time. Additional organisms isolated included *Corynebacterium species*, *Methicillin-Resistant Staphylococcus aureus*, *Propionibacterium acnes*, *Enterobacter aerogenes*, and *Cryptococcus neoformans*.

Only one of our criteria for the diagnosis of ventriculitis, the ratio of glucose in the CSF:serum proved to be statistically significant when this ratio is less than 0.5 or 1:2 (p=0.0298). Approximately 21% of patients without ventriculitis had a glucose CSF:serum ratio <0.5, as compared to 50% of patients with confirmed ventriculitis. While statistically significant, this

was not found to be a reliable predictor of ventriculitis (p=0.0539). It was noted that patient who were diagnosed with ventriculitis were twice as likely to have a glucose CSF:serum ratio of less than half.

Upon further investigating additional criteria for suspected ventriculitis, the ratio of CSF WBC:RBC had inconsistent findings. As noted in Table 4, our patients with confirmed ventriculitis were over twice as likely to have a ratio above 1:250, when compared to patients without ventriculitis. Pfausler et al[23], reported an ability to diagnose ventriculitis up to three days prior to conventional diagnosis (positive CSF culture) by calculating the ratio of leucocytes to erythrocytes in CSF and leucocytes to erythrocytes in the peripheral blood, which they defined as the cell index (CI). However, Pfisterer et al[24], suggest that an upward trend in CSF leukocytosis should lead the physician to be suspicious of a contamination. As shown in Table 4, our study found a marginally significant association between the CSF WBC:RBC ratio and diagnosis of ventriculitis (p=0.0866).

While the levels of protein in the CSF have classically been a valuable indicator, care must be taken when looking at mean values for this parameter. As Table 4 indicates, our mean CSF protein value for all patients combined is 184.27 mg/dL with a standard deviation of 296.04. The mean protein value for all 3 categories is similarly high (all above 100mg/dL), with large standard deviations. This is a result of several outlying values, which have skewed the mean value for this parameter. Out of 123 patients combined, 12 patients had CSF protein levels of >500mg/dL and 5 of these were greater than 1000mg/dL, which causes a misleading elevation in our mean values for this parameter (as reflected in the large standard deviation). Although we feel most of these outliers were likely due to contamination of the CSF sample with tissue debris, we have shown the mean and standard deviation nonetheless for statistical completeness.

We acknowledge that this was a retrospective observational study, and with that comes certain limitations. The exact timing of temperature and of GCS documentation to match with one and two days prior to onset of ventriculitis was difficult to verify and sometimes unavailable. In addition, even though a standard sterile neurosurgical technique was mandated for CSF collection, human error is always a factor. We feel this may have been reflected in the outlying CSF protein values>500mg/dL. We also recognize that the patient population with ventriculitis was small and therefore limited our statistical analysis due to a small sample size. We propose a continuation of this study in a prospective manner that will allow for more meticulous documentation and collection of necessary data points. We aim to look at additional parameters at various intervals to see if they would be more reliable in predicting the onset of ventriculitis in patients with in-situ EVDs, thus allowing for the optimal timing of CSF collection in these patients.

Conclusion

We feel confident that this study demonstrates no reliable CSF, serum, or clinical parameters that can effectively predict the development of ventriculitis in an EVD patient. As shown in Table 1, we examined a variety of CSF parameters, including the presence of

organisms, protein, glucose, and ratios of WBC to RBC. We also included other factors such as the presence of organisms at other sites (non-CSF), temperature, a decline in GCS as well as WBC in the serum. These parameters were collected and studied for up to 2 days prior to the day of CSF collection. While the mean ratio of glucose in the CSF was shown to be statistically significant, at a value of less than 1:2, this parameter failed to show a statistical difference between ventriculitis vs. non-ventriculitis patients (p=0.0539).

In conclusion, we found that the various CSF, serum, and clinical variables that we studied were not reliable predictors for the development of ventriculitis. In the future, we plan on carrying this study forward in a prospective manner, to help us determine the optimal timing of CSF collection for patients with in-situ EVDs. However, at this time, we feel validated in our departmental protocol of CSF collection in monitoring of ventriculitis. Thus, we will continue to draw CSF samples on patients with in-situ EVDs on our previously established schedule of Monday/Wednesday/Friday for as long as the EVD remains in place.

Reference:

1. Holloway KL, et al. Ventriculostomy Infections: the Effect of Monitoring Duration and Catheter Exchange in 584 Patients. *J Neurosurg* 85. (1996): 419-426.
2. Bota, DP, et al. Ventriculostomy-Related Infections in Critically Ill Patients: A 6-year Experience. *J Neurosurg* 103. (2005): 468-472.
3. Williams TA, et al. Decrease in Proven Ventriculitis by Reducing the Frequency of Cerebrospinal Fluid Sampling from Extraventricular Drains. *J Neurosurg* 115. (2011): 1040-1046.
4. Muttaiyyah S, et al. Clinical Parameters Do Not Predict Infection in Patients with External Ventricular Drains: A Retrospective Observational Study of Daily Cerebrospinal Fluid Analysis. *Journal of Medical Microbiology* 57. (2008): 207-209.
5. Mikhaylov Y, et al. Efficacy of Antibiotic-Impregnated External Ventricular Drains in Reducing Ventriculostomy-Associated Infections. *Journal of Clinical Neuroscience* 21. (2014): 765-768.
6. Worley E, Astle S, Watson JC (August 25, 2015) Prospective Evaluation of Ventriculostomy Infections. Cureus 7(8): e312. DOI 10.7759/cureus.312
7. Mayhall CG, et al. Ventriculostomy-Related Infections. *NEJM* 310. (1984): 553-9.
8. Raman M, et al. A Meta-Analysis of Ventriculostomy-Associated Cerebrospinal Fluid Infections. *BMC Infectious Disease*. DOI 10.1186/s12879-014-0712-z
9. Mounier R, et al. (November 10, 2015) From the Skin to the Brain: Pathophysiology of Colonization and Infection of External Ventricular Drain, a Prospective Observational Study. PLOS ONE. DOI: 10.1371/journal.pone.0142320
10. Aucoin PJ, et al. Intracranial Pressure Monitors: Epidemiologic Study of Risk Factors and Infection. *Am J of Medicine* 80. (1986): 369-76.

11. Wright K, et al. Rates and Determinants of Ventriculostomy-Related Infections During a Hospital Transition to Use of Antibiotic-Coated External Ventricular Drains. *Neurosurg Focus* 34 (2013): 1-5.
12. McCarthy PJ, et al. International and Specialty Trends in the Use of Prophylactic Antibiotics to Prevent Infectious Complications After Insertion of Extraventricular Drainage Devices. *Neurocrit Care* 12. (2010): 220-224.
13. Lozier AP, et al. Ventriculostomy-Related Infections: A Critical Review of the Literature. *Neurosurgery* 51. (2002): 170-182.
14. Bogdahn U, et al. Continuous-Pressure Controlled, External Ventricular Drainage for Treatment of Acute Hydrocephalus – Evaluation of Risk Factors. *Neurosurgery* 31. (1992): 898-904.
15. Clark WC, et al. Complications of Intracranial Pressure Monitoring in Trauma Patients. *Neurosurgery* 25. (1989): 20-24.
16. Pfisterer W, et al. Early Diagnosis of External Ventricular Drainage Infection: Results of a Prospective Study. *J Neurol Neurosurg Psychiatry* 74. (2003): 929-932.
17. Roitberg BZ, et al. Bedside External Ventricular Drain Placement for the Treatment of Acute Hydrocephalus. *Br J Neurosurg* 15. (2001): 324-327.
18. Alleyne CH, et al. The efficacy and Cost of Prophylactic and Perioprocedural Antibiotics in Patients with External Ventricular Drains. *Neurosurgery* 47. (2000): 1124-1129.
19. Brown E, et al. The Management of Neurosurgical Patients with Postoperative Bacterial or Aseptic Meningitis or External Ventricular Drain-Associated Ventriculitis. *Br J Neurosurg* 14. (2000): 7-12.
20. Wong GK, et al. Failure of Regular External Ventricular Drain Exchange to Reduce Cerebrospinal Fluid Infection: Result of a Randomized Controlled Trial. *J Neurol Neurosurg Psychiatry* 73. (2002): 759-761.
21. Lyke KE, et al. Ventriculitis Complicating Use of Intraventricular Catheters in Adult Neurosurgical Patients. *Clin Infect Dis* 33. (2001): 2028-2033.
22. Zingale A, et al. Infections and Re-Infections in Long-term External Ventricular Drainage: A Variation Upon a Theme. *J Neurosurg Sci* 43. (1999): 125-133.
23. Pfausler B, et al. Cell Index - a New Parameter for the Early Diagnosis of Ventriculostomy (External Ventricular Drainage)-Related Ventriculitis in Patients with Intraventricular Hemorrhage. *Acta Neurochir (Wein)* 146. (2004): 477-481.
24. Pfisterer W, et al. Early Diagnosis of External Ventricular Drain Infection: Results of a Prospective Study. *J Neurol Neurosurg Psychiatry* 74. (2003): 929-932.

Markedly improved success rate of endoscopically assisted third ventriculostomy is achieved by routine placement of external lumbar drain

Watkins, Justen, D.O., Cabanne, Marc, D.O., Miulli, Dan, D.O., FACOS

Department of Neurosurgery, Arrowhead Regional Medical Center, Colton, California, USA

Key Words: endoscopically assisted third ventriculostomy, lumbar drain, hydrocephalus, ventriculoperitoneal shunt, ventricular shunt, ventricles, cerebrospinal fluid

Abbreviations: ETV, ELD, CSF, VPS, CLD, ETVSS, ICH, IVH

ABSTRACT

Hydrocephalus is a major cause of patient decreased quality of life and high health care financial burden in the US and throughout the world. The placement of ventricular shunts (VPS) has proven to be a safe treatment for hydrocephalus, but is associated with a high complication rate leading to a lower quality of life and continued financial burden for patients, their families, and society as a whole.

The endoscopically assisted third ventriculostomy (ETV) has been practiced as an alternative to ventricular shunting since the 1990's. Success rates vary widely and there are many factors which contribute to the varying success rates. The ETV procedure has the potential to alleviate much of the overall quality of life issues and some of the financial burdens associated with hydrocephalus provided success rates can be increased and the procedure and management techniques are adopted more widely. Common techniques have been published in the past which report associated improvements in success rates amongst individual surgeons.

Here we report a novel perioperative technique and management strategy that displays a higher than reported success rate. Our methods and results show potential to significantly improve overall ETV success rates if reproduced and subsequently adopted widely.

We retrospectively studied records of 22 adult patients (n=22) with hydrocephalus who were treated with an ETV procedure. Routinely, we placed an external lumbar drain (ELD) post-operatively which was continued for a minimum of two days. There was a 95.4% success rate at 30 days. The overall success rate was 81.8%. This is significantly higher than the average of the predicted success scores calculated by the ETVSS (71.8%). It is also significantly higher than previous studies' reported ETV success rates in adults.

We propose additional similar studies be performed to test reproducibility of increased success rates using our technique, ideally through a prospective, randomized, multi-center trial.

BACKGROUND

Hydrocephalus is a well described condition without medical treatment[15,18,28,33]. Debilitating and life threatening in its effects, this disease has been historically formidable for physicians to treat[5,9,28,30]. As modern Neurosurgery has matured over the last century many options for treatment have blossomed and thus millions of patients have benefited. As surgical procedures and implantation of permanent devices such as ventricular shunts have become mainstream treatment modalities since the mid-20th century[24], the overall monetary cost of this disease on societies has consequently increased[4,]. Ventricular shunting operations and revisions account for over a billion US dollars per year of medical expenditure in the United States alone[31]. Furthermore, patients living with hydrocephalus, and their families, continue to suffer decreased quality of life and financial burdens resulting from the frequent complications attributable to the inherent difficulties associated with this disease and the contemporary treatments[29,30,32].

Successful treatment of hydrocephalus by third ventriculostomy was first described in the 1920's[23]. Endoscopically assisted third ventriculostomy (ETV), developed in the 1990's utilizing modern technology to more safely perform the original technique, has become a commonly used treatment for many forms of hydrocephalus either as a primary treatment and alternative to ventricular shunting, or as a secondary treatment following shunt failure[14,18].

The achievement of increased ETV success rates has the potential to drastically improve patients' quality of life and reduce individual, familial, and societal financial burdens[4,29,31]. ETV failure has been described as the persistence or recurrence of clinical hydrocephalus which requires surgical treatment following an ETV procedure on a patient[5,17]. ETV success and failure has been extensively reported and ETV failure predictive factors with an ETV success scoring system for childhood hydrocephalus (ETVSS) have been proposed, reproduced, and widely adopted[6,13,14,17,22,27]. Differences in success rates varying across the age spectrum have been identified[8,10]. Case series along with associated success rates of ETV's are frequently published, and the rates vary widely (from 60.9 - 78.3% in our literature review)[1,3,6,10,12,13,16,19,22].

As was recently reported by Ozisik, et al., ETV success rates can be improved by augmentation of caudal cerebrospinal spinal fluid (CSF) flow through continuous lumbar drainage (CLD) facilitated with placement of an external lumbar drain (ELD)[2]. The rationale behind this practice is based on 2 hypothesis. First, continuous caudal CSF flow naturally promotes patency of the stoma created by an ETV through an inhibiting effect on regrowth of Liliequist's membrane. Second, in cases where increased protein in the CSF is a contributing factor to decreased absorption at the cortical subarachnoid space, drainage and removal of CSF would theoretically aid in clearance of and decrease in CSF protein, therefore increasing the likelihood of ETV success.

OBJECTIVE

We analyzed the practice of routinely placing an ELD in conjunction with ETV, which is in contrast to previously published practices which only selectively place an ELD when ETV is performed or after sign of failure[2,26]. Noting that ETV success rates have shown improvement with occasional, selective use of ELD, we hypothesized that routine ELD placement and CLD on all patients undergoing ETV would demonstrate even higher success rates in a significantly measurable way. The increased success rates would be in comparison to average predicted ETVSS and reported success rates of previously published standard ETV case series.

METHODS

PRE-REVIEW:

This was a retrospective, multi-surgeon, single-institution study to evaluate the effectiveness of routine ELD placement and CLD following ETV procedure on patients with hydrocephalus. Our primary hypothesis was that routine ELD placement on patients undergoing ETV procedure would lead to an increased ETV success rate. The study was approved by the institution's Internal Review Board (IRB). All applicable laws (HIPPPA and state and local laws) and regulations were strictly observed during the study. It was performed with volunteer efforts alone; no financial or tangible material compensation was involved.

INCLUSION CRITERIA:

Inclusion criteria was any patient with hydrocephalus who underwent an ETV procedure with routine ELD over the previous six (6) years (2009 - 2015). All patients received an ELD in conjunction with the performance of the ETV, and the ELD was placed in the operating room immediately following the ETV procedure. There was no pre-screening or selection criteria for placement of the drain.

ENDOSCOPICALLY ASSISTED THIRD VENTRICULOSTOMY:

All patients were treated and managed at the sponsoring institution. Informed consent was obtained from each patient and next-of-kin prior to every procedure. The ETV procedures were performed under similar conditions, i.e. inpatient scheduled surgery performed in the operating theater, under general anesthesia, with sterile procedures and equipment. In all cases, a RIGHT sided approach was used and a small burr hole was made with a port placed through the meninges and brain parenchyma to access the ventricular space. Then a video scope was inserted and manipulated by the surgeon. The third ventricle was identified, entered, and a stoma was created in the floor of the third ventricle anterior to the maxillary bodies.

LUMBAR DRAIN:

All patient's received an ELD as a routine placement immediately following ETV procedure and prior to exiting the operating room. The ELD's were kept in place for a minimum of 48 hours post ETV, and were discontinued solely at the discretion of the surgeon. Basis for discontinuation were a combination of indicators including the patient's neurologic examination, CSF protein levels, and head computer tomography imaging studies. Neither laboratory nor imaging studies were routinely obtained for determination of discontinuation of ELD.

The lumbar drain was uniformly managed on all patients immediately following placement. All patients were admitted to intensive care units following surgery. The ELD was kept open and the level was titrated to achieve a drainage goal of 10-15 mL per hour in a continuous fashion (i.e. not an intermittent, hourly drainage). After 48 hours, the ELD could be discontinued if the patient was neurologically stable. In most cases, the ELD was removed after the 48 hour period. A minority of patients received continued drainage for an extended period due to persistence or development of new neurologic symptoms. In all cases, the ELD was successfully weaned over several days.

ETV FAILURE:

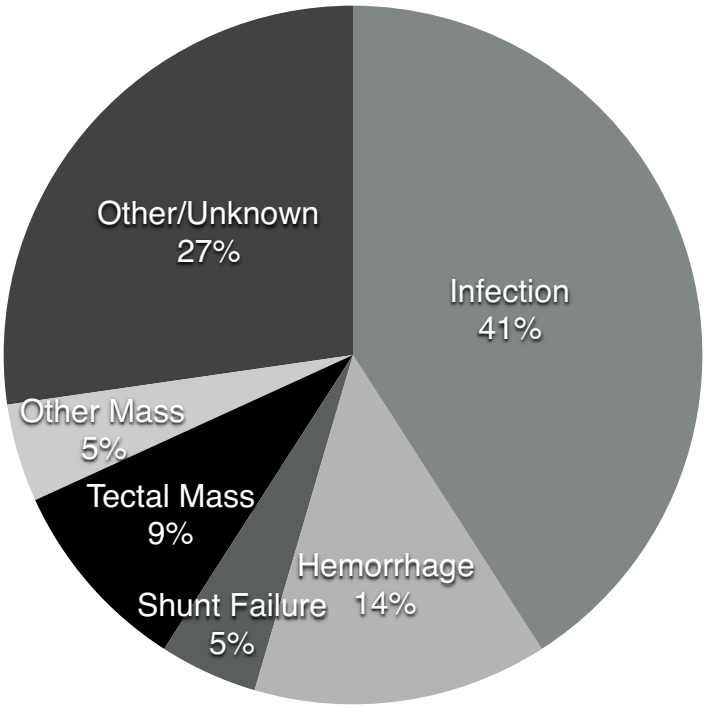
ETV failure for this study was defined as persistence or return of Hydrocephalus which required intervention with a repeat ETV or placement of a permanent ventricular shunt. Additionally, we analyzed ETV failure on a stratified basis. ETV failure was identified as either *EARLY* (<30 days) or *LATE*(<30 days) failures. We measured the days to ETV failure as being days after initial ETV when a repeated intervention was performed (EVD, repeat ETV, or VPS).

DATA COLLECTION AND ANALYSIS:

A database search was conducted using the Neurosurgery census from January 2009 and ending in November 2015. All medical records from patients during that time period who underwent an ETV were reviewed. Reviews focused on each patient's history of present illness, diagnostic information and assessments, treatments rendered, and response to treatments, including failures and need for re-treatment. All patients meeting inclusion criteria were included in the study. The data which was collected included age, sex, diagnosis and etiology (including pathology, cytology, microbiology, and laboratory studies), ETVSS, verification of performance of ETV and ELD, time of ELD drainage post-op, final CSF protein level taken just prior to removal of the drain, and any invasive procedure(s) performed to treat hydrocephalus following ETV (excluding initial ELD).

Characteristics of patients in the failure group were analyzed included etiology. The ETVSS for the failure group and the success group was analyzed and the respective median value for each was reported, along with the overall mean ETVSS for all patients.

Chart 1 - Etiology of Hydrocephalus



The time to ETV failure was calculated and recorded for each ETV failure patient. The final protein levels were analyzed for the failure group and reported as a mean value. The EARLY and LATE failure rates were analyzed separately and overall failure rate was calculated as a percentage. The overall failure rate was compared with previously reported failure rates.

RESULTS

A total of 25 adult patients underwent ETV's during the time period reviewed. Age range includes 18 - 66 years. There were a total of seven (7) females (32%) and 15 males (68%). Three (3) patients (aged 18, 38, and 60 years) were excluded from the study due to ELD not being placed immediately post-op. The study included a total of 22 patients (n=22) with ages ranging from 23 - 66 years. The mean age was 46 years. Etiologies of Hydrocephalus (see Chart 1) requiring ETV were varied and included post-infectious (9 = 40.9%), ICH/IVH (3 = 13.6%), ventricular shunt failure (1 = 4.5%), non-tectal mass (2 = 9.1%), tectal mass (1 = 4.5%), other/unknown cause (6 = 27.3%). The calculated ETVSS for all patients in the study ranged from 60 - 80%, with a mean of 71.8%. The failure group's median ETVSS was 60%. The success group's median ETVSS was 80%. The number of ELD days was also recorded and ranged from 2 to 28 days, with a median ELD days of 2.5.

Amongst the 22 patients in our study group, there were a total of 18 successes which did not need subsequent surgical treatment for hydrocephalus. Additionally there were a total of four (4) ETV failures (see Table 1). The most common etiology of hydrocephalus corresponding to a failed ETV was infection, specifically, neurocysticercosis (responsible for 3 failures). There was one lone failure with a separate etiology of intraventricular hemorrhage (IVH). Amongst the failures group, the final CSF protein levels taken just prior to failure ranged from 33 - 293 grams per deciliter (gm/dl). The mean final CSF protein level amongst the failure group was 63 gm/dl. The days to failure were recorded as ranging from 28 - 94 days following ETV. The median days to ETV failure was 35.5 days. Only one (1) patient had an EARLY failure (within 30 days). This corresponds with a 95.4% success rate at 30 days. The days to failure were 28 for the single case of EARLY failure. Additionally, there were a total of three (3) LATE failures (after 30 days), with the days to failure amongst the LATE failures ranging from 32 - 94 days. The total ETV failures compared to overall ETV cases corresponds to an overall ETV success rate of 81.8% (18 of 22).

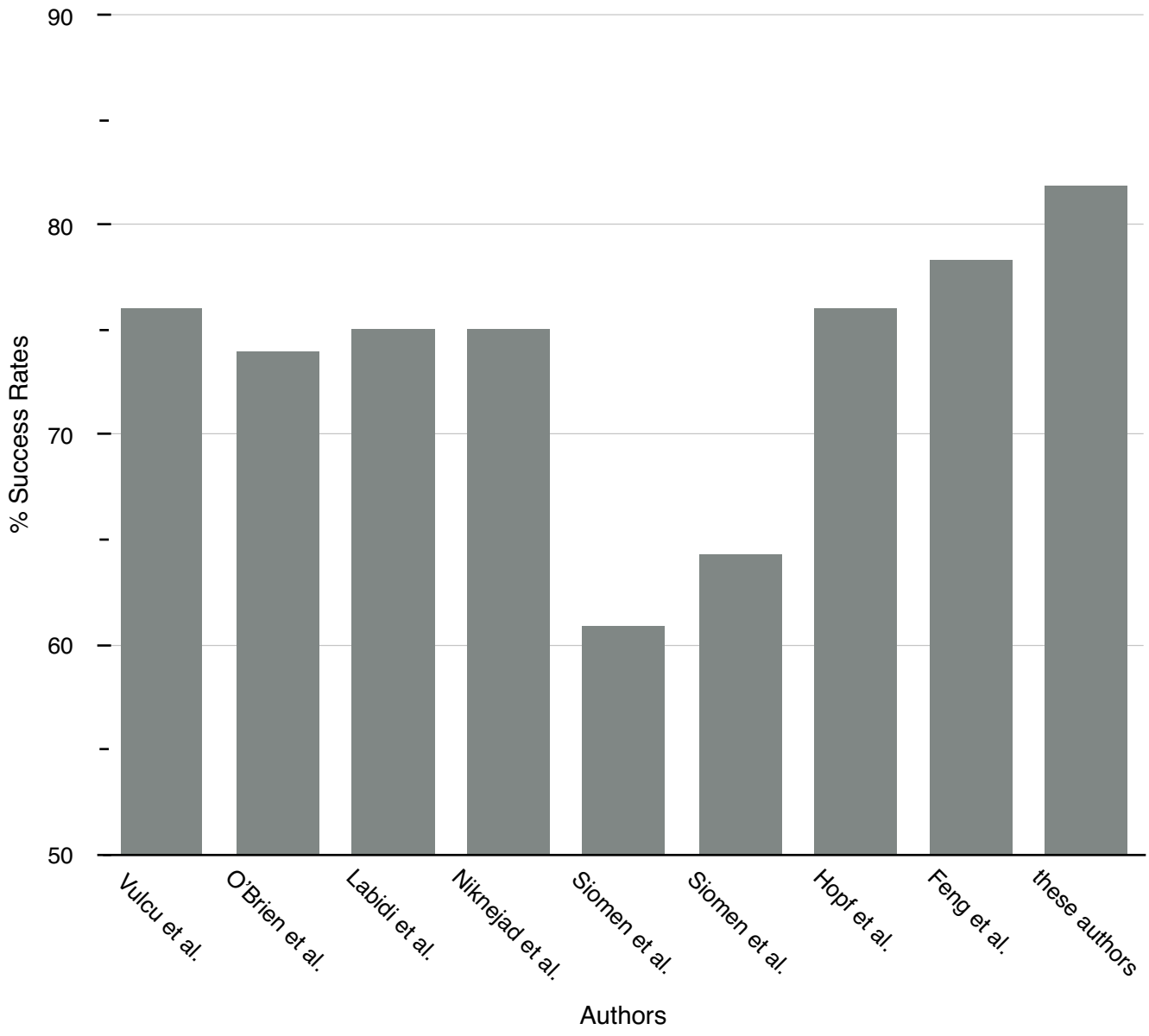
DISCUSSION

Previously reported adult patient primary ETV case series' success rates[1,3,6,8,11,12,16] vary from 60.9 - 78.3% (see Chart 2). The mean reported success rate from these papers is 72%. The most commonly reported finding in multiple studies is that ETV's performed for tumors and anatomical obstructive hydrocephalus correlate with higher success rates than when performed for hemorrhages and infections[11,12]. Our study reports a series with 54.5% of the cases being caused by either infection or hemorrhage (see Chart 1). Taken into account the etiology, following the standard technique, our success rate would likely be substantially smaller than has been previously reported.

Table 1 - Series of patients who received ETV's with routine ELD placement and results

Patient #	Age	Sex	ELD placed in OR	ETV failure?	Days until failure	Days of ELD	Cause of HCP	Final CSF protein
1	25	M	y	n		2	cerebral coccidiodomycoses	293
2	66	F	y	n		14	ICH no IVH	63
3	61	F	y	y	32	6	IVH	unknown
4	36	M	y	n		28	cysticercosis	101
5	54	F	y	n		4	cysticercosis	unknown
6	37	M	y	n		2	unknown	unknown
7	43	F	y	n		3	cysticercosis	unknown
8	30	M	y	n		2	unknown	41
9	46	M	y	n		2	unknown	37
10	29	M	y	y	39	2	cysticercosis	51
11	23	F	y	n		2	unknown	109
12	59	F	y	n		2	aneurysmal SAH	50
13	56	M	y	y	94	4	cysticercosis	104
14	66	F	y	n		2	unknown	211
15	35	M	y	y	28	2	cysticercosis	33
16	60	M	y	n		4	malignant melanoma	128
17	55	M	y	n		2	neurosyphlis	128
18	55	M	y	n		4	enraptured AVM	128
19	52	M	y	n		3	benign tectal mass	<4
20	24	M	y	n		5	VPS failure	98
21	53	M	y	n		2	malignant melanoma	unknown
22	46	M	y	n		3	cryptococcal meningitis	187

Chart 2 - Comparison of overall ETV success rates between published case series



Author	Success Rate
Vulcu et al.[1]	76
O'Brien et al.[3]	74
Labidi et al.[6]	75
Niknejad et al.[8]	75

Siomen et al. A[11]	60.9
Siomen et al. A[11]	64.3
Hopf et al.[12]	76
Feng et al.[16]	78.3
these authors	81.8

The method of routine ELD placement with CLD following ETV described in this paper is unique, as none of the previously published papers reporting ETV case series described similar methods. Only one (1) paper by Ozisik, et al.[2], studied the selective use of ELD's following ETV, and some of the ELD's were placed at varying times post-operatively. The paper did report an overall decrease in ETV failure through select use of ELD. Interestingly, their overall ETV success rate was equal to ours (81.8%).

Further comparison of results between these two studies yielded additional information. They reported that their results indicated a majority of failures occur within 30 days post-op (EARLY failures). In analysis of the failure group in our study, the majority of failures (75%) occurred LATE (after 30 days). While not immediately provable, this would suggest that our methods have the effect of markedly *reducing* EARLY failures (EARLY success rate of 95.4%).

LIMITATIONS

This study has several limitations. First, it is a retrospective review. A future study would ideally be performed in a prospective manner. Additionally, as all of the patients received a routine ELD post-operatively, there was no internal control group and the study was not randomized. Subsequent studies would be benefited by providing randomization to serve as a control mechanism. Another limitation identified was that although there were multiple primary surgeons performing the operations and overseeing the ELD management, the records that were reviewed were from a single institution. An improvement for this limitation would include a multi-center analysis. Further limitations include relatively small study size, unrecorded variations in decision criteria for discontinuing the ELD, disproportionately lower number of females, and the absence of pediatric patients included in the study.

CONCLUSIONS

Avoidance of ventricular shunt placement in patients with hydrocephalus would lead to improved quality of life and decreased financial burden on patients, their families as well as society. Innovations in ETV techniques and perioperative management strategies that increase success rates have the potential to drastically improve these measures.

Here, we have described a technique and perioperative management strategy that is shown to significantly increase the success rate of ETV procedures on patients. We propose that additional studies be performed following this protocol and reporting of the results. A prospective, randomized, multi-center trial could feasibly be conducted. Ultimately, if the results are reproduced with significant numbers, wider adoption of this technique could potentially translate into substantial improvement of patient, familial, and societal burdens.

REFERENCES

1. Sonja Vulcu, MD, Leonie Eickele, MD, Giuseppe Cinalli, MD, Wolfgang Wagner, MD, and Joachim Oertel, MD. Long-term results of endoscopic third ventriculostomy: an outcome analysis. J Neurosurg. Published online July 31, 2015; DOI:10.3171/2014.11.JNS14414.

2. Ozisik P, Roth J, Beni-Adani L, Constantini S. Continuous spinal drain following endoscopic third ventriculostomy: a proposal to change the definition of failure. Child's Nervous System. 2011 Nov;27(11):1973-8.

3. O'Brien DF, Javadpour M, Collins DR, Spennato P, Mallucci CL. Endoscopic third ventriculostomy: an outcome analysis of primary cases and procedures performed after ventriculoperitoneal shunt malfunction. J Neurosurg. 2005 Nov;103(5 Suppl):393-400.

4. Garton, Hugh J.L. M.D., M.H.Sc.; Kestle, John R.W. M.D., M.Sc.; Cochrane, D. Douglas M.D.; Steinbok, Paul M.B., B.Sc. A Cost-effectiveness Analysis of Endoscopic Third Ventriculostomy. Neurosurgery. 2002 Jul;51(1):69-78.

5. Fabiano, Andrew J. MD; Doyle, Kristina; Grand, Walter MD. Delayed Stoma Failure in Adult Communicating Hydrocephalus After Initial Successful Treatment by Endoscopic Third Ventriculostomy: Case Report. Neurosurgery. 2010 Jun;66(6):e1210-11.

6. Moujahed Labidi, MD, Pascale Lavoie, MD, MSc, FRCSC, Geneviève Lapointe, MD, FRCSC, Sami Obaid, MD, Alexander G. Weil, MD, FRCSC, Michel W. Bojanowski, MD, FRCSC, and André Turmel, MD, MSc, CSPQ. Predicting success of endoscopic third ventriculostomy: validation of the ETV Success Score in a mixed population of adult and pediatric patients. J Neurosurg. Published online July 24, 2015; DOI: 10.3171/2014.12.JNS141240.

7. Xu R, McCrea HJ, Hoffman CE, Souweidane MM, Greenfield JP. The Impact of Endoscopic Third Ventriculostomy on Shunt Revision Rate: A 14-Year Experience at a Single Institution. World Neurosurgery. 2015 Sep;84(3):677-680.e1.

8. Niknejad HR, Depreitere B, De Vleeschouwer S, Van Calenbergh F, Van Loon J. Results of endoscopic third ventriculostomy in elderly patients ≥65 years of age. Clinical Neurology and Neurosurgery. 2015 Mar;130:48-54.

9. Bouras T, Sgouros S. Complications of endoscopic third ventriculostomy. World Neurosurgery. 2013 Feb;79(2 Suppl):S22.e9-12.

10. Beems T, Grotenhuis JA. Is the success rate of endoscopic third ventriculostomy age-dependent? An analysis of the results of endoscopic third ventriculostomy in young children. Child's Nervous System. 2002 Nov;18(11):605-8.

11. Siomin V, Cinalli G, Grotenhuis A, Golash A, Oi S, Kothbauer K, Weiner H, Roth J, Beni-Adani L, Pierre-Kahn A, Takahashi Y, Mallucci C, Abbott R, Wisoff J, Constantini S. Endoscopic third ventriculostomy in patients with cerebrospinal fluid infection and/or hemorrhage. J Neurosurg. 2002 Sep;97(3):519-24.

12. Hopf NJ, Grunert P, Fries G, Resch KD, Perneczky A. Endoscopic third ventriculostomy: outcome analysis of 100 consecutive procedures. Neurosurgery. 1999 Apr;44(4):795-804; discussion 804-6.

13. Brockmeyer D, Abtin K, Carey L, Walker ML. Endoscopic third ventriculostomy: an outcome analysis. Pediatric Neurosurgery. 1998 May;28(5):236-40.

14. Jenkinson, Michael D., Ph.D., Caroline Hayhurst, F.R.C.S.(SN), Mohammed Al-Jumaily, M.R.C.S., Jothy Kandasamy, M.R.C.S, Simon Clark, Ph.D., Conor l. Mallucci, F.R.C.S.(SN). The role of endoscopic third ventriculostomy in adult patients with hydrocephalus. J Neurosurg. 2009 May;110(5):861-66.

15. Harold L. Rekate, M.D., Trimurti D. Nadkarni, M.Ch., and Donna Wallace, R.N., M.S., C.P.N.P. The importance of the cortical subarachnoid space in understanding hydrocephalus. J Neurosurg, Collections. 2012 May;116(5):1-11.

16. Feng H, Huang G, Liao X, Fu K, Tan H, Pu H, Cheng Y, Liu W, Zhao D. Endoscopic third ventriculostomy in the management of obstructive hydrocephalus: an outcome analysis. J Neurosurg. 2004 Apr;100(4):626-33.

17. García LG, López BR, Botella GI, Páez MD, da Rosa SP, Rius F, Sánchez MA. Endoscopic Third Ventriculostomy Success Score (ETVSS) predicting success in a series of 50 pediatric patients. Are the outcomes of our patients predictable? Childs Nerv Syst. 2012 Aug;28(8): 1157-62.

18. Timothy W. Vogel, M.D., Biji Bahuleyan, M.D., Shenandoah Robinson, M.D., and Alan R. Cohen, M.D. The role of endoscopic third ventriculostomy in the treatment of hydrocephalus. Journal of Neurosurgery: Pediatrics. 2013 Jul;12(1):54-61.

19. Appelgren T, Zetterstrand S, Elfversson J, Nilsson D. Long-term outcome after treatment of hydrocephalus in children. Pediatr Neurosurg. 2010;46(3):221-6.

20. Abhaya V. Kulkarni, PhD, James M. Drake, FRCSC, Conor L. Mallucci, FRCS(SN), Spyros Sgouros, FRCS(SN), Jonathan Roth, MD, Shlomi Constantini, MD, MSc. Endoscopic Third Ventriculostomy in the Treatment of Childhood Hydrocephalus. The Journal of Pediatrics. 2009 Aug;155(2):254-59e2.

21. Armen Melikian, Anton Korshunov. Endoscopic Third Ventriculostomy in Patients with Malfunctioning CSF-Shunt. World Neurosurgery. 2010 Oct-Nov;74(4-5):532-37.

22. Concezio Di Rocco, Paolo Frassanito, Luca Massimi, Gianpiero Tamburrini. Prediction of Outcome of Endoscopic Third Ventriculostomy. World Neurosurgery. 2013 Nov;80(5):509-11.

23. Mixter WJ: Ventriculoscopy and puncture of the floor of the third ventricle. Boston Med Surg J. 1923;188:277–78.

24. Nulsen FE, Spitz EB. Treatment of hydrocephalus by direct shunt from ventricle to jugular vain. Surg Forum. 1951;399–403

25. Cinalli G, Spennato P, Ruggiero C, Aliberti F, Zerah M, Trischitta V, Cianciulli E, Maggi G. Intracranial pressure monitoring and lumbar puncture after endoscopic third ventriculostomy in children. Neurosurgery. 2006 Jan;58(1):126-36; discussion 126-36.

26. Chowdhry SA, Cohen AR. Intraventricular neuroendoscopy: complication avoidance and management. World Neurosurgery. 2013 Feb;79(2 Suppl):S15.e1-10.

27. Kulkarni AV, Drake JM, Kestle JRW, Mallucci CL, Sgouros S, Constantini S Predicting who will benefit from endoscopic third ventriculostomy compared with shunt insertion in childhood hydrocephlalus using the ETV Success Score. J Neurosurg Pediatr. 2010;6:310–315.

28. Rahme R1, Bojanowski MW. Internal hydrocephalus, external hydrocephalus, and the syndrome of intracerebral cerebrospinal fluid entrapment: a challenge to current theories on the pathophysiology of communicating hydrocephalus. Med Hypotheses. 2010 Jan;74(1): 95-8.

29. Chevis N. Shannon, M.B.A., Dr.P.H., Tamara D. Simon, M.D., M.S.P.H., Gavin T. Reed, M.P.H., Frank A. Franklin, M.D., Ph.D., Russell S. Kirby, M.S., Ph.D., Meredith L. Kilgore, Ph.D., and John C. Wellons, III, M.D. The economic impact of ventriculoperitoneal shunt failure. J Neurosurg Pediatr. 2011 Dec; 8(6): 593–599.

30. Korinek, Anne-Marie MD, MSc; Fulla-Oller, Laurence MD, MSc; Boch, Anne-Laure MD, PhD; Golmard, Jean-Louis MD, PhD; Hadiji, Bassem MD; Puybasset, Louis MD, PhD. Morbidity of Ventricular Cerebrospinal Fluid Shunt Surgery in Adults: An 8-Year Study. Neurosurgery. 2011 Apr;68(4):985-995.

31. Patwardhan, Ravish V. M.D.; Nanda, Anil M.D. Implanted Ventricular Shunts in the United States: The Billion-dollar-a-year Cost of Hydrocephalus Treatment. Neurosurgery. 2005 Jan; 56(1):139-145.

32. Kulkarni AV1, Shams I. Quality of life in children with hydrocephalus: results from the Hospital for Sick Children, Toronto. J Neurosurg. 2007 Nov;107(5 Suppl):358-64.

33. Dandy, Walter E., M.D.; Blackfan, Kenneth D., M.D. An experimental and clinical study of internal hydrocephalus. JAMA. 1913;61(25):2216-2217.

Proper Education on Spinal Orthotics, A Way to Minimize Associated Complications – A multi-center study at three trauma hospitals

Christopher Elia DO[1], Katie Huynh DO[1], Fanglong Dong, PhD[2], Dan Miulli DO[1]
1: Riverside University Health System – Medical Center Moreno Valley, CA
2: Western University of Health Sciences, 309 E 2nd St, Pomona, CA, 91709

Corresponding Author: Christopher Elia,

Key Words:
Orthotic brace, pressure ulcer, trauma patient, cervical collar, bracing

Journal:
Journal of trauma, Spine, Journal of orthopedic and spine trauma, Jorunal of trauma nursing, Orthopedic Nursing

Abstract

Introduction
Methods
Results: A total of 237 nurses completed the presentation. The biggest improvement in the ability to correctly identify braces is TLSO, increased from 77.2% to 97.9% ($p<0.0001$), followed by the ability to identify LSO increased from 80.2% to 100% ($p< 0.0001$), and CTO increased from 87.3% to 99.6% ($p=0.0005$). Regarding the clinical knowledge, the right answer to the question *whether or not halo vest needed to be removed for CPR* increased from 45.6% to 98.3% ($p<0.0001$), and the right answer to the question *whether or not TLSO brace need to be worn at all times in patients with spinal precautions* increased from 64.6% to 96.2% ($p<0.0001$). Nurse reported their comfort level of taking care of patients with spinal precautions increased from 90.7% to 100% before and after the presentation.

Introduction

Spinal orthotic bracing is a common modality in which we treat non-operative spinal fractures as well as operative fractures in which the patient/family requests non-surgical options. Despite being a non-surgical option of treatment, it is not without associated risks. Moreover, the risks increase when ancillary staff as well as novel resident physicians are not educated on proper use of spinal orthotics. There have been many studies on the efficacy of different bracing systems but special consideration needs to be accounted for in certain population such as pediatrics, the elderly and the morbidly obese (1).

Complications associated with cervical-thoracic orthotics (CTO) have been reported causing stage III pressure ulcers on the chin within 6 days of application, which also correlates with literature review on craniofacial pressure associated with all types of CTOs. Moreover, complications are higher in September through December (2). Common misunderstandings, such as placing patients with Minerva CTO braces with cervical hyperextension, can lead to dysphagia, aspiration and even respiratory arrest and death (3).

Cervical collar and HALO immobilization carry increased risk of developing pressure ulcers and require close monitoring to prevent such events [7]. Length of time in cervical immobilization has been associated with increased incidence of skin breakdown and pressure ulcer formation [8,9]. A previous study found that the most

common cause of skin breakdown trauma patients was positional pressure with cervical collars being the second leading cause of skin breakdown [10].
Misuse of other orthotics such as lumbosacral orthotic (LSO) braces and thoracolumbosacral orthotic (TLSO) braces can lead to decubitus ulcer formation when the brace is not properly applied or removed when indicated. HALO vests, although useful in treatment of unstable cervical spine fractures may also be associated with severe complications including, but not limited to respiratory arrest when improperly applied and managed (4). Complications associated with spinal orthotics are associated with the intrinsic nature of the devices as well as iatrogenic. The objectives planned are to minimize these complications by properly educating treatment teams involved in the care of these patients and to identify common misunderstandings associated with spinal orthotics.

Methods

A retrospective chart review on spinal trauma patients who suffered complications associated with spinal orthotics was conducted to identify common complications and morbidities associated with spinal orthotic use. It was found that there were common misunderstandings regarding proper use of spinal orthotics that lead to complications such as decubitus ulcer formation, dysphagia, dyspnea, aspiration, and in extreme cases, cardiopulmonary arrest.
This information was compiled and a corrective/action plan was surmised with the goal being to prevent further spinal orthotic-associated complications. Data was compiled into a presentation and provided 239 critical-care nurses at three level-II trauma centers with a brief in-service spinal orthotic course as well as pre-test and post-test assessments. Questions involved in the assessment were kept simple to identify common misunderstandings regarding spinal orthotics. Pictures of commonly used orthotics were displayed in a multiple choice matching format. Other questions were kept relatively simple with true/false answer choices. Participants were identified according to numbers to elicit more honest responses to the questions. After the post-test a short discussion was held and feedback was obtained. Common misconceptions regarding spinal orthotic bracing were identified. Participants were then shown spinal orthotics and instructed on their proper utilization. A review of pressure points was conducted, emphasizing the need to assess all pressure points each shift. Some of the high yield points included over-extensions in CTO causing dysphagia/aspirations and showing staff how to adjust a halo vest to allow for CPR without removing the vest.

Results

A total of 237 critical-care nurses from three level-II trauma centers were assessed in our pre-test and post-test assessment. The first component of the quiz is to correctly differentiate braces. Figure 1 presented the analysis results. All (100%) nurses correctly cervical collar and halo vest before and after the brief in-service spinal orthotic presentation. The biggest improvement in the ability to correctly identify braces is TLSO, increased from 77.2% to 97.9% ($p<0.0001$), before and after the presentation, respectively. The ability to correctly identify LSO increased from 80.2% to 100% ($p< 0.0001$), before and after the presentation, respectively. Lastly, the ability to correctly identify CTO increased from 87.3% to 99.6% ($p=0.0005$), before and after the presentation, respectively.
Nurses were also queries about their clinical knowledge of managing patients. The analysis results were presented in Figure 2. Two most significant improvement were detected. The first improvement is regarding whether or not halo vest needed to be removed for CPR. Before the presentation, 45.6% of nurses answered this question correctly. This is in contrast to the 98.3% of right answers after the presentation ($p<0.0001$). The second significant improvement is regarding whether or not TLSO brace need to be worn at all times in patients with spinal precautions. The right answer percentage increased from 64.6% to 96.2% ($p<0.0001$) before and

after the presentation, respectively. Additionally, the percentage of cervical collar could cause pressure ulcers increased from 99.2% to 100%. The percentage of right answers to the question *"cervical spine need to be stabilized when transferring/rolling a patient if they already have a cervical collar on"* increased from 96.6% to 100%.

Nurses were also queried about their comfort level of taking care of patients with spinal precautions. Before the presentation, 90.7% (215 of 237) feel comfortable taking care of patients with spinal precautions. This percentage increased to 100% after the presentation.

Discussion

Spinal orthotics are commonly used in spine trauma patients and serve as a useful modality in the conservative management of this patient population. Despite the benefits of spinal orthotics they must be used judiciously, especially in the elderly population to prevent associated complications.

The above results are concerning and suggest that there may be a void in properly educating trauma nursing staff regarding proper use of spinal orthotics. With proper nursing education the associated complications may be minimized. Although there is mention of respiratory arrest in literature in patients with spinal orthotics, such as CTO braces, this raises the question as to the affects of iatrogenic-causes of complications.

Halo fixation appears to also have much associated confusion amongst trauma ancillary staff. Halo fixation intrinsically leads to a decrease in pulmonary vital capacity of 10-30% leading to restricted pulmonary function (5). Although daily pin-site care is required to prevent pin site infection, other complications such as scapular pressure sores have been reported in elderly patients with relatively kyphotic thoracic spines (6). Therefore, it is critical that the treatment team be properly educated on proper use of halo fixation to minimize iatrogenic contribution of complication rates. Proper use of fitting and how to perform CPR on this patient population should also be incorporated into routine critical-care nursing education. We believe that incorporating these measures into practice will lead to lower complications associated with spinal orthotics.

Conclusion

In this assessment of 239 critical care nurses at three level-two trauma centers the authors discovered that there are many misunderstandings regarding fundamentals of proper utilization of spinal orthotics. The rate of co-morbidities associated with spinal orthotics can be minimized by properly educating our nursing staff on proper use of spinal orthotics. Orthotic technicians should be integrated into the teaching of patients, ancillary staff, and patients' families regarding proper orthotic use and fitting. Incorporating the poly-trauma team into spinal orthotic education may also minimize associated complications.

Designating a nurse educator to provide in-service educational teachings on proper use of spinal orthotics (bi)annually may also help prevent associated complications. Moreover, having an annual spine orthoses didactic lecture for trauma, neurosurgery, and orthopedic spine surgery may help lower associated complications of spinal orthotics.

References

1. Alberts LR, Mahoney CR, & Neff JR. Comparison of the Nebraska collar a new prototype cervical immobilization collar, with three standard models. Journal of Orthopaedic Trauma, 1998;12(6):425-430

2. Plaisier B, Gabram SGA, Schwartz RJ, & Jacobs LM. Prospective evaluation of craniofacial pressure in four different cervical orthoses. Journal of Trauma, 1994:37(5): 714-720

3. Damadi AA, Saxe AW, Fath JJ, & Apelgren KN. Cervical spine fractures in patients 65 years or older: a 3-year experience at a level 1 trauma center. Journal of Trauma, 2008;65:745-748

4. Lewallen RP, Morrey BF, & Cabanela ME. Respiratory arrest following posteriorly displaced odontoid fractures: case reports and a review of the literature. Clinical Orthopaedics and Related Research, 1984;188:187-190
5. Lind B, Bake B, Lundqvist C, Nordwall A: Influence of halo vest treatment on vital capacity. Spine 12:449–452, 1987
6. Glaser JA. Complications associated with the halo-vest: A review of 245 cases. J Neurosurg 65:762-769, 1986
7. Walker J: Pressure ulcers in cervical spine immobilization: a retrospective analysis. J Wound Care 21(7):323-326, 2012
8. Powers J, Daniels D, McGuire C, Hilbish C: The incidence of skin breakdown associated with the use of cervical collars. J Trauma Nurs 13(4):198-200, 2006
9. Webber-Jones JE, Thomas CA, Bordeaux RE Jr: The management and prevention of rigid cervical collar complications. Orthop Nurs 21(4):19-25, 2002
10. Watts D, Abrahams E, MacMillan C, Sanat J, Silver R, VanGorder S, Waller M, York D: Insult after injury: pressure ulcers in trauma patients. Orthop Nurs 17(4), 1998

Figure 1: Percentage of Nurses who identify the braces correctly

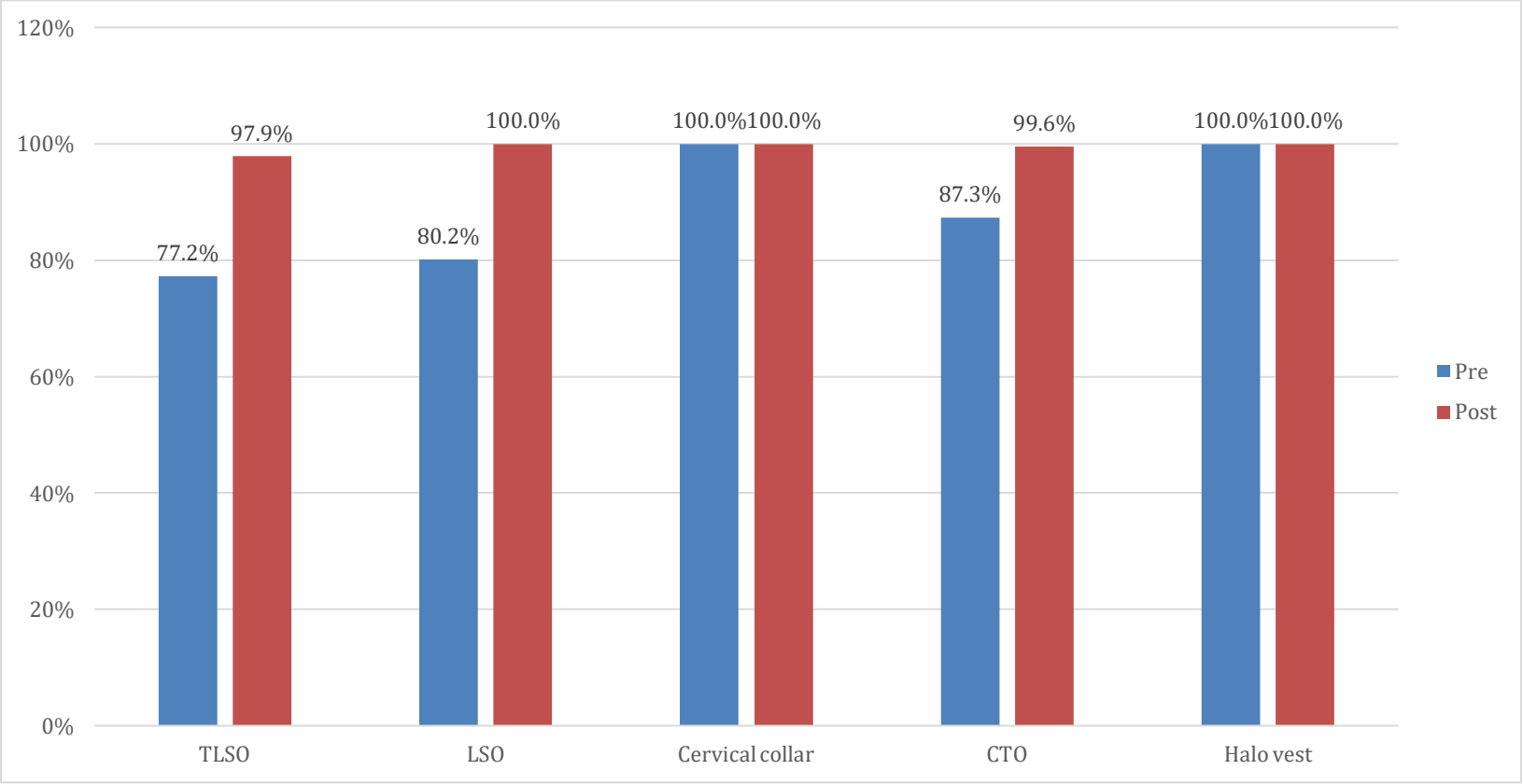
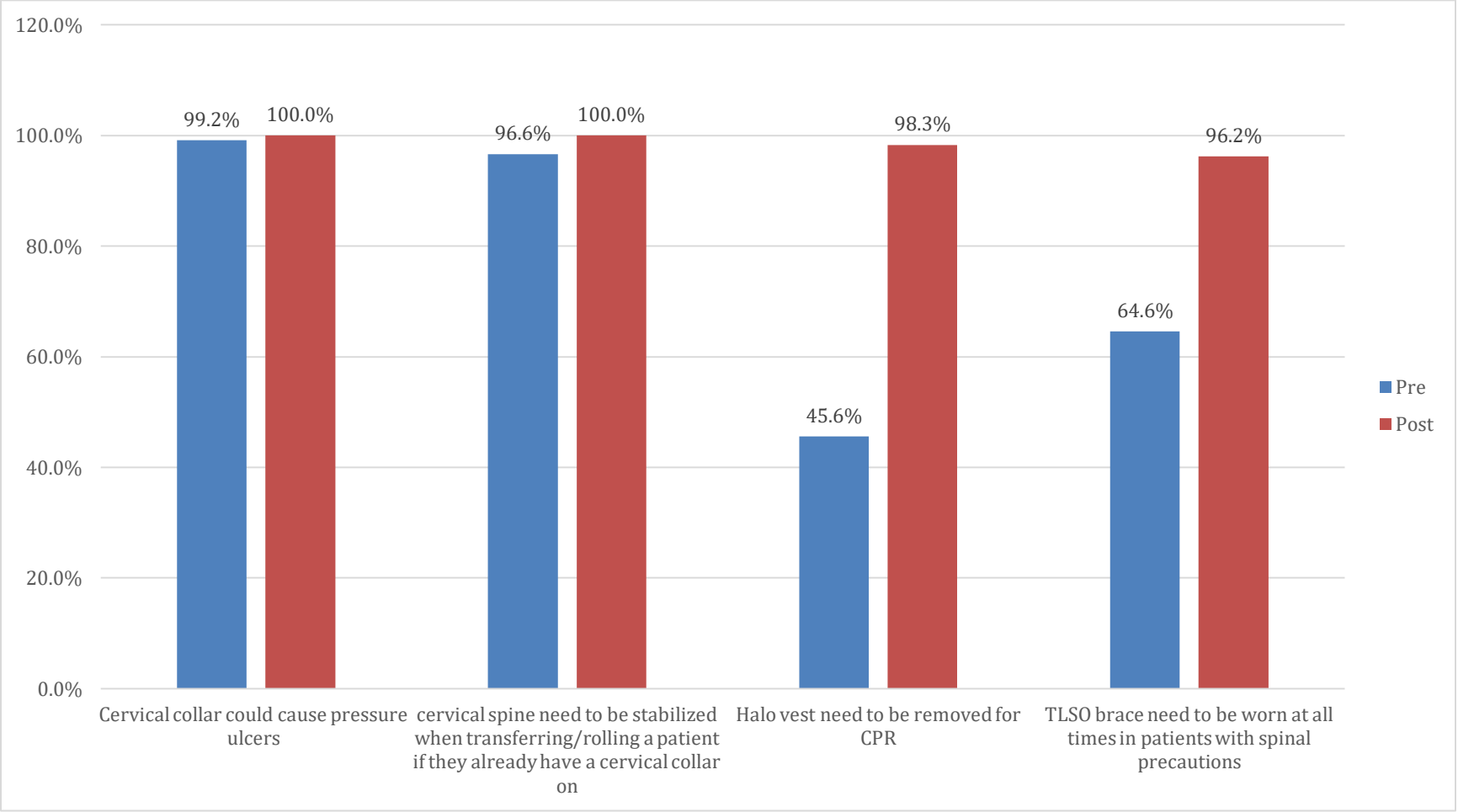


Figure 2: Percentage of nurses who answered the questions correctly



Appendix A: Pre and Post-quiz

Pre-test quiz

Q1-5: Matching: Match the following images with the associated type of brace

A. Thoracic-lumbar-sacral orthotic (TLSO) brace
B. Lumbar-sacral orthotic (LSO) brace
C. Cervical collar
D. Cervical-thoracic orthotic (CTO)
E. Halo vest

Q6. Do you feel comfortable taking care of patients with spinal precautions? (Y/N)

Q7. Does a Halo vest need to be removed for CPR? Y/N

Q8. Does a TLSO brace need to be worn at all times in patients with spinal precautions? (Y/N)

Q9. Does the cervical spine need to be stabilized when transferring/rolling a patient if they already have a cervical collar on? (Y/N)

Q10. Cervical collar could cause pressure ulcers of:

A. chin
B. occiput
C. clavicle area
D. all of the above

Post Test Quiz

Q1-5: Matching: Match the following images with the associated type of brace

A. Thoracic-lumbar-sacral orthotic (TLSO) brace
B. Lumbar-sacral orthotic (LSO) brace
C. Cervical collar
D. Cervical-thoracic orthotic (CTO)
E. Halo vest

Q6. Do you feel **MORE** comfortable taking care of patients with spinal precautions? (Y/N)

Q7. Does a Halo vest need to be removed for CPR? Y/N

Q8. Does a TLSO brace need to be worn at all times in patients with spinal precautions? (Y/N)

Q9. Does the cervical spine need to be stabilized when transferring/rolling a patient if they already have a cervical collar on? (Y/N)

Q10. Cervical collar could cause pressure ulcers of:

A. chin
B. occiput
C. clavicle area
D. all of the above

Q11: What can be done to minimize pressure ulcers for cervical collars?

A. Make sure the patient's neck is hyper-extended
B. Examine the skin around the collar and bony prominences every shift
C. Make sure the collar is loosely fitting
D. Taking the collar off when transferring the patient
E. B and C are both correct

Q12: LSO/TLSO brace must be worn at all times, even when laying flat in bed? (T/F)

Q13: Improper fitting CTO brace can lead to:

A. Respiratory distress
B. Difficulty swallowing
C. Pressure ulcers
D. All of the above

Appendix B: Figures for quiz question 1 through 5.

Immediate Label-Free Evaluation of Human Brain Tumor Biopsies with Confocal Reflectance Microscopy

Joseph Georges, DO, PhD[2,7*], Jennifer M. Eschbacher, MD[1*], Aqib H. Zehri, MD[1*], Mohammadhassan Izady Yazdanabadi, BS[3], Nikolay L. Martirosyan, MD, PhD[2], Emily Szeto, BS[4], Catherine Y. Seiler, PhD[5], Michael A. Mooney, MD[2], Katherine Y. Goehring, HT[1], Kendall R. Van Keuren-Jensen, PhD[6], Mark C. Preul, MD[2], Stephen W. Coons, MD[1], Shwetal Mehta, PhD[4], Peter Nakaji, MD[2]

Departments of [1]Neuropathology and [2]Neurosurgery
Barrow Neurological Institute
St. Joseph's Hospital and Medical Center
Phoenix, Arizona

[3]School of Life Sciences
Arizona State University
Tempe, Arizona

[4]Department of Neurobiology
Barrow Brain Tumor Research Center
Barrow Neurological Institute
St. Joseph's Hospital and Medical Center
Phoenix, Arizona

[5]The Biobank Core
Barrow Neurological Institute
St. Joseph's Hospital and Medical Center
Phoenix, Arizona

[6]Division of Neurogenomics
Translational Genomics Institute
Phoenix, Arizona

[7]Department of Neurosurgery
Philadelphia College of Osteopathic Medicine
Philadelphia, Pennsylvania

*These authors contributed equally to this work.

ABSTRACT

Background: Frozen sections are often utilized for intraoperative diagnostics. However, frozen section diagnosis can introduce artifacts into tissue biopsies and limit molecular analysis. Without microscopic confirmation of tissue adequacy, missampling can compromise patient care by resulting in inadequate material for diagnosis and molecular studies. A reliable simple-to-use method for intraoperative tissue evaluation that does not compromise specimen integrity would be valuable.

Objective: Investigate confocal reflectance microscopy (CRM) as a novel imaging modality for the quick and reliable screening of central nervous system (CNS) tissue.

Methods: We prospectively evaluated neoplastic and non-neoplastic CNS lesions with CRM to determine cellularity, architecture, and morphological characteristics in rodent xenograft tissue and 65 patients undergoing biopsy between February 2014 and November 2015. All sample images were reviewed by a neuropathologist and compared to hematoxylin-and-eosin–stained sections using either the frozen section slide made directly from the tissue or the matched clinical frozen section slide. Architectural patterns of tumor growth, cellularity, and surrounding normal cellular structures were routinely visualized in neoplastic and non-neoplastic lesions.

Results: CRM routinely contrasted architectural patterns of tumor growth, cellularity and surrounding normal cellular structures in neoplastic and non-neoplastic lesions. The observed histopathological features seen in CRM images were consistent with the tissue diagnosis and matched the hematoxylin-and-eosin–stained section. In addition, RNA isolated from the frozen tissue after CRM imaging retained high RNA integrity, suggesting that CRM does not alter tissue properties for molecular studies.

Conclusion: Our data indicate that CRM is a useful tool for rapidly screening patient biopsies for diagnostic adequacy, molecular studies, and biobanking.

INTRODUCTION

Over 150,000 Americans were diagnosed with a CNS tumor in 2015 [1-3]. For many of these patients, treatment required surgical resection and medical therapy. Biopsies taken during surgery are often rapidly processed to provide preliminary diagnoses for guiding surgical course. Intraoperative biopsies are also obtained to determine adequacy of tissue for diagnosis in non-resectable lesions. The diagnosis is then rendered following fixation and processing of often limited tissue. With the advent of personalized medicine, patient tissue is increasingly biopsied for patient molecular studies and determination of appropriate targeted pharmacotherapies. Harvesting tissue biopsies representative of a patient's tumor is critical for proper surgical and medical management. Current approaches such as stereotactic biopsy can mis-sample tumor, leading to procurement of nonrepresentative tissue and inaccurate diagnoses. (1, 2) In some cases, tissue is collected for storage in a biorepository for future retrieval for clinical research. Surgeon sampling error can result in the collection and distribution of nonrepresenative tissue, propagating downstream costly scientific error in clinical studies.

Frozen section is the clinical standard intraoperative histopathological technique for tissue assessment. However, frozen section diagnoses are time-consuming and can decrease quality and quantity of tissue for evaluation. Frozen section preparation requires embedding tissue in optimal cutting temperature compound (OCT), followed by freezing and sectioning with a specialized instrument (cryostat) before a slide is generated for staining. Following frozen section analysis, remaining tissue is typically fixed and processed for evaluation and diagnosis. However, sectioning of tissue for frozen section analysis can

leave little residual tissue for permanent diagnostic evaluation and performance of specials stains, which are both often required for a specific final diagnosis. Freezing of tissue introduces ice crystals, an especially common problem with edematous brain and spinal cord tissue, which can compromise histopathologic evaluation, and negatively influence post-hoc immunohistochemical stain quality. A technique which rapidly screens tissue biopsies without tissue loss from sectioning or introduction of frozen section artifacts could improve the diagnostic yield of tissue samples and increase the likelihood of collecting representative samples.

Confocal microscopy is an optical imaging modality which allows visualization of tissue at differing depths through optical sectioning. This technique provides images of thin sections of tissue without sectioning by spatially filtering visualized photons from outside the focal plane. Confocal microscopy coupled with fluorescent contrast agents is becoming increasingly utilized as a clinical tool in gastrointestinal medicine, dermatology, gynecology, and ophthalmology (3-9). This imaging modality provides real-time information of cellular morphology and tissue structures.

Confocal reflectance microscopy (CRM) is a label-free confocal imaging modality which generates high resolution molecular images without fluorescent dyes or high energy light sources required for traditional confocal fluorescence imaging. In place of fluorescent dyes, CRM generates contrast by collecting back-scattered photons after they have interacted with cells and tissues. We hypothesize CRM will provide a safe and rapid means for screening brain tumor biopsies appropriate for biobanking and generate sufficient contrast the differentiate specific human brain tumors. In this study we first utilize CRM to assess tissue cellularity from rodent glioma models, then evaluate alterations to the molecular integrity of tissue imaged with CRM. Next, we test CRM on clinical samples with a pathology-based CRM system. Our data illustrate CRM's potential both for screening clinical biopsies prior to biobanking and for generating rapid intraoperative diagnoses. Our goal is to determine CRM's efficacy as a screening technique that will improve the quality of tissues collected and biobanked for brain tumor patients and evaluate the effectiveness of CRM for rapidly identifying histopathological features from fresh human brain tumor biopsies.

METHODS

Rodent xenografts

Nude rats were acquired from Charles River Laboratories. Rats (n=5) were anesthetized by intramuscular injection of a mixture of 10 mg/kg xylazine and 80 mg/kg ketamine (Wyeth, Madison, NJ) and placed in a small animal stereotactic headframe (Model 900, David Kopf Instruments, Tujunga, CA). A 10-mm incision was made starting between the animal's eyes to expose bregma. A bur hole was made 3.5 mm lateral to bregma. Human glioma cells (U251; ATCC) were infused at a depth of 4.5 mm below the surface of the brain after the syringe (Hamilton) was advanced 5.0 mm to create a 0.5-mm pocket. The cell suspension was infused using a UMP3-1 UltraMicroPump microinjector (WPI, Sarasota, FL) set to a volume of 10 μL with an infusion rate of 3.00 μL/minute. The needle was withdrawn 2 minutes after the injection to minimize backflow of the cell suspension. The bur hole was covered with bone wax, the skin incision was sutured, and the rats were allowed to recover.

Rodent tissue

Twenty-eight days after implantation, rodent xenografts were deeply anesthetized using xylazine and ketamine (previously described). They were immediately decapitated, and their brains were removed. Immediately, coronal slices (350 μm thick) were cut from the cerebrum on a Leica VT1200 vibratome and placed in artificial cerebrospinal fluid (aCSF) containing the following (in mM): 126 NaCl, 26 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$ and 10 glucose, pH 7.4. Slices were then fixed in 4% paraformaldehyde at 4 degrees Celsius overnight and washed three times with phosphate buffered saline (PBS). Three tumor-containing slices per animal were incubated with DAPI for 45 minutes at room temperature, rinsed three times with PBS, and placed into number 1.5 glass bottom dishes (MatTek) for imaging. A coefficient of determination analysis was used to compare cells identified with CRM to cells labeled with DAPI.

Rodents utilized for molecular experiments (n=2) were deeply anesthetized using xylazine and ketamine and rapidly decapitated. Whole brains were placed in ice cold aCSF and sectioned into 1mm coronal sections using a rodent brain block. The cerebrum from each section was blocked into 4 equivalent sections. Two sections were immediately frozen in liquid nitrogen (LN2) for reference. At 15, 30, 45, 60, 90, and 120minutes two sections were placed into glass bottom dishes. At each time point, the cortex, corpus callosum, and caudate/putamen from one section was imaged with CRM. As a control, one section was simultaneously placed on the stage of the microscope but not imaged. Each section was frozen in LN2 and stored at -80° C for assessment of DNA, RNA, and protein.

Xenograft imaging

All samples were imaged in uncoated No.1.5 glass-bottom dishes (MatTek corp). CRM was conducted with a Zeiss invereted 710 laser scanning confocal microscope equipped with a 40x/1.2NA water emersion objective. For reflectance imaging, a 633-nm diode laser was raster scanned across the sample and reflected photons were collected by tuning the emission filters to allow photons with the same wavelength of the incident laser passage to the photomultiplier tube. For DAPI imaging, samples were excited with a 405-nm diode laser and 430-490nm emission collected. The confocal aperture was set to one Airy unit for all imaging. The laser and gain values were set to fill the dynamic range of the photomultiplier tube, and the frame size was set to sample at nyquist. Images were collected in 12-bit format with linear processing.

DNA Isolation & Analysis

DNA was isolated from brain tissue using the QIAamp DNA Mini (Qiagen), per manufacter's instructions. DNA was quantified using the Nanodrop Spectrophotometer (Thermo Scientific). Samples were loaded in equal concentrations (100 ng) in a 1% agarose gel with ethidium bromide and imaged on an Alpha Imager (Alpha Imager).

RNA Isolation and Analysis

Tissue was homogenized in 500 ul Ambion's Cell Disruption Buffer (Life Technologies) and subsequently isolated using Ambion's *mir*Vana kit (Life Technologies), per manufacturer's instructions. RNA concentrations were determined using the Nanodrop Spectrophotometer (Thermo Scientific) and provided information for the dilutions necessary to remain within the dynamic range of the Bioanalyzer. The integrity of the RNA (RNA integrity number; RIN)was assessed using Agilent 2100 Bioanalyzer

Nanochips (Agilent Technologies). RIN of 1 suggests strong degradation while RIN greater than 8 suggest minimal degradation.

Western Blot Analysis

Frozen tissue was sectioned on dry ice and protein lysate was made by placing brain sections into 750 ul Ambion's Cell Disruption Buffer (Life Technologies); triturated using RNase-free pipettes; and sonicated using Covaris Sonolab at 2 X 5% for 5 seconds; 2 X 20% for 15 seconds; 2 X 20% for 15 seconds; 2 X 5% for 5 seconds (Covaris Inc).

Protein concentrations were quantified by BCA (Pierce, Thermo Scientific) and 18 ug/lane was loaded in 4-12% NuPage Bis-Tris gels (Invitrogen) and run using NuPage electrophoresis reagents (Invitrogen). Protein was transferred onto Novex nitrocellulose membrane (Invitrogen) and thereafter incubated for an hour in blocking solution consisting of 5% bovine serum albumin (Sigma Aldrich) in tris-buffered saline with 0.1% Tween (Thermo Fisher Scientific). Primary antibodies were incubated for 12 hours at 4 C while secondary HRP-conjugated antibodies were incubated for 1 hour at room temperature. Blots were probed for AKT (1:1000; Cell Signaling; Cat#: 9272) and GAPDH (1:60,000; Millipore; Cat#: AB2302). Horseradish peroxidase (HRP)-conjugated secondary antibodies were Anti-Rabbit (1:2000; Cell Signaling; Cat#: 7074**)** and Anti-Chicken (Millipore; Cat#:12-341).

Blots were developed by using Pierce SuperSignal Chemiluminescent Substrate (Thermo Fisher Scientific) per manufacturer's instructions. Protein signal was detected on film (General Electric).

Clinical samples

Sixty-five patients who underwent surgical resection of suspicious masses or lesions involving the CNS between Febuary, 2014 and November, 2015 at Barrow Neurological Institute of St. Joseph's Hosptial and Medical Center were recruited for this study. Patient's ages ranged from 23 years to 82 years. This study has been reviewed and approved by an Institutional Review Board (IRB) at St. Joeseph's Hosptial and Medical Center (**IRB#10BN130**). Confocal imaging was performed in the Department of Pathology at the time of biopsy.

We utilized CRM to histopathologically interrogate fresh brain tumor biopsies. Sampled neoplasms included: pituitary adenomas, gliomas, schwannomas, meningiomas, giant cell tumor, treatment effect, metastatic tumors, reactive non-neoplastic processes, and central neurocytoma (see Table 1). Fresh human biopsies were collected in the operative theatre and transported to pathology. All 58 tissue specimens were received fresh and subsequently imaged with CRM. Following imaging, 35 cases were embedded in OCT, and a frozen section slide of the imaged side was prepared utilizing a cryostat and hematoxylin and eosin stained slides. The remainder of samples (23) were divided from the clinical frozen section tissue, imaged and snap frozen in liquid nitrogen.

Images were collected by a histotechnologist (KG) with confocal microscopy training and a resident physician with a background in molecular imaging (JG). Images were collected at a remote time period and independent from histopathological analysis by the neuropathologist.

All sample images were reviewed by a neuropathologist and compared to hematoxylin and eosin stained sections. Images were either compared with the frozen section slide made directly from the tissue or

with the matched clinical frozen section slide. Time for collection of images for all specimens was less than 5minutes.

Clinical biopsy imaging

Biopsies were placed in uncoated No.1.5 glass-bottom dishes (MatTek corp) for imaging. No tissue processing or staining was performed prior to CRM imaging. All CRM imaging was performed with a Zeiss inverted 710 laser scanning confocal microscope. Images were acquired with a 20x/0.8NA air objective and a 40x/1.2NA water emersion objective. Reflectance images were acquired by raster-scanning the sample with a 633-nm diode laser and collecting reflected photons of the same incidence wavelength. The confocal aperture was set to one Airy unit for all imaging. The laser and gain values were set to fill the dynamic range of the photomultiplier tube and frame size was set to sample at Nyquist. Images were collected in 8-bit format. Simulated low-magnification images were generated by utilizing Zeiss' tiling function which generates a large volume image by stitching together multiple highly resolved and magnified images. Following CRM, biopsies were marked and processed for standard histopathological assessment with H&E staining. Reflectance images were assessed for pathoneumonic features and compared to their corresponding H&E images. All image processing was performed utilizing linear functions within NIH ImageJ.

Clinical biopsy molecular integrity

To evaluate whether CRM affected RNA integrity from clinical samples, 20 cases were randomly selected to evaluate the impact that CRM may have on RNA integrity. Of these samples, eight tissue samples were immediately frozen after imaging in liquid nitrogen for future RNA analysis. These tissue samples included pituitary adenoma (1), schwannoma (2), meningioma (1), glioblastoma (2), and low grade astrocytoma (2). 12 samples were frozen in OCT following imaging, and a slide was prepared from the imaged surface. OCT was cut away from the frozen tissue block, and the sample was stored in liquid nitrogen for future RIN anayalsis. These 12 cases included schwannoma (2), meningioma (5), ependymoma (2), pituitary gland (1), pituitary adenoma (1). Specimens embedded in OCT prior to imaging included frozen tissue were homogenized using liquid nitrogen cooled Mini Mortar (Bel-Art™ Sceinceware). Total RNA was isolated using the PureLink® RNA mini kit (ThermoFisher Scientific). Briefly, lysis buffer was added to homogenized tissue and lysed by repeated tituration. The lysate is centrifuged at ~2,600 x g for 5 minutes at room temperature. One volume of 70% ethanol is added, vortexed and passed through the Spin Cartridge to bind RNA to the column. Bound RNA is washed with Wash Buffer I and II and RNA eluted in RNAase-free water in a fresh RNAase-free collection tube. RNA integrity was analyzed on TapeStation (Agilent Technologies) using the High Sensitivity D1000 screen tape.

Statistical analysis

Coefficient of correlation (R^2 value) was determined between DAPI stained nuclei and nuclei detected by CRM using Graphpad Prizm. Differences were considered statistically significant for probability of less than .05. The Agilent 2100 Bioanalyzer provided an RNA integrity number (RIN) calculated algorithmically by including the 28s/18s ribosomal RNA bands, the region before the peaks, signal areas, and intensities. An elevated threshold baseline and a decreased 28s:18s ratio are both indicative of RNA degradation while high 28S and 18S ribosomal RNA peaks as well as a small amount of 5s RNA or a RNA number of greater than 7.5 are indicative of intact RNA. Results are expressed as the means and mean

square error (SEM) data with normal distribution compared by one-way analysis of variance and student's t-test.

RESULTS

Rodent tissue

Reflectance imaging differentiates neoplastic cellular tumor from acellular tissue.

To investigate the potential of reflectance imaging for differentiating cellular tumor biopsies from acellular biopsies, we imaged acute slices generated from rodents intracranially implanted with human glioma cells. We incubated slices with DAPI to label all cell nuclei, then sequentially imaged the slices with CRM and LSCM (n=15 slices from 5 animals). We collected five images per acute slice and compared cells identified with CRM to cells identified with DAPI. We found CRM provided definitive contrast between cell nuclei, cytoplasm, and extracellular tissue. CRM adequately contrasted normal brain cytoarchitecture, such as cell bodies, axons and blood vessels (Fig 1). Within tumor regions, CRM provided contrast to visualize hypercellular regions and relatively acellular regions with isolated cell populations (Fig2. A-C & E-G). In hypercellular and acellular regions we found CRM contrast correlated with r^2=0.97 and r^2=.098; respectively, to cells labeled with DAPI (Fig1. D&H).

Reflectance imaging does not alter molecular characteristics of rodent biopsies

To determine if CRM alters the molecular characteristics of interrogated tissue, we examined DNA, RNA, and protein from tissue imaged with CRM to tissue immediately frozen for analysis and compared that with tissue that had reflectance imaging and delayed freezing time. Although the typical time from surgical resection to reception in pathology and assessment using CRM typically takes 15 minutes, we tested out to 180 minutes. Neoplastic tissues are heterzyogous in terms of cellularity and gene and protein expression and may yield inter-specimen molecular variability. Therefore, we conducted these experiments on control tissue harvested from rodent normal brain. DNA quality was assessed in CRM imaged samples, which showed no difference compared to immediately frozen controls. Discrete DNA bands were detectable up to 180 minutes after extraction (Fig. 2B) suggesting no degradation of DNA elements. RNA integrity number (RIN) was generated to determine the integrity of isolated RNA. RIN number was comparably the same between control and CRM imaged groups (Figure 2C), indicating no effect of CRM on RNA integrity of imaged samples. RNA integrity remained relatively the same up to 120 minutes post-biopsy. There was a slight decrease over time in RIN value that was similar for both the control and CRM tissue, most likely due to RNases within the tissue over time. Protein kinase B (AKT), a protein involved in GBM pathogenesis, was examined for potential damage post-imaging with CRM. Western blot analysis of extracted tissue showed that up to120 minutes post-extraction, discrete AKT bands were detectable and contained similar density to control samples (Fig. 2E).

Clinical Samples

Histologic features

Pituitary

Eight pituitary adenomas and 3 normal pituitary glands were evaluated. Pituitary adenomas demonstrated sheets of round to oval cells with prominent nuclei and a moderate amount of brightly reflective cytoplasm. Pituitary adenomas subtypes included 6 gonadotroph adenomas, one adrenocorticotroph adenoma and one prolactinoma. Three biopsies from normal pituitary glands demonstrated small lobules of pituitary cells. Normal pituitary cells appeared smaller with less pleomorphic nuclei on CRM than neoplastic pituitary epithelial cells. (Figures 4&5).

Gliomas

15 gliomas were imaged, including -1 well diffententiated astrocytoma, 1 protoplasmic astrocytoma, 1 anaplastic oligodendroglioma, 1 anaplastic oligoastrocytoma, 6 glioblastomas, 3 ependymoma and 1 subependymoma. Except for the subependymoma, all gliomas demonstrated visible cell bodies on CRM. Nuclei, however, were often difficult to distinguish. Astrocytomas showed atypical cells, some of which exhibited abundant and prominent cytoplasmic processes (Figure 6). Possible focal necrosis was identified on one glioblastoma, similar to the matched HE section, characterized by noncellular regions which lacked reflectance. Glioblastomas showed enlarged cell bodies, often with apparent brightly reflective processes (Figures 6&7). Microvascular proliferation was not identified on HE or CRM images. One oligodendroglioma showed an infiltrative architectural growth pattern. Tumor cell nuclei appeared enlarged, round , and pleomorphic as seen on the corresponding HE image (Figure 8). One subependymoma exhibited prominent cystic spaces, similar to microcysts identified on the matched HE section. Fibrillary stroma was especially prominent and showed increased reflectance (Figure 9). One ependymoma showed sheets of cellular tumor. Perivascular pseudo rosettes were not seen (compare to HE), although they were identified on the matched HE slide.

Schwannomas

Four schwannomas were identified, which exhibited reflectance in a fasicular pattern consistent with interlacing neoplastic spindle cells (Figure 10). The tumor tissue in all four cases appeared reflective. Tumor nuclei were difficulat to visualize in all four schwannomas. In two cases, scattered small cells showed brightly reflective cytoplasm suggestive of interspersed macrophages. Cavernous areas lacking reflectance were interspersed throughout the tumor, consistent with tumor vasculature.

Meningiomas

Sixteen meningiomas were visualized, including two atypical meningiomas (WHO Grade II). All sixteen meningiomas showed architectural features on CRM consistent with a diagnosis of meningioma, including lobular and fibrous growth patterns (Figure 11). Whorls were often identified. Refractile collagen was seen in most tumors. Eight of sixteen (50%) tumors showed at least focally visible tumor nuclei, which appeared bright on CRM. When visible, tumor cellularity appeared similar to matched HE sections. Round non reflective structures suggestive of psammoma bodies were occasionally seen, similar to the corresponding HE images. Two atypical meningiomas demonstrated prominent cells with evident nuclei. The atypical tumors appeared to demonstrate a more diffuse growth pattern than lower grade meningiomas. Other atypical features identified on HE sections were not visualized on CRM (hypercellularity, small cell foci).

Metastasis.

Metastatic tumors from breast, tonsil and lung were evaluated. All three tumors showed evident cell bodies with visible nuceli. One metastatic breast tumor showed lobules of atypical cells (Figure 12) with prominent nuclei. One metastatic lung adenocarcinoma showed sheets of atypical cells. One metastatic squamous cell carcinoma from the tonsil showed sheets of atypical cells with visible nuclei.

Treatment Effect

Three treated glioblastoma showed sheets of fibrillary reflective material consistent with necrosis and treatment effect (Figure 13). No tumor cells were identified on CRM or matched HE slides. One metastatic colon tumor did not show identifiable cellularity consistent with treatment effect observed on HE images.

TOTAL IMAGES

A total of 304 images were collected for all tumors and non-neoplastic lesions, consisting of 221 CRM images of regions thought to be representative of tumor and 83 tile images. On retrospective analysis by the neuropathologist, 211 of 221 images (95.5%) and 81 of 83 tiled images (98%) demonstrated architectural or cytological features characteristic of the diagnosis, for a total of 292 reprentative images of 304 total image (96%).

RNA INEGRITY

Tissue samples frozen immediately following CRM showed preservation of RNA integrity within ranges >7 (range: 6.8-8.8; See Table2). All tissue samples embedded in OCT prior to snap freezing showed RIN values <7 (range: not detected – 6.8).

DISCUSSION

Confocal reflectance microscopy is a safe and rapid technique for assessing cellularity of fresh tissue biopsies. This imaging modality can be immediately utilized on fresh tissue samples without application of exogenous contrast agents, and without altering the molecular characteristics of examined tissues. CRM can provide a much needed tool for neurosurgery-neuropathology teams by maximizing the quality of tissue samples collected during surgical resection. Here, we show the utility of this imaging modality for interrogating histopathology of fresh human CNS tumor and nonneoplastic biopsies. In this study, CRM immediately generated images from fresh tissue without sectioning or staining.

Label free images acquired from CRM provided valuable real-time histopathological information from tissue specimens. All specimens exhibited architectural characteristics consistent with the tumor diagnosis. All imaged samples exhibited features which corresponded with the hematoxylin and eosin

stained slides which were identifiable as lesional or neoplastic tissue. Surrounding normal structures could be ascertained, including vasculature and fibrous tissue. CRM contrasted noncellular tissue such as gliotic brain and necrosis, which appeared similar to HE slides. Normal pituitary gland exhibited small lobules of cells, similar to pituitary gland histology, while adenomas showed sheets of cells, with loss of normal lobularity.

Patterns of tumor growth and nuclear atypia were often visible. All cases of pituitary adenoma, central neurocytoma, paraganglioma, metastatic carcinoma and glioma demonstrated visible tumor nuclei. While half of meningiomas showed visible tumor nuclei, none of the schwannomas demonstrated visible nuclei, a feature which may be helpful in the diagnosis of schwannoma with CRM. Although tumor cells and nuclei were not visible in all specimens, tumor architecture characteristic of the tumor diagnosis was identifiable in all cases. Schwannomas demonstrated fibrous and fasicular architecture, while meningiomas frequently demonstrated fibrous or lobuluar phenotypes.

We found CRM did not alter the DNA, RNA, or protein that could be extracted and quantified from biopsies screened up to 2 hours after resection. Tissue samples frozen following CRM showed preservation of RNA integrity within ranges (>7) reported to be acceptable for gene array studies, with most values >8, considered acceptable for all molecular studies (11), suggesting that CRM does not alter RIN values; however larger sample sizes are needed to validate these results.

Mis-sampling of tissue intraoperatively can lead to under-grading of tumors, one of the most commonly reported sources of error in neuropathology frozen section analysis (12-15). Secondary to difficulty in distinguishing edematous brain and necrotic tissue from tumor, intraoperative consult with the pathologist is often requested by the surgeon to determine the diagnostic usefulness of biopsied tissue. The frozen section is a clinical standard for generating intraoperative diagnoses. It has secondary utility for screening tissue samples from biorepositories prior to distribution. Though useful, the frozen section protocol is time-consuming, generates tissue artifacts from freezing, and loses valuable tissue with sectioning. CRM, in contrast can generate images from fresh biopsies within seconds without freezing, sectioning, staining or further processing. Therefore, CRM does not produce the freezing artifacts common with frozen sections, and can generate diagnostic images for immediate feedback as to adequacy of tissue biopsy. CRM does not involve embedding specimens in a medium, such as OCT (Optimal cutting temperature compound), which is necessary when embedding specimens for frozen sections. CRM also does not subject tissue to fluorescent dyes. Furthermore, we found CRM was capable of producing useful images from sub-millimeter sized tissue samples; samples too small for frozen section processing. Compared to current clinical standards, CRM provided equally useful information in a shorter time frame.

Sampling error in the selection of tissue can also have detrimental effects on tissue collected for research studies, biobanking, and clinical trials. Patient tissue is increasingly being selected intraoperatively or fresh in the pathology gross room for molecular studies, which can affect treatment course and provide predictive and prognostic value. The misrepresentation of nonneoplastic tissue as tumor (or vice versa) can have costly and potentially disastrous effects on study results and patient treatment. In our study, CRM did not alter RNA integrity, and images correlated with the HE slides. These results suggest that CRM can serve as a useful tool to rapidly screen tissue samples for adequacy for research studies, patient molecular studies, clinical trials and biobanking.

In our study, CRM images were collected by a histotechnologist and a resident physician trained to use the confocal microscope. Ninety-six percent of images collected demonstrated histopathological features consistent with the diagnosis. One advantage of CRM image acquisition is the relative ease and rapidity by which images can be captured and stored. Personnel without formal clinical pathology training were able to select areas of tissue to image which corresponded with the tissue diagnosis. In the few cases where images were nonrepresenative of the tissue, other representative images were collected. In addition, all cases had tiled images stored for analysis.

The ability to digitally store images has implications for clinical diagnosis and biobanking. Images can be rapidly captured by trained operating room or gross room staff for intraoperative clinical use, in lieu of frozen section. These images can be digitally transmitted to a pathologist at a remote location for confirmation of tissue adequacy for diagnosis. Tissue can then be fixed in formalin and processed for permanent hematoxylin and eosin slides to be read at a later date by the pathologist. Tissue stored in a biobank can have matched CRM images stored in a digital catalog or library. The images can be recalled for evaluation by the researcher prior to tissue distribution to ascertain adequacy of tissue. Likewise, CRM images of patient tissue sent for molecular analysis can be digitally transmitted to laboratories or to centers for clinical trials.

Current limitations for CRM include imaging depth and equipment costs. In our studies, CRM provided detailed images from tissue depths of 200-300μm. This limited the amount of information attained from larger biopsies. However, in sub-millimeter thick tissue samples, CRM provided images from the majority of the tissue volume. Furthermore, CRM requires point scanning imaging systems which are routinely available in the basic sciences, but may be cost-prohibitive for smaller pathology departments. The cost of these systems is likely to decrease as imaging technologies advance.

CRM shows promise as a screening tool for the selection of representative tumor for use in diagnostics, biobanking, and molecular studies. CRM can provide rapid feedback as an intraoperative tool to guide clinicians in the selection of diagnostic material, thereby avoiding errors in diagnosis based on non-representative tissue. This can be especially useful, as patients with unresectable tumors are often biopsied to determine diagnosis and treatment. Time for frozen section diagnosis can also result in prolonged patient anesthesia, while CRM can be performed in a matter of seconds. When frozen section is not used during stereotactic needle biopsy, the surgeon risks re-biopsy of the patient to obtain diagnostic tissue. A method which can rapidly assess tissue without introducing artifact can be helpful for avoiding error in diagnosis, and reducing anesthesia time. In addition, CRM can assure the banking of representative tissue for research studies. Images can be stored on a digital database and released to researchers with the matched corresponding tissue. Lastly, the accurate selection of tumor tissue is necessary for molecular studies and patient clinical trials, where treatment is often based on the results of these studies. In summary, label-free optical-imaging approaches, such as CRM are emerging methods for microscopically interrogating tissue for diagnostic adequacy, without sectioning or staining, while preserving tissue integrity for molecular studies.

REFERENCES

1. Davis FG, Dolecek TA, McCarthy BJ, et al.: Toward determining the lifetime occurrence of metastatic brain tumors estimated from 2007 United States cancer incidence data. *Neuro Oncol* 14:1171-1177, 2012.
2. Ostrom QT, de Blank PM, Kruchko C, et al.: Alex's Lemonade Stand Foundation Infant and Childhood Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2007-2011. *Neuro Oncol* 16 Suppl 10:x1-x36, 2015.
3. Siegel RL, Miller KD, Jemal A: Cancer statistics, 2015. *CA Cancer J Clin* 65:5-29, 2015.
4. Jackson RJ, Fuller GN, Abi-Said D, et al.: Limitations of stereotactic biopsy in the initial management of gliomas. *Neuro Oncol* 3:193-200, 2001.
5. Karstensen JG, Klausen PH, Saftoiu A, et al.: Molecular confocal laser endomicroscopy: a novel technique for in vivo cellular characterization of gastrointestinal lesions. *World J Gastroenterol* 20:7794-7800, 2014.
6. Benati E, Argenziano G, Kyrgidis A, et al.: Melanoma and naevi with a globular pattern: confocal microscopy as an aid for diagnostic differentiation. *Br J Dermatol*, 2015.
7. Georges J, Zehri A, Carlson E, et al.: Label-free microscopic assessment of glioblastoma biopsy specimens prior to biobanking [corrected]. *Neurosurg Focus* 36:E8, 2014.
8. Hartmann D, Ruini C, Mathemeier L, et al.: Identification of ex-vivo confocal scanning microscopic features and their histological correlates in human skin. *J Biophotonics*, 2015.
9. Kang D, Schlachter SC, Carruth RW, et al.: Comprehensive confocal endomicroscopy of the esophagus in vivo. *Endosc Int Open* 2:E135-140, 2014.
10. Kap M, Oomen M, Arshad S, et al.: Fit for purpose frozen tissue collections by RNA integrity number-based quality control assurance at the Erasmus MC tissue bank. *Biopreserv Biobank* 12:81-90, 2014.
11. Spivak CJ, Pirouzmand F: Comparison of the reliability of brain lesion localization when using traditional and stereotactic image-guided techniques: a prospective study. *J Neurosurg* 103:424-427, 2005.
12. Su P, Liu Y, Lin S, et al.: Efficacy of confocal laser endomicroscopy for discriminating colorectal neoplasms from non-neoplasms: a systematic review and meta-analysis. *Colorectal Dis* 15:e1-12, 2013.
13. Tan J, Quinn MA, Pyman JM, et al.: Detection of cervical intraepithelial neoplasia in vivo using confocal endomicroscopy. *BJOG* 116:1663-1670, 2009.
14. Zoeller GK, Benveniste RJ, Landy H, et al.: Outcomes and management strategies after nondiagnostic stereotactic biopsies of brain lesions. *Stereotact Funct Neurosurg* 87:174-181, 2009.
15. Meyer M, Keith-Rokosh J, Reddy H, et al.: Sources of error in neuropathology intraoperative diagnosis. *Can J Neurol Sci* 37:620-624, 2010.
16. Chandrasoma PT, Smith MM, Apuzzo ML: Stereotactic biopsy in the diagnosis of brain masses: comparison of results of biopsy and resected surgical specimen. *Neurosurgery* 24:160-165, 1989.

FIGURES

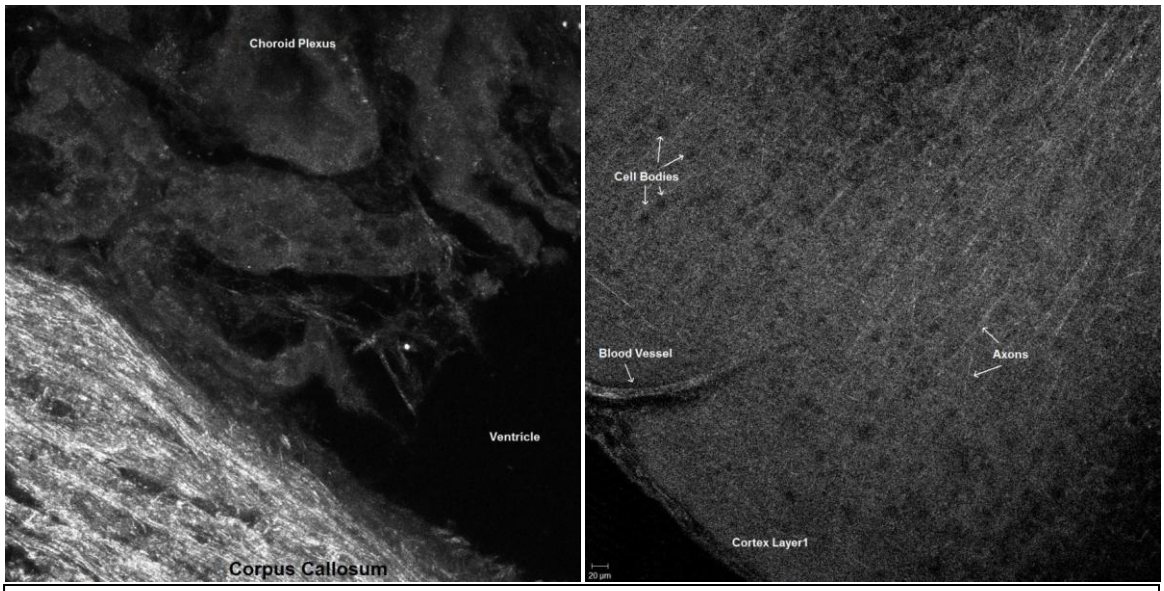


Figure 1. Label-free imaging of rodent acute slices from normal brain.
Cortex (Left). Confocal reflectance microscopy contrasts cell bodies, axons, and blood vessels in normal brain. Cell bodies appear as multiple hypointense circular regions within the tissue; note typical lack of cell bodies contrasted in layer 1 of the cortex. Myelinated axons are visualized as hyperintense fibers extending from cell bodies. **Ventricle and corpus callosum (Right).** White matter tracts in the corpus callosum generate a hyperintense reflectance signal. Individual cell bodies are contrasted in the choroid plexus. A lack of signal is appreciated from the fluid-filled ventricle. Scale bar equals 20μm.

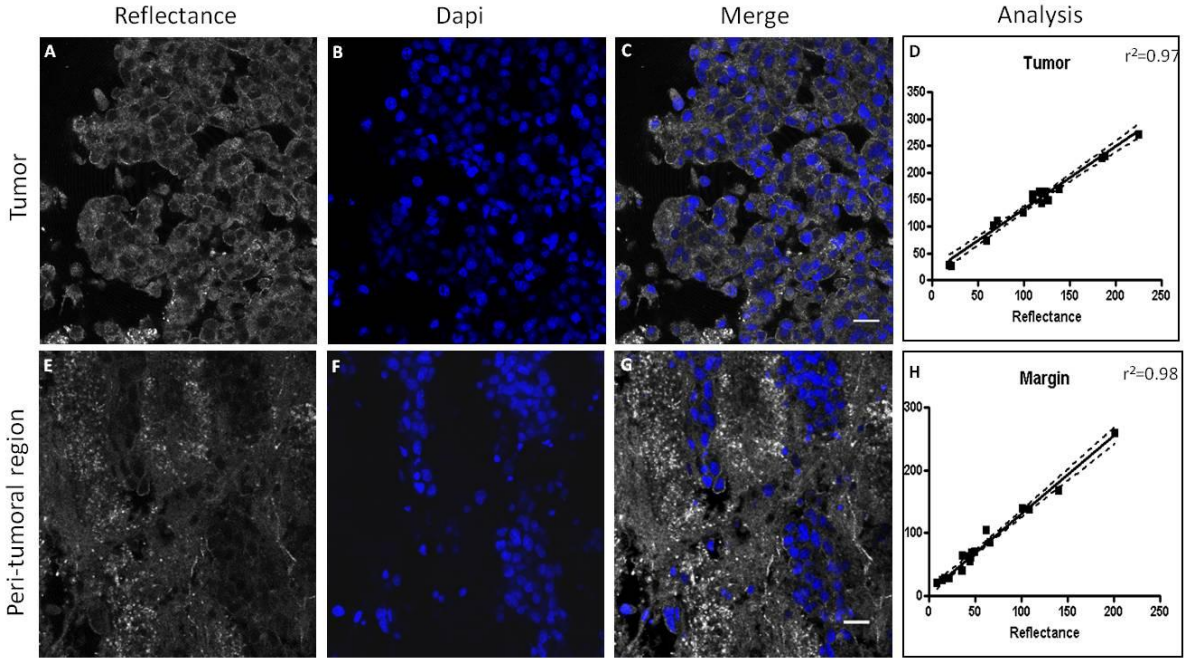


Figure2: Reflectance confocal imaging identifies cellular tumor in rodent acute slices.
Cellular Tumor (A-D). (A) Reflectance image of rodent xenograft tumor region; note shading of cell nuclei. (B) Dapi stain of identical tumor region identifies all cells in the field of view. (C) Overlay shows location of cells contrasted by reflectance to cells labeled with dapi. (D) Plot of coefficient of determination and confidence intervals for tumor cells identified by reflectance imaging. r^2=0.97. **Cellular tumor and acellular tissue interface (E-H).** (E) Reflectance image of cellular tumor and adjacent acellular region from rodent xenograft; note isolated cell populations. (F) Fluorescence confocal image of identical region labeled with dapi. (G) Overlay of reflectance and fluorescence images from tumor and peritumoral tissue interface. (H) Plot of coefficient of determination and confidence intervals for cells identified by reflectance imaging at tumor and interface acellular tissue interface. r^2= 0.98. Scale bar equals 20μm

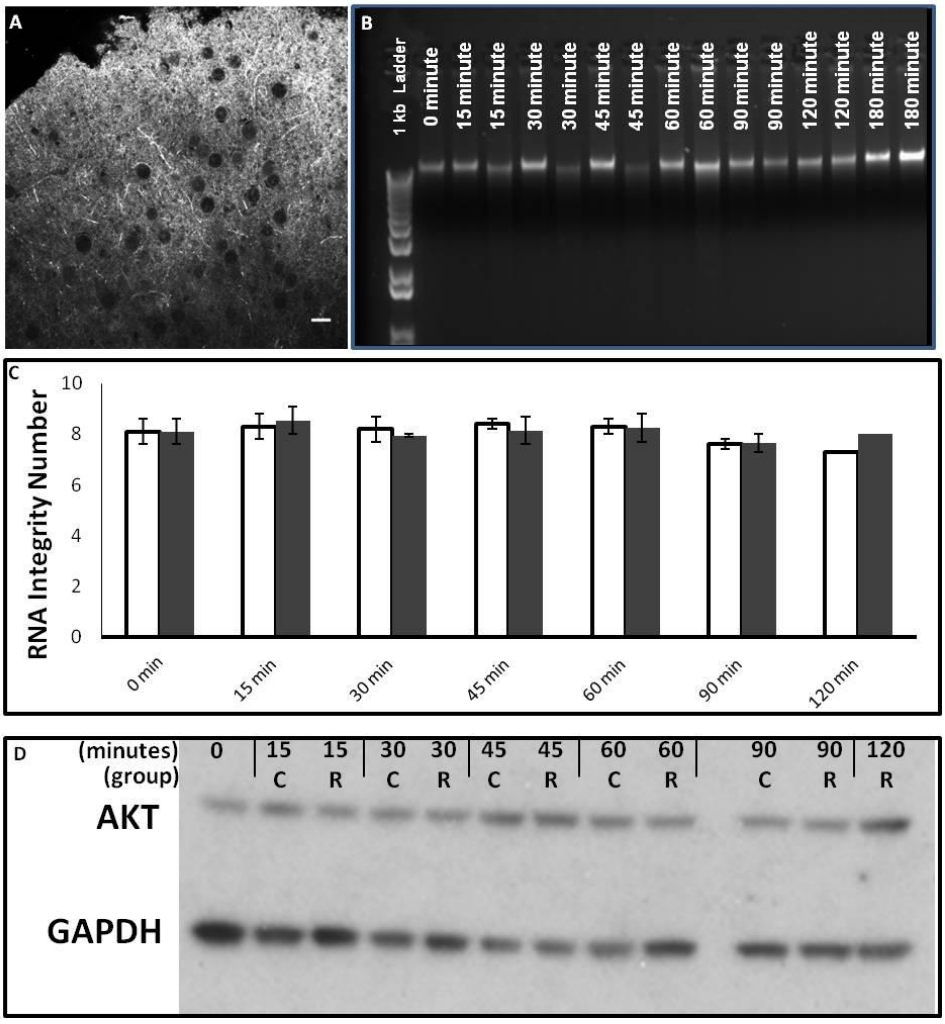


Figure 3: CRM does not alter DNA, RNA, or protein of examined rodent tissue. **A)** CRM image of fresh rodent brain tissue at cerebral cortex 90 minutes post-excision. Scale bar equals 20μm. **B)** Gel electrophoresis of genomic DNA extracted from control and CRM imaged biopsies; note similar bands across time course. C=control and R= CRM. between imaged and control biopsies. **C)** Time course comparison of RNA integrity for all imaged and control samples; note similar RNA integrity for all samples. **D)** Western blot analysis showing expression of AKT over 120-minute time course. Protein signal did not degrade over time course or when exposed to CRM. R= Confocal Reflectance Microscopy, C= Control.

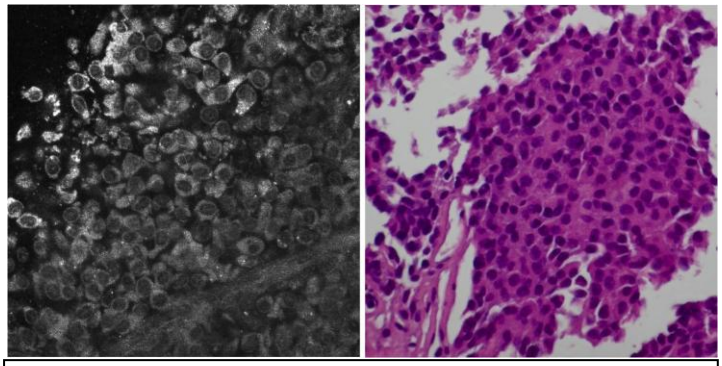


Figure 4: Confocal reflectance microscopy image (x200) of a pituitary adenoma on the left and the matched frozen section image (hematoxylin and eosin, ×400) of the same tumor on the right.

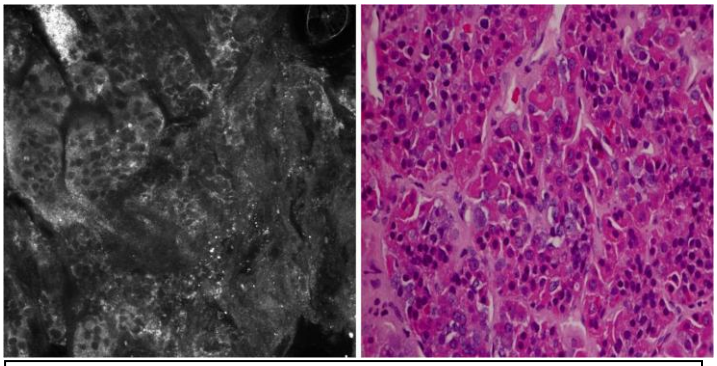


Figure 5: Confocal reflectance microscopy image (x200) of a normal adenohypophysis on the left and the matched frozen section image (hematoxylin and eosin, ×400) of the same tumor on the right.

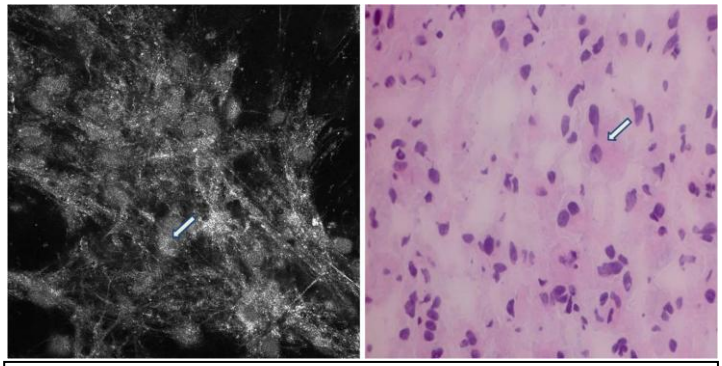


Figure 6: Confocal reflectance microscopy image (x200) of a well-differentiated astrocytoma on the left and the matched frozen section image (hematoxylin and eosin, ×400) of the same tumor on the right. Nuclei are vaguely visible in some of the tumor cells (arrows) in the confocal image.

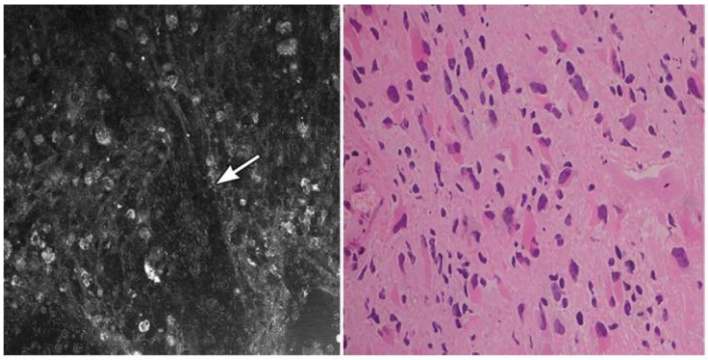


Figure 7: Confocal reflectance microscopy image(x200) of a glioblastoma on the left and the matched frozen section image (hematoxylin and eosin, ×400) on the right. Both show infiltrating tumor cells. The arrow denotes a vessel that contains erythrocytes.

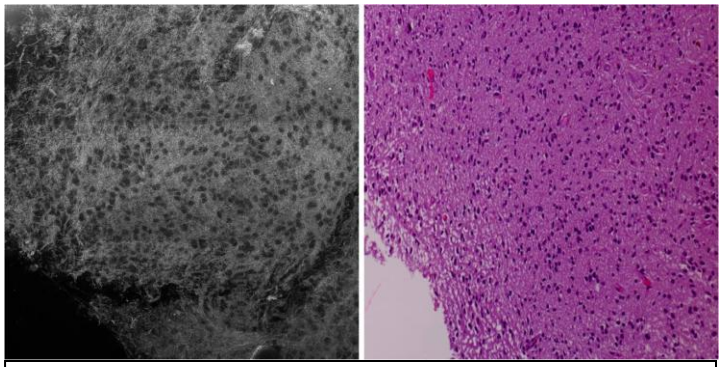


Figure 8: Confocal reflectance microscopy (x200) image of an oligodendroglioma on the left and the matched frozen section image (hematoxylin and eosin, ×200) on the right. Both show infiltrating tumor cells.

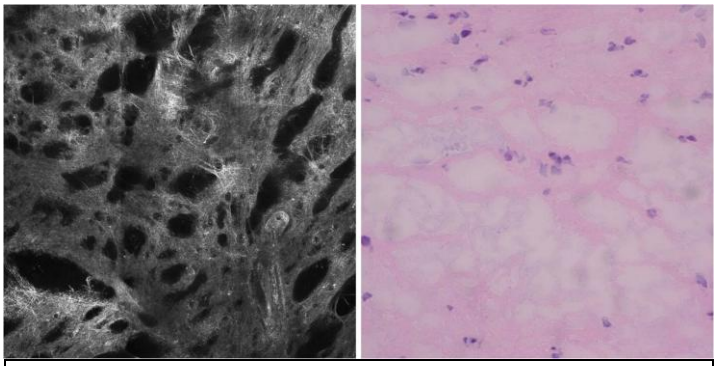


Figure 9: Confocal reflectance microscopy image (x200) of a subependymoma on the left and the matched frozen section image (hematoxylin and eosin [H&E], ×200) on the right. Myxoid microcysts are seen in the H&E images that correspond to the dark regions in the CRM image.

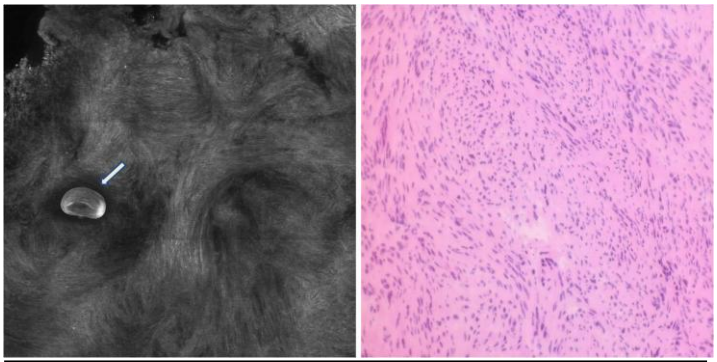


Figure 10: Confocal reflectance microscopy image (x200) of a schwannoma on the left and the matched frozen section image (hematoxylin and eosin, ×200) on the right. The fascicular architecture of the tumor is evident, as is a blood vessel (*arrow*).

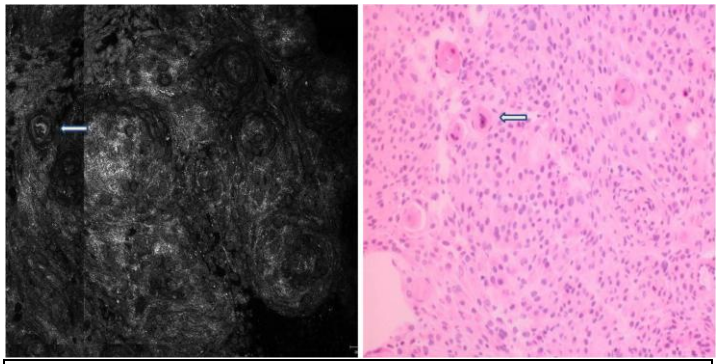


Figure 11: Confocal reflectance microscopy image (x200) of a transitional meningioma on the left and the matched frozen section image (hematoxylin and eosin, ×200) on the right. Nuclei are visible in the confocal image, as are psammoma bodies (arrows) and lobules of tumor cells.

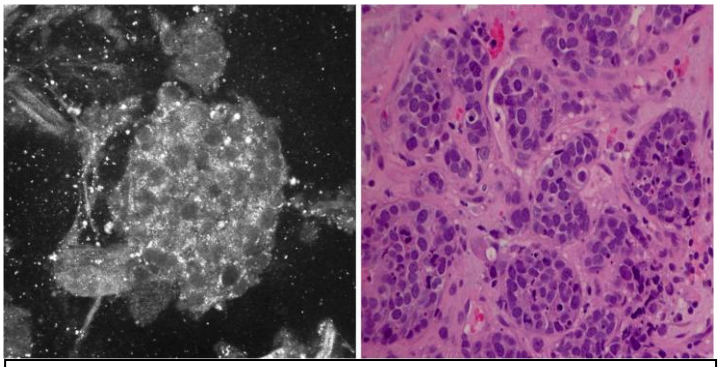


Figure 12: Confocal reflectance microscopy image (x200) of a metastatic breast carcinoma on the left and the matched frozen section image (hematoxylin and eosin, ×200) on the right.

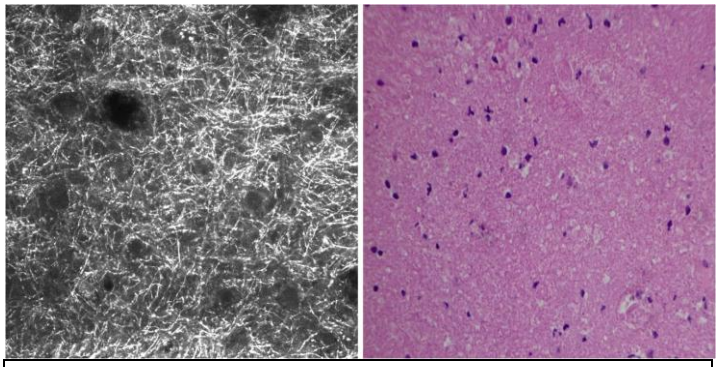


Figure 13: Confocal reflectance microscopy image (x200) on the left and the matched frozen section image (hematoxylin and eosin, ×200) on the right. Both show treatment effect in a glioblastoma.

Table 1. Tumor Types in 65 Patients

Tumor Type	No. of Tumors
Gliomas (n=15)	
Glioblastoma	6
Astrocytomas, grade II	1
Protoplasmic Astrocytoma	1
Ependymoma, grade II	3
Oligodendroglioma, grade II	1
Oligoastrocytoma, grade III	1
Oligodendroglioma, grade III	1
Subependymoma	1
Meningioma (n=25)	
Transitional	12
Fibrous	1
Meningothelial	6
Psammomatous	1
Metaplastic	1
Atypical	4
Adenoma (n=10)	
ACTH	1
Gonadotrophin	6
Prolactin	1
Null (nonsecreting)	1
Mixed prolactin/growth hormone	1
Pituitary gland	5
Schwannoma	4
Treatment effect (n=3)	
Colon adenocarcinoma	1
Glioblastoma	2
Metastatic carcinoma (n=3)	
Breast	1
Squamous cell	1
Lung	1

Abbreviations: ACTH, adrenocorticotropic hormone.

Table 2. RNA Integrity Values

Diagnosis	RNA Integrity Value
Glioblastoma	8.8
Glioblastoma*	8.5
Transitional Meningioma	8.4
Schwannoma	8.3
Well-differentiated astrocytoma	7.9
Pituitary adenoma	7.8
Schwannoma	7.4

*Two glioblastoma specimens were evaluated, and the values for both are shown.

Bedside twist drill aspiration of cerebral abscess less than 2.5cm in size: A case series and discussion

Tyler Carson D.O.[1,2], Dan E. Miulli D.O.[1,2]

[1]Department of Neurosurgery, Riverside University Health System-Medical Center, Moreno Valley, CA, USA. [2]Department of Neurosurgery, Arrowhead Regional Medical Center, Colton, CA, USA.

Dr. Tyler Carson, Department of Neurosurgery, Riverside University Health System Medical Center, 26520 Cactus Avenue, Moreno Valley, CA 92555. Tel. 1- 951-486-4460.

Abstract

Background: Intracranial abscess remains a potentially deadly condition despite development of newer antibiotics and improved surgical methods. Many studies exist evaluating the surgical indications for abscess drainage and it has been generally accepted that intracranial abscesses greater than 2.5 cm may best be treated with surgical intervention followed by antibiotic therapy. More recently studies have shown good results with stereotactic aspiration of abscesses to 1cm in size. Furthermore, a recent case series in 2014 of 103 cases of bedside twist drill aspiration of cerebral abscess >2.5cm showed a good recovery in 83.4% of cases.

Methods: This case series looks at the benefits of bedside twist drill aspiration of peripherally located brain abscess less than 2.5cm in size.

Results: In our series of patients, all had been placed on broad spectrum antibiotics and had negative blood and CSF cultures. Our bedside biopsy resulted in de-escalation of antibiotics in 2 of the 3 patients and decreased hospital length of stay.

Conclusion: In patients with peripherally located brain abscesses less than 2.5cm in size bedside twist drill drainage is a safe and reasonable approach. Positive identification of infective pathogen allows for a decreased hospital length of stay and de-escalation of antibiotics regiment resulting in significant reduction of hospital costs and resources. This is of benefit to the patient as well as the health system.

Keywords: Brain, abscess, aspiration, evacuation, surgical, bedside

Introduction:

Intracranial abscess remains a potentially deadly condition despite development of newer antibiotics and improved surgical methods. Cerebral abscess affects 1 in 10,000 hospital admits or 1,500 to 2,500 people per year in the US. [1] Persons with poor socioeconomic status and those residing in underdeveloped countries are at even high risk.[2] In the case of HIV or other immunosuppressed individuals the incidence jumps to 10xs the average or 1 in 1,000 hospital admissions and therefore undiagnosed HIV infection should always be worked up in patients presenting with cerebral abscess.[4,5] In fact, patients with predisposing condition such as HIV/immunosuppression, recent craniotomy or traumatic brain injury constitute 86% of cases.[4,5] The most common pathogen is Streptococcus and Staphylococcus species which compromised 34% and 18% respectively of 5,894 cases of cultured bacteria abscess.[5] Other opportunistic infections such as toxoplasmosis gondii and Mycobacterium tuberculosis as well as fungi and parasites are also of concern especially given the frequency of HIV and immunosuppressed individuals afflicted by the condition. [4]

Presenting symptoms have classically been described as a triad of fever, headache and nausea, however this classic triad is present in on 20% of patients. [5,6] In fact Helweg-Larson et al showed that of 102 patients with pyogenic brain abscess 39% had no fever, 26% had normal CRP and 49% had no leukocytosis on presentation. [6] Often patient with underlying immunosuppression may not mount an appropriate response to such infection and altered mental status, personality changes or subtler neurologic findings may be the only clue to diagnosis. MRI can be helpful in determination of cerebral abscess versus necrotic tumor. Both tend to be ring enhancing lesions, however abscesses show restricted water diffusion as indicated by hyperintensity on DWI and hypointensity on ADC sequences. [7] With improved medical and surgical treatment patient outcome has improved; Mortality has decreased from 40% to 10% over the past 5 decades, while the rate of patients with full recovery increased from 33% to 70%. [5]

Treatment for cerebral abscess relies on both surgical and non-surgical approaches. The classic study by Rosenblum et al showed that patients may be successfully treated non-surgically with small abscess. [8] 3 cm was suggested cutoff of size for which medical management alone was not recommend. Best outcomes for medical treatment alone are small size, early cerebritis stage, early administration of antibiotic and early clinical improvement. [9] With improved surgical techniques such as stereotactic guidance some have suggested non-surgical treatment in abscess only 1.5 cm in size or less. [10] However, each individual patient situation should be taken into consideration. For example, deep seeded abscess in the cerebritis stage greater than 3cm in size may be poor surgical candidate. Furthermore, patients with superficial abscess in the capsular stage less than 3 cm in size may with no known source may benefit from surgical drainage for diagnostic purposes. A trend towards minimally invasive drainage and medical management with serial MRI brain is emerging. [11,12,13] Regardless of whether medical treatment alone or a combination of surgical resection followed by medical therapy, the success of treatment hinges

on isolation of the causative organism. [14] Medical management success, whether in combination with surgical drainage or not, relies on antimicrobial therapy that not only diffuses into the CSF but also has bactericidal activity on the causative organism. [15] Because of this, identification of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) from culture of either the abscess itself or the suspected source is a necessity.

The two main surgical options for brain abscess are excision versus aspiration. A retrospective comparison between the two approaches between 1990-2008 performed by Ratnaike et al showed a 6.6% mortality for patients who underwent aspiration compared to a 12.7% mortality for those undergoing excision of abscess. [16] A recent review by Zhai et al suggests resection of superficially located encapsulated brain abscesses versus aspiration showed a high rate of neurologic improvement at 1 month, but no significant difference at 3 months from surgery in either neurologic outcome or mortality. [17] Indeed many studies have compared the efficacy and safety of aspiration to open surgery [16,17,18,19,20] and each may have its usefulness depending on the specific condition and presentation of the patient.

This paper looks at the role for aspiration of cerebral abscess via twist drill craniotomy, specifically for patients with abscess less than 2.5 cm in size and in whom no source had been identified. A recent case series by Singh et al details their experience with 103 cases of pyogenic brain abscess > 2.5 cm in size that were treated with aspiration via twist drill craniotomy. [21] They report a 4.8% mortality, with 92.1% having only mild or moderate disability. Our series of 3 patients to this point in time show similar efficacy and results.

Materials and Methods:

All patients had received a CT head upon admission with a significant finding of lesion(s) concerning for intracranial abscess based on patient history. Using CT reconstructions and imaging software the entry point for each biopsy site was calculated using fixed boney anatomy. Entry point was selected to be orthogonal to skull surface and traversing as little cortex as possible. Specifically, the entry point was calculated by measuring the distance above the external auditory meatus (EAM), the distance posterior from the Nasion corresponding to 90-degree angle to the entry site and the distance from the midline to the entry point. This site was then clipped of hair and marked with a 1cm x 1cm square metal marker and in one case an MR-Spot® marker. Follow-up imaging after marker placement confirmed entry point, adjustments to entry point were made as necessary.

After entry point was confirmed we proceeded with surgical aspiration of the abscess at bedside using a twist drill method. Patients received versed preoperatively and local anesthetic of 5-10 cc of 1% lidocaine with epinephrine. A 2 cm linear incision was made and the hand twist drill was utilized to access the intracranial space. Dura was palpated and opened with an 11 blade and a brain needle (Dandy ventricular needle) was advanced orthogonal to the skull to a depth corresponding the abscess depth noted on CT/MRI imaging. Fluid from abscess was aspirated

and sent for pathology and microbiology studies. The incision was then closed with suture or staple and patient was sent for a post-operative CT to confirm that there were no hemorrhage and correct site was accessed.

Results and Discussion:

All patients presented with headache. 2/3 presented with fever. One presented with seizure. The results of each individual patient are detailed in Table 1. Images of each abscess associated with these patients on admission can be seen in figures 1-3.

	Age/Sex	Presentation	Abscess size	Blood cx	CSF from LP	Aspirate culture	Changes to medical treatment	Glasgow Outcome Score
1	31 M	Headache, confusion	3 cm	-	-	No Growth	Open crani, negative pathology and microbiology	4
2	55 M	Headache, fever, focal motor seizure, hemiparesis	1.5 cm	-	-	MRSA	Changed to single antibiotic	5
3	40 M	Headache, fever, facial numbness	2.5 cm	-	-	Staph epi + Proprionibacterium Ances	Changed to single antibiotic	5

Table 1 – Results from patients undergoing bedside aspiration of cerebral abscess

Of the 3 patients who received bedside twist drill aspiration of cerebral abscess, a positive identification of the infective agent was able to be made in 2 cases. This was despite each having had blood cultures drawn prior to antibiotic therapy and both having had lumbar punctures performed with negative cultures. Additionally, each had already been placed on Vancomycin, Flagyl and Ceftriaxone prior to biopsy and identification of infective agent.

Based on the final MIC/MBC results from cultures both patient 2 and 3 were able to have their antibiotics de-escalated to a single agent (Vancomycin in both cases) per infectious disease recommendations. Both patients were able to be discharged with a PICC line and home antibiotic therapy. This resulted in decreased cost to the patient and health system in terms of fewer medications, fewer trips for home health to administer IV medications and less inpatient hospital days. Upon follow-up, both patients had good outcomes without any residual neurologic deficit.

Patient 1 did not have a positive result from biopsy of suspected abscess and underwent open craniotomy for evacuation and tissue biopsy. Of note this patient had multiple intracranial lesions and known cavitary lung lesion. The results from a lung biopsy as well as open biopsy of brain

were negative for malignant tissue or infection. Patient was discharged to inpatient rehab and referred to outside hospital for rheumatology and infectious disease work-up.

Based on our institutional experience patients with peripherally located brain abscesses less than 2.5cm in size and peripherally located can be safely treated with bedside twist drill aspiration. Positive identification of infective pathogen allows for a decreased hospital length of stay and de-escalation of antibiotics regiment resulting in significant reduction of hospital costs and resources. The ability to perform this procedure at bedside rather than utilizing valuable operating room time and expense as well as subjecting the patient to the risks of general anesthesia is also of benefit to the patient as well as the health system.

Given the favorable outcome we intend to use this method of bedside twist drill aspiration in certain situations at our institution. The ability to safely obtain a diagnosis in patients who may otherwise be treated solely with broad spectrum antibiotic therapy for many weeks is of great benefit. Furthermore, at an extremely busy county medical center where resources are limited the ability to perform this at bedside is tremendously helpful for maintaining hospital resources.

References:

1. Mathisen GE, Johnson JP. Brain abscess. Clin Infect Dis. 1997 Oct. 25(4):763-79.
2. Nathoo N, Nadvi S S, Narotam P K, et al. Brain abscess: management and outcome analysis of a computed tomography era experience with 973 patients. World Neurosurg. 2011 May-Jun;75(5-6):716-26; discussion 612-7. doi: 10.1016/j.wneu.2010.11.043
3. The, U. K. C. H. I. V. C. S. S. C. (2011). HIV-associated central nervous system diseases in the recent combination antiretroviral therapy era. European Journal of Neurology 18(3): 527-534.
4. Brouwer M C, et al. (2014). Brain Abscess. New England Journal of Medicine 371(5): 447-456.
5. Brouwer M C, Coutinho J M, van de Beek D. Clinical characteristics and outcome of brain abscess: systematic review and meta-analysis. Neurology. 2014 Mar 4;82(9):806-13. doi: 10.1212/WNL.0000000000000172. Epub 2014 Jan 29.
6. Helweg-Larsen J, Astradsson A, Richhall H, Erdal J, Laursen A, Brennum J. Pyogenic brain abscess, a 15 year survey. BMC Infectious Diseases. 2012;12:332. doi:10.1186/1471-2334-12-332.
7. Leuthardt E C, Wippold F J, Oswood M C, Rich K M. Diffusion-weighted MR imaging in the preoperative assessment of brain abscesses. Surg Neurol. 2002 Dec;58(6):395-402.
8. Mark L. Rosenblum, et al. (1980). Nonoperative treatment of brain abscesses in selected high-risk patients. Journal of Neurosurgery 52(2): 217-225.

9. Greenberg, M. S., Cerebral Abscess In: Handbook of Neurosurgery. 7th edition. New York, Thieme Publishers New York, 2010; p. 350-356.
10. Obana W G, Rosenblum M L. Nonoperative treatment of neurosurgical infections. Neurosurg Clin N Am. 1992 Apr;3(2):359-73.
11. Sharma B S, Gupta S K, Khosla V K. Current concepts in the management of pyogenic brain abscess. Neurol India 2000;48:105
12. Alvis Miranda H, Castellar-Leones SM, Elzain MA, Moscote-Salazar LR. Brain abscess: Current management. Journal of Neurosciences in Rural Practice. 2013;4(Suppl 1):S67-S81. doi:10.4103/0976-3147.116472.
13. Lu C H, Chang W N, Lui C C. Strategies for the management of bacterial brain abscess. J Clin Neurosci. 2006 Dec:13(10):979-85. Epub 2006 Oct 23.
14. Hakan T. Management of bacterial brain abscess. Neurosurgery Focus. 2008;24(6):E4. Doi:3171/FOC/2008/24/6/E4.
15. Garvey G. Current concepts of bacterial infections of the central nervous system. Bacterial meningitis and bacterial brain abcess. Journal of Neurosurgery. 1983 Nov;59(5):735-44.
16. Ratnaike T E, Das S, Gregson B A, Mendelow A D. A review of brain abscess surgical treatment – 78 years: aspiration versus excision. World Neurosurgery. 2011 Nov;76(5):431-6. Doi: 10.1016/j.wneu.2011.03.048.
17. Zhai Y, Wei X, Chen R, et al. Surgical outcome of encapsulated brain abscess in superficial non-eloquent area: A systematic review. Br J Neurosurg. 2016 Feb;30(1):29-34. Doi 10.3109/02688697.2015.1109059. Epub 2015 Nov 16.
18. Duma C M, Kondziolka D, Lunsford L D. Image-guided stereotactic management of non-AIDS-related cerebral infection. Neurosurg Clin N Am. 1992 Apr;3(2):291-302.
19. Kariev MK, Kadyrbekov R T, Akhmediey N M, et al. Zh Vopr Neirokhir Im N N Burdenko. 2001 Apr-Jun;(2); discussion 20-1.
20. Stevens D C, Asfora W T. 8-year-old patient with multipule large cerebral abscesses successfully treated with stereotactic aspiration: case report and literature review. S D Med. 2013 Oct;66(10):420-3
21. Singh I, Rohilla S, Kumawat M. Twist drill aspiration of pyogenic brain abscesses:our experience in 103 cases. J Neurol Surg A Cent Eur Neurosurg. 2014 May;74(3):189-94. Doi: 10.1055/s-0033-1342933. Epub 2013.

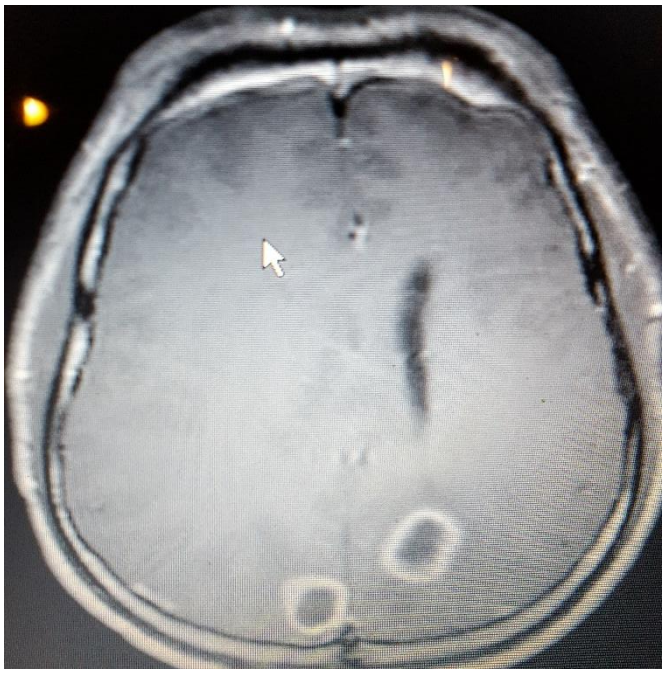


Figure 1 – Patient #1 MRI brain on admission. T1WI + contrast.

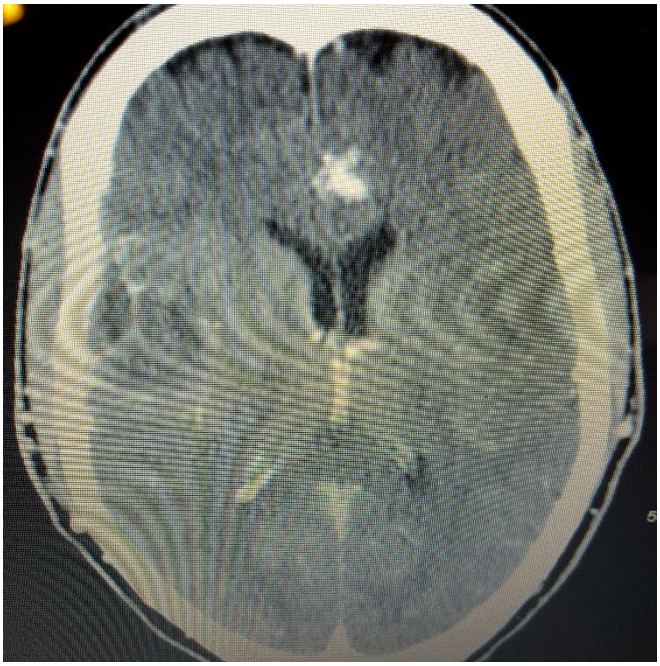


Figure 2 – Patient #2 CT brain with contrast on admission

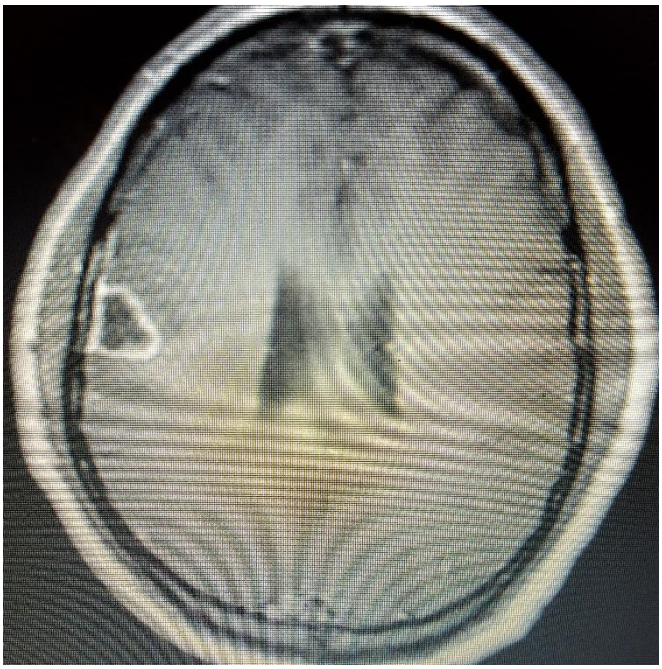


Figure 3 – Patient #3 MRI brain on admission T1WI + contrast