The Direct Administration of Isopropyl Alcohol to Rat Astrocytes

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Abstract

Anesthesia providers have been cautious when applying isopropyl alcohol (IPA) prep pads specifically as an antiseptic for ports on neuraxial catheters. After re-bolusing a neuraxial catheter with medication, there has been concern that a residual amount of IPA travels to the patient and causes neurolysis. The literature review analyzed various neurolytic agents and their purposes when applied to clinical practice. IPA is not used as a neurolytic agent in clinical practice or even reported as an accidental agent in the literature. There is a need for continued research of IPA and its effects on cells. The purpose of this study was to determine if there is neurolysis caused by a direct application of 70% IPA to rat astrocyte cells at varying time intervals running from 5-60 minutes following trypan blue exclusions and hemocytometer analysis. The outcome was that alcohol concentration decreased as dry time increased after scrubbing the epidural catheter port with an IPA pad. The project resulted incomplete as it did not reach the steps of administering the found alcohol concentrations onto rat astrocytes for neurolysis. The results of this scholarly project provided evidence needed to assess the safety of scrubbing an epidural catheter port with a 70% IPA prep pad and possibly allow health organizations to develop policies based on the results and for further research.

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Introduction

Healthcare providers understand the importance of disinfecting catheter hubs prior to accessing patient intravascular, central venous, and other peripheral lines to reduce bacterial contamination. During clinical practice, nurse anesthetists may need to administer a bolus of a local anesthetic through an epidural injection port, but there is controversy regarding disinfecting the epidural catheter port with an isopropyl alcohol (IPA) prep pad and the potential for neurolysis followed by a bolus dose. There is a wide variety of neurolytic agents in the literature, including but not limited to ethyl alcohol, phenol (carbolic acid), and glycerol for treating cancer pain, spasticity, vertebral hemangiomas, and neuralgia. The literature excludes the effects of IPA as a neurolytic agent, leading to a need for further research to identify if scrubbing the hub of epidural catheter with 70% IPA causes neurolysis.

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Significance and Background

The Centers for Centers for Disease Control and Prevention (CDC, 2016) and The Joint Commission (TJC, 2013) recommend disinfecting hubs and ports on catheter lines that are intravascularly inserted due to the potential for infection. However, there are no guidelines or policies for disinfecting the port of an epidural catheter when re-bolusing a patient with a local anesthetic. There is a gap in the literature supporting the use of the aseptic technique on epidural catheters for medication administration, which can lead to controversy in clinical practice. A concern some clinicians have with scrubbing the hub of an epidural catheter prior to medication administration is the introduction of IPA into the epidural space, possibly causing neurolysis. Alcohol has been traditionally found to be associated with neuritis by damaging nerves through denaturing proteins and fatty substance extraction (Amr et al., 2018; Boyce, 2018; Choi et al., 2016; Koyyalagunta et al., 2016). Certain forms of alcohol and phenol are used to intentionally cause neurolysis as a treatment method for interventional management of cancer pain, nerve spasticity, and vertebral hemangioma (D'Souza & Warner, 2022; Goyal et al., 1999; Kim et al., 2015).

There is no established standard for the minimum requirement of alcohol or phenol to cause neurolysis (Poddar et al., 2016). The gap in literature also does not define the effects of IPA on nerves in the epidural space. This lack of evidence creates significant questions for certified registered nurse anesthetists (CRNA). First, is there any residual IPA going through the epidural catheter following a bolus of medication that will reach the epidural space? Second, if it reaches the epidural space, is it enough to cause neurolysis? Careful attention must be given to aseptic precautions because it is equally important to consider the risks of nerve cell injury

(Campbell et al., 2014). The goal of this scholarly project was to determine if IPA will cause neurolysis when directly applied to rat astrocyte cells. Using alternative cells to analyze this phenomenon more closely can improve understanding of IPA's effect on human cells.

PICOT Search Format Questions

Two questions were generated in PICOT format that guided the systematic review of the literature (Melnyk & Fineout-Overholt, 2023). The first question addressed the problem: When analyzing the practice of neuraxial techniques (P), does the introduction of isopropyl alcohol to cells (I) cause cell neurolysis (O)? The second question addressed the problem: In rat astrocyte cells (P), does the concentration at the end of an epidural catheter after disinfecting the injection port with a 70% isopropyl alcohol pad (I), following trypan blue exclusions and hemocytometer analysis cause neurolysis (O), during varying time intervals from 5 to 60 minutes (T)?

Search Strategy and Results

The search strategy included the Pubmed database and Google Scholar. A total of 23,842 articles were initially retrieved. Through title, abstract, and methods evaluation, in addition to the implementation of solutions that cause neurolysis, 31 articles met inclusion and exclusion criteria. Search limits were English language and date of article publication (1940-2022). Key search terms and MeSH combinations include *alcohol* AND *neurolysis, epidural AND alcohol AND neurolysis, alcohol ablation, chemical neurolysis, alcohol neurolysis complication, alcohol epidural injection.* MeSH terms included alcohol, epidural, and neurolysis.

Grade Criteria

The literature was evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) criteria (Brozek et al., 2009). Initially, the rating of evidence was a moderate 3. The studies included case reports, randomized clinical trials,

statistical analysis, retrospective studies and evaluations, retrospective meta-analyses, and systematic reviews. These studies represented a wide range of methodologies. Methodological flaws included convenience sampling, which contributed to selective sample sizes. Inconsistencies demonstrated in the studies were subjective data and unclear delineation of results, further decreasing the criteria by a point. Some of the evidence gave confidence intervals and small p values, which increased the GRADE by one point.

Indirectness was seen in the type of neurolytic agent administered in the studies and the variability of diseases, decreasing grade criteria by a point. Imprecision included purposive sampling and many small sample sizes, which led the quality of evidence to decrease by a point. No clear evidence of publication bias was present in the studies. A large magnitude of the effect was clear in the evidence, which increased the score of the literature by a point. Given the final GRADE criteria score of the evidence being a very low 2, it is recommended that further research be conducted regarding alcohol and neurolysis.

Literature Review and Synthesis of Evidence

The current practice of scrubbing the hub of central venous and peripheral intravascular catheters is to use a 70% IPA prep pad or apply an IPA–impregnated protective cap, which guards against external contamination for every line entry to reduce the risk of central line-associated bloodstream infections (CLABSIs) (Boyce, 2018; CDC, 2016; TJC, 2013). As it relates to anesthesia, this same aseptic technique is controversial when applied to epidural catheter ports. There is a difference in practice amongst anesthesia providers when scrubbing the hub of an epidural port before re-bolusing a dose of medication.

There is a concern that introducing IPA into the epidural space could possibly cause neurolysis. The extent to which providers believe this practice is detrimental and harmful is

based on ablation neurolysis data; those techniques use significantly higher volumes of ethanol, phenol, glycerol, and other neurolytic agents to induce neurolysis purposefully. This review described the evidence that informs these assumptions as a way of framing the need for additional research to determine whether hesitation related to the use of IPA in epidural catheter ports is warranted.

Neurolysis

The literature specifically references ethyl alcohol (absolute alcohol, dehydrated alcohol, or ethanol) as being a well-established agent used in purposefully induced neurolysis (Campbell et al., 2014; Koyyalagunta et al., 2016; Rangel Jaimes et al., 2022). Alcohol neurolysis is the irreversible damage of nerve cells by denaturing proteins and removing cholesterol, phospholipids, and cerebroside from the neural membranes causing precipitation of mucoproteins (Amr et al., 2018; Boyce, 2018; Choi et al., 2016 & Koyyalagunta et al., 2016; Rangel Jaimes et al., 2022). This also leads to interference with cellular metabolism and disruptions of cytoplasmic integrity, damaging both the Schwann and nerve cells, resulting in Wallerian degeneration (Amr et al., 2018; Boyce, 2018; Choi et al., 2016; Koyyalagunta et al., 2016; Rangel Jaimes et al., 2022). Through direct contact, the alcohol triggers sympathetic denervation, leading to a response of inflammation and necrosis (Al-Jumah et al., 2020). Neurolysis can cause long-term effects such as lesions, paralysis, vertebral collapse, paresthesia, and relief of pain (Goyal et al., 1999; Kang et al., 2020; Kim et al., 2015).

In neurolytic ablation, nerve regeneration may occur based on various factors, such as the thickness of the nerves and the sympathetic tones. Patients will have to receive frequent therapies to ablate the nerves to alleviate their pain, as the nerve destruction is not permanent. These studies have shown in their results that after 3-6 months, the patient's pain will reoccur (Choi et

al., 2016; D'Souza & Warner, 2022; Goyal et al., 1999; Kim et al., 2015; Koyyalagunta et al., 2016). Campbell et al. (2014) and Rangel Jaimes et al. (2022) suggest there are no randomized controlled trials that evaluate the efficacy of this treatment and require further research to address risks of neurological damage and the exact dose necessary to achieve adequate pain control.

While nerve damage occurs by many methods outside of neurolytic agent exposure, peripheral nerves naturally repair themselves through Schwann cell utilization and transcription factors, but central nervous system axons cannot (Choi et al., 2016). The nature of central nervous system tissue yields poor regenerative capability attributing to why many spinal cord injuries can be devastating and long-lasting (Choi et al., 2016). This is central to the controversy around the use of an IPA prep pad prior to medication administration through an epidural catheter.

Neurolytic Agents

Alcohol is a water-soluble, hypobaric solution that disseminates rapidly from the injected site and can be painful upon injection (Koyyalagunta et al., 2016). Effects can depend on the route for the administration of the neurolytic agent. Administration can occur intrathecally, peripherally, or intramuscularly. In addition to the use of ethyl alcohol, phenol, and glycerol are other agents that are used to deliberately cause neurolysis in the treatment of refractory cancer pain, vertebral hemangiomas, spasticity, and neuralgia (Goyal et al., 1999).

The literature identified various volumes, concentrations, and medications needed to produce therapeutic neurolysis. Amr et al. (2018) used a total volume of 20 ml (12 ml of 100% alcohol, 6 ml of 2% lidocaine, and 2 ml of 8 mg dexamethasone) divided into two injectable doses of 10 ml for each side of T11 vertebra. Other studies suggest the use of 30 ml injections of absolute alcohol directly into the hemangiomatous cavity to achieve neurolysis (Goyal et al.,

1999). Koyyalagunta et al. (2016) identified the use of 24.73 ± 8.89 ml of alcohol and 20.24 ± 5.05 ml of phenol to produce neurolysis. The phenol mixture comprised 10% phenol in 20% glycerin, while the alcohol mixture was 98% dehydrated ethanol (Koyyalagunta et al., 2016). Regarding neurolysis in the use of epidurals, an injection of 10-15 ml of 50-95% alcohol was used to achieve pain relief in patients (Odom, 1940; Poddar et al., 2016). This suggests that in neuraxial anesthesia, large and repeated doses of neurolytic agents are needed to cause significant effects, such as relief of pain from cancer.

By contrast, several studies indicate that smaller volumes with varying levels of concentrations are successful in achieving neurolysis. Leung et al. (2021) and Khawaja and Scrivani (2020) note that a much lower dose of phenol achieved neurolysis when given in a 5% concentration and volumes ranging from 0.5 ml to 3 ml. Leung et al. (2021) continue to point out that alcohol concentrations included 50% or 75% in volumes of 0.5-1 ml to achieve treatment. One ml of a higher concentration of 99.5 % dehydrated alcohol is also used as a relief of pain from intractable intercostal neuralgia of neurolysis (Kang et al., 2020), and in a case report regarding direct administration of 2 ml of 96% absolute alcohol into the intrathecal space, relief of pain was achieved up to 6 weeks (Rangel Jaimes et al., 2022). Ultimately, the concentration of alcohol is directly proportional to the degree of nerve injury (Kang et al., 2020; Wang et al., 2011).

Accidental neurolysis has been seen in agents such as in chlorhexidine. There have been cases where a whole syringe (8 ml) of 0.5% chlorhexidine in alcohol was injected into the epidural space and another where a bupivacaine syringe contaminated with 0.5% chlorhexidine in alcohol greater than 0.1 ml was injected spinally which led to chronic adhesive arachnoiditis and, ultimately, paraplegia (Campbell et al., 2014).

Although varying ranges of alcohol concentrations are used for neurolysis, there are no definitive minimum concentrations established to cause neurolysis (Miller, 2013; Poddar et al., 2016). The studies described in this review emphasize the use of ethyl alcohol or phenol as opposed to IPA, indicating a literature gap regarding the use of IPA as a neurolytic agent. Current outcomes from indirect studies are integral for determining the use of IPA. Determining if IPA is safe for use as a disinfectant on epidural catheters without causing neurolysis could reduce infection rates or refute the use of IPA on epidural catheters. Guidelines and policies can be created in light of new research.

Project Aims

The purpose of this project was to identify whether the amount of IPA at the end of an epidural catheter after scrubbing the port with 70% IPA prep pad at varying dry time intervals of 0-20 seconds would lead to neurolysis in the biochemistry lab at AdventHealth University (AHU) Orlando, Florida. The scholarly project's specific objectives were as followed:

1. Identify and utilize resources needed to conduct and assist in the research at AdventHealth University (AHU) Chemistry and Microbiology Laboratory (lab).
2. Determine the concentration of IPA with gas chromatography (GC) after scrubbing the hub with a 70% IPA pad and flushing 18 ohms water through an epidural catheter at different dry time intervals of 0,10,20 seconds.
3. Use GC to create the correct concentration of IPA found in the previous objective to administer to rat astrocyte cells directly.
4. To identify the quantity of dead rat astrocyte cells using trypan blue exclusion per hemocytometer analysis of neurolysis in as 5, 20, 40, and 60 minute periods.

Methods

Design

This scholarly project used a quantitative research design method with the assistance of an outside research lab to determine the concentration of IPA after scrubbing the hub with a 70% IPA pad and flushing the epidural catheter with 18 ohms water using GC. The outside lab utilized its resources to identify low concentrations of IPA using the GC machine.

Setting

The setting for the innovation was at the AHU Biochemistry Science Lab, located in Orlando, Florida, in addition to a third-party laboratory in Altamonte Springs, Florida.

Sample Methodology

There were no participants in this scholarly project, as this project used IPA solutions as the independent variables. There were no risks or discomforts due to no human participants.

Data Collection and Instruments

The project co-investigators collected the results. Three samples were created for each of the three different dry times. Those solutions were then tested under GC to detect any identifiable concentrations across the varying dry times. The GC is a very sensitive method of identifying and quantitating toxic alcohols (Kemble & Cervinski, 2020). A comprehensive chromatogram laid out detailed results of the findings.

The third-party lab supplied 40 ml vials. These vials were used to store the sample of 18 ohms water flushed through the epidural catheter after scrubbing the hub of the epidural catheter for 30 seconds and waiting at dry times of 0, 10, and 20 seconds. Ten ml of 18-ohm water was used in the syringe and flushed through the catheter. An additional 30ml of 18-ohm water was used to fill the vile and eliminate any head space. In between each sample, the syringe and the

epidural catheter were rinsed with water to remove any possible residual alcohol. The vials were then sealed and delivered to the third-party lab for testing.

The hemocytometer was used along with trypan blue exclusion criteria to determine the death number of the astrocytes. Prior to IPA administration on the rat astrocyte cells, the concentration of IPA was determined using GC to formulate the solution. GC is a very sensitive method of identifying and quantitating toxic alcohols (Kemble and Cervinski, 2020).

A hemocytometer is most frequently used to count cells. The mammalian cells would be placed on eight 1x1 mm areas on the two panels of the hemocytometer (Grishagin, 2015). A commercial cell counter or a manual counter (‘‘clicker’’) may be utilized while looking into the eyepiece of the microscope (Grishagin, 2015).

Data Analysis

Microsoft Excel was utilized to record the total amount of IPA in parts per billion found in each sample vial. A curve was created to illustrate the trend of the results. The third-party laboratory provided an analytical report. Appendix B and C were created to show the trend in results. This non-human subject research does not require informed consent or any protection of patient health information. Therefore, the data information was stored on a personal AdventHealth University account as a protected document accessed only by co-investigators and project chair. It will be available for 7 years and then destroyed as per AdventHealth’s Scientific Review and Committee by AH IT.

Planning and Procedures/Limitations

Planning

The former primary stakeholder was Dr. Martin Rivera, the project chair. The current primary stakeholder was Dr. Jill Mason. Dr. Sebastian Farrell assisted with the sample collection

and interpretation of the analytical report. Dr. Farrell also established the relationship with the third-party laboratory, all lab resources, and training required to obtain the samples. Dr. Erik Williams is a certified registered nurse anesthetist and was the project mentor and an end-user. The budget and supplies are on Appendix E

Implementation

Forty milliliter (ml) vials were used to capture the samples. An epidural catheter port was scrubbed with a 70% IPA pad for 30 seconds. A 20 ml syringe with 10 ml of 18 ohms water was then connected to the epidural catheter after waiting 0 seconds, 10 seconds, and 20 seconds using a stopwatch. The 10 ml of 18-ohm water in the syringe was flushed through the catheter into the 40 ml vial. An additional 30ml of 18-ohm water was used to fill the vial and eliminate any head space. In between each sample, the syringe and the epidural catheter were rinsed with water to remove any possible residual alcohol, and a new 70% IPA pad was used for each dry time. The vials were then sealed and delivered to the third-party lab for testing.

Barriers and Facilitators

One major barrier to the project was the inability of AHU GC machine to detect small amounts of IPA in a reliable manner. This barrier was solved by identifying a research lab capable of detecting small amounts of alcohol in ppb with their GC. Anticipated barriers were the scheduling of lab times as well as the availability of the staff and key players. Facilitating factors included the lab locations and key stakeholders which are on the AHU campus. Dr. Farrell was instrumental in finding the correct laboratory and facilitating lab time to collect the samples.

Anticipated Limitations

Due to the timeline barrier, rat astrocytes were not utilized in this project. Anticipated

limitations included preserving the cells in sustainable conditions such as constant atmospheric pressure and temperature to ensure the correct quantity is a limiting factor. Other limitations was ensuring that the rat astrocyte cells and IPA solution remained free of contamination. Regarding the direct application of IPA onto the cells, there was a chance of a localization effect. This localization effect could cause neurolysis on the cells in contact with the high concentration and no cell death further away from the high concentration. Limitations to the tools are that the hemocytometer method of counting cells has faults such as uneven cell distribution, subjective interpretation of the criteria met to determine if a cell is in the counting space, contamination of the device, and inconsistent filling rates of the hemocytometer (Ongena et al., 2010).

Procedures to Sustain

Frequent communication was required to accomplish the end result of acquiring a reliable GC to identify small amounts of IPA. The communication involved identifying the appropriate lab sites willing to take our samples and produce results. This required staying in contact with the labs and Dr Farrell updating us on their progress. Additionally, remaining consistent in recreating sample concentrations at AHU lab was necessary to avoid deviations and errors. In order to sustain the intervention, further research and analysis must be conducted to identify more data points and to identify the exact amount of isopropyl alcohol that causes neurolysis on rat astrocytes.

Timeline

The experiment was anticipated to have a duration of 2 years. This scholarly project required submission to the Institutional Review Board (IRB) and the Scientific Review Committee (SRC) in the fall of 2022. Experimentation and data collection began in the biochemistry lab of January 2023 and continued until the end of April 2024. The data from this

project was collected in Fall 2023. The information was disseminated in the Spring of 2024. Refer to Appendix F for the final timeline, in which there were no major discrepancies between the initial proposed timeline and the final.

Results/Findings

For the sake of interpretation of the data, 1mcg/L= 1 parts per billion (ppb). The results for the samples were as followed: DT0A, B,C 3530 mcg/L, DT10A,B,C 2850mcg/L, and DT20A,B,C 2190mcg/L (See Appendix E).

Discussion and Implications

The amount of alcohol identified in each sample was inversely proportional to the labeled dry time. Given that the amount of alcohol decreased as dry time increased, it can be extrapolated that the alcohol level would be at zero ppb at approximately 53 seconds. From the 0-second dry time to the 10-second dry there was a 19.3% decrease in alcohol, dry time 10 to 20 seconds showed a decrease of 23%, and dry time 0 to 20 seconds there was a 37.9% decrease. By extrapolating data from the created diagrams, it was possible to predict that IPA would be fully evaporated, demonstrating a 100% decrease between 0 seconds and 53 seconds of dry time, as illustrated in the forecast curve (see Appendix C).

With these findings, scrubbing the epidural catheter port for at least 30 seconds and waiting 1 minute, there will be no alcohol injected into the patient. This could be a safe way to disinfect the potentially contaminated injection port in the instance it becomes disconnected from a continuous infusion.

The initial PICOT question addressed in this project was unable to be achieved due to a time barrier. Although the detected amount of IPA was not applied to the rat astrocytes directly, the data collected still has some significance because of the relationship between dry times and

the amount of alcohol, suggesting that waiting the full 53 seconds eliminates the risk of neurolysis for the patient.

The impact of this evidence-based practice project can assist providers in discerning safe methods for their own anesthesia practice. Should institutions adapt this new information upon further research, policies can be created to address the cleansing of epidural catheter ports when disconnecting and administering medication.

Applicability to Practice

The current guideline to provide disinfection to an epidural catheter after a witnessed disconnection within 8 hours of insertion as provided by the American Association of Nurse Anesthesiology (AANA) includes cleaning a portion of the catheter that is 10 inches (25.4 cm) from the disconnected end by submerging it into povidone-iodine for 3 minutes, letting the catheter completely dry, cutting it in the center of the sterile area with sterile scissors, and then reconnecting it (American Association of Nurse Anesthesiology, 2012). These current guidelines identify an epidural catheter as a critical item, in that it contacts sterile body cavities and, therefore, necessitates sterility at the time of use (American Association of Nurse Anesthesiology, 2012). There is no evidence to refute the use of 70% IPA prep pads to disinfect the epidural catheter port before re-bolusing with medication.

This scholarly project examined whether the use of a 70% IPA prep pad on an epidural catheter port led to neurolysis, a concern providers assume based on data from ablation neurolysis. The profession would benefit from clearer evidence on the safety of using 70% IPA when disinfecting the epidural catheter port. The scholarly project requires further implementation of the next steps to finalize conclusions on the effects of the alcohol concentrations found.

Theoretical Framework

The scholarly project implemented the theoretical framework of Plan, Do, Study, and Act. This type of framework involves quality improvement concepts (Christoff, 2018). This framework was best suited regarding the scholarly project because the results could influence a change in healthcare to improve outcomes.

The Plan was to determine if an idea can be tested for improvement (Chen et. al., 2021) The "plan" phase is to determine if IPA is going through the epidural catheter port after scrubbing it with an IPA pad. The amount found at the end of various dry times would have been placed on rat astrocyte cells to determine neurolysis. There is a gap in the literature regarding the effects of residual alcohol through an epidural catheter.

The "do" phase was to put an experiment into action and gather information (Chen et. al., 2021). The project was started in the Fall of 2022 and achieved results in the Fall of 2023. The "study" phase was to gain an understanding of the data (Chen et. al., 2021). After reviewing the acquired information from the GC, the results could then be interpreted. There was a decrease in IPA going through the alcohol as the dry time increased. The "act" phase would involve the decision to make changes for improvement (Chen et al., 2021). This scholarly project has the potential to support certain decisions for guideline changes. The findings will be disseminated in the spring of 2024, and further recommendations can be made.

Conclusion and Limitations

The primary limitations in this project, which contributed to a prolonged investigation period, included the lack of reliability of the GC machine and equipment provided within AHU laboratory. The AHU laboratory's GC had many faults associated with it such as leaks in the lines of the machine, lack of helium to operate the machine appropriately, and detection of

erroneous gasses. Repairs to the machine and correction of these faults caused delays in collecting accurate data. Other limitations include having too small of a sample size to be able to conclude that there was a linear curve, which would have proven an alcohol concentration of zero at a certain time on the x axis if more data points were applied. The scholarly project required more samples and data points to provide efficacy in the results.

This scholarly project was able to achieve some of its objectives, and the data can be deduced in a fashion where further research may not be required. The initial goal of identifying the amount of alcohol that passes through the epidural catheter after scrubbing the hub was achieved and can be used to recommend that it is safe after waiting at least 53 seconds between scrubbing with IPA and administration of medication. The alcohol was not tested on rat astrocytes, as time did not permit further investigation. If this scholarly project were to continue, the detected amounts of IPA would have been directly applied to rat astrocyte cells to study the effect and determine potential cell death.

Dissemination

The conclusive evidence acquired at the end of this scholarly project (by April 2024), was disseminated to AHU in Orlando, Florida. The scholarly project is posted on the AHU website after it was completed and approved by the faculty. The information was presented in charts in Microsoft Excel and Microsoft PowerPoint. The information was also presented on a visual poster per the requirements of the DNAP program at AHU.

References

Al-Jumah, R., Urits, I., Viswanath, O., Kaye, A. D., & Hasoon, J. (2020). Radiofrequency ablation and alcohol neurolysis of the splanchnic nerves for a patient with abdominal pain from pancreatic cancer. *Cureus*, *12*(10), e10758. https://doi.org/10.7759/cureus.10758

American Association of Nurse Anesthesiology. (2012). *Infection control guide for certified registered nurse anesthetists.* https://www.aana.com/docs/default-source/practice-aana-com-web-documents-(all)/professional-practice-manual/infection-control-guide-for-crnas-archived.pdf?sfvrsn=e8ff45b1_4

Amr, S. A., Reyad, R. M., Othman, A. H., Mohamad, M. F., Mostafa, M. M., Alieldin, N. H., & Hamed, F. A. (2018). Comparison between radiofrequency ablation and chemical neurolysis of thoracic splanchnic nerves for the management of abdominal cancer pain, randomized trial. *European Journal of Pain (London, England)*, *22*(10), 1782–1790. https://doi.org/10.1002/ejp.1274

Avelar-Freitas, B. A., Almeida, V. G., Pinto, M. C., Mourão, F. A., Massensini, A. R., Martins-Filho, O. A., Rocha-Vieira, E., & Brito-Melo, G. E. (2014). Trypan blue exclusion assay by flow cytometry. *Brazilian Journal of Medical and Biological Research*, *47*(4), 307–315. https://doi.org/10.1590/1414-431X20143437

Boyce, J. M. (2018). Alcohols as surface disinfectants in healthcare settings. *Infection Control and Hospital Epidemiology*, *39*(3), 323–328. https://doi.org/10.1017/ice.2017.301

Brozek, J. L., Akl, E. A., Alonso-Coello, P., Lang, D., Jaeschke, R., Williams, J. W., Phillips, B., Lelgemann, M., Lethaby, A., Bousquet, J., Guyatt, G. H., Schünemann, H. J., & GRADE Working Group (2009). Grading quality of evidence and strength of recommendations in clinical practice guidelines. Part 1 of 3. An overview of the GRADE approach and

grading quality of evidence about interventions. *Allergy*, *64*(5), 669–677. https://doi.org/10.1111/j.1398-9995.2009.01973.x

Bryda E. C. (2013). The mighty mouse: the impact of rodents on advances in biomedical research. *Missouri medicine*, *110*(3), 207–211.

Campbell, J. P., Plaat, F., Checketts, M. R., Bogod, D., Tighe, S., Moriarty, A., & Koerner, R. (2014). Safety guideline: Skin antisepsis for central neuraxial blockade. *Anesthesia, 69*(11), 1279-1286. https://doi.org/10.1111/anae.12844

Centers for Disease Control and Prevention. (2016, September 26). *Hemodialysis central venous catheter scrub-the-hub protocol*. U. S. Department of Health and Human Services. https://www.cdc.gov/dialysis/prevention-tools/scrub-protocols.html

Chen, Y., VanderLaan, P. A., & Heher, Y. K. (2021). Using the model for improvement and plan-do-study-act to effect smart change and advance quality. *Cancer Cytopathology*, *129*(1), 9-14.

Choi, E., Im, S. M., Yoo, H. S., Nahm, F. S., Kim, Y. H., Choe, G., & Lee, P. B. (2017). The neurological safety of an epidurally administered lipo-PGE1 agonist in rats. *Regional anesthesia and pain medicine*, *42*(1), 75–81. https://doi.org/10.1097/AAP.0000000000000520

Choi, E. J., Choi, Y. M., Jang, E. J., Kim, J. Y., Kim, T. K., & Kim, K. H. (2016). Neural ablation and regeneration in pain practice. *The Korean Journal of Pain*, *29*(1), 3–11. https://doi.org/10.3344/kjp.2016.29.1.3

Christoff, P. (2018). Running PDSA cycles. *Current problems in pediatric and adolescent health care*, *48*(8), 198-201.

D'Souza, R. S., & Hooten, W. M. (2022). Neurolytic blocks. In *StatPearls*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK537360/

D'Souza, R. S., & Warner, N. S. (2022). Phenol nerve block. In *StatPearls.* Treasure StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK525978/#_NBK525978_pubdet_

Goyal, M., Mishra, N. K., Sharma, A., Gaikwad, S. B., Mohanty, B. K., & Sharma, S. (1999). Alcohol ablation of symptomatic vertebral hemangiomas. *American Journal of Neuroradiology*, *20*(6), 1091–1096.

Grishagin, I. V. (2015). Automatic cell counting. *Analytical biochemistry*, *473*, 63–65. https://doi.org/10.1016/j.ab.2014.12.007

Kang, J., Liu, Y., Niu, L., Wang, M., Meng, C., & Zhou, H. (2020). Anesthesia upstream of the alcoholic lesion point alleviates the pain of alcohol neurolysis for intercostal neuralgia: A prospective randomized clinical trial. *Clinics*, *75*, e1296. https://doi.org/10.6061/clinics/2020/e1296

Kemble, D. J., & Cervinski, M. A. (2020). A Single-Column gas chromatography method for quantifying toxic alcohols. *The Journal of Applied Laboratory Medicine, 5*(2), 300-310.

Khawaja, S. N., & Scrivani, S. J. (2020). Utilization of neurolysis in management of refractory head and neck cancer-related pain in palliative patients: A retrospective review. *Journal of Oral Pathology & Medicine*, *49*(6), 484–489. https://doi.org/10.1111/jop.13058

Kim, B. H., No, M. Y., Han, S. J., Park, C. H., & Kim, J. H. (2015). Paraplegia following intercostal nerve neurolysis with alcohol and thoracic epidural injection in lung cancer patient. *The Korean Journal of Pain*, *28*(2), 148–152. https://doi.org/10.3344/kjp.2015.28.2.14

Koyyalagunta, D., Engle, M. P., Yu, J., Feng, L., & Novy, D. M. (2016). The effectiveness of

alcohol versus phenol based splanchnic nerve neurolysis for the treatment of intra-abdominal cancer pain. *Pain Physician, 19*(4), 281-292. https://doi.org/10.36076/ppj/2019.19.281

Leung, K. Y., Chan, W. K., Man, W. K., & Ko, C. H. (2021). Comparison of alcohol and phenol neurolysis in children with spasticity: A pilot matched controlled trial. *Hong Kong Journal of Paediatrics*, *26*, 141-148.

Miller, B. (2013). Arachnoiditis: Are we accusing the wrong agent(s)? *Anesthesia, 68*(4), 423. https://doi.org/10.1111/anae.12183

Melnyk, B., & Fineout-Overholt, E. (2023). *Evidence-based practice in nursing & healthcare: A guide to best practice* (5th ed.). Wolters Kluwer.

Odom, C. B. (1940). Epidural anesthesia in résumé and prospect. *Anesthesiology*, *1*,119-120. https://doi.org/10.1097/00000542-194007000-00032

Ongena, K., Das, C., Smith, J. L., Gil, S., & Johnston, G. (2010). Determining cell number during cell culture using the Scepter cell counter. *Journal of Visualized Experiments,* (45), e2204. https://doi.org/10.3791/2204

Poddar, K., Dasgupta, S., & Gulati, R. (2016). Epidural alcohol neurolysis – A good option for cancer pain management in developing countries. *Journal of Anesthesia & Critical Care: Open Access*, *6*(5), 00244. https://doi.org/10.15406/jaccoa.2016.06.00244

Rangel Jaimes, G. W., Ortega Agón, K. A., & Cediel Carrillo, X. J. (2022). Intrathecal neurolysis for the management of refractory pain in a patient with terminal oncological disease: Case report. *Pain Management, 12*(5), 569-577. https://doi.org/10.2217/pmt-2021-0097

Swerdlow, M. (1978). Intrathecal neurolysis. *Anesthesia*, *33*(8), 733–740.

https://doi.org/10.1111/j.1365-2044.1978.tb08472.x

The Joint Commission. (2013). Preventing central line-associated bloodstream infections: Useful tools, an international perspective. https://www.jointcommission.org/-/media/tjc/documents/resources/health-services-research/clabsi-toolkit/clabsi_toolkit_tool_3-21_scrub_the_hubpdf.pdf?db=web&hash=79BF0D29BD4AAF13DEC3C3DE5AB90494&hash=79BF0D29BD4AAF13DEC3C3DE5AB90494

Wang, B. B., Wang, B. L., Cao, Z. Y., & Wang, Y. (2011). Effects of perineurial block of sciatic nerve with different concentrations of alcohol on structure and function of the nerve and its innervated muscles in rats (Chinese). *Chinese Journal of Physical Medicine and Rehabilitation, 33*(12), 891-897.

Appendix A

<table>
<tr><td colspan="6">References
Al-Jumah, R., Urits, I., Viswanath, O., Kaye, A. D., & Hasoon, J. (2020). Radiofrequency Ablation and Alcohol Neurolysis of the Splanchnic Nerves for a Patient With Abdominal Pain From Pancreatic Cancer. Cureus, 12(10), e10758. https://doi.org/10.7759/cureus.10758
Kim, B. H., No, M. Y., Han, S. J., Park, C. H., & Kim, J. H. (2015). Paraplegia following intercostal nerve neurolysis with alcohol and thoracic epidural injection in lung cancer patient. The Korean journal of pain, 28(2), 148–152. https://doi.org/10.3344/kjp.2015.28.2.14</td></tr>
<tr><td>Purpose</td><td>Variables</td><td>Setting/Subjects</td><td>Measurement and Instruments</td><td>Results</td><td>Evidence Quality</td></tr>
<tr><td>Study One:
Evaluate radio frequency ablation and alcohol neurolysis on the improvement of pain
Study two:
Receive an epidural to provide treatment of intercostal nerve neurolysis and evaluate pain
Design
Study one: case report

Study two: case report</td><td>Study one:
Independent: solution of 8 mL (100% ethyl alcohol) & 2 mL (contrast)
Dependent: relief of pain from RFA and alcohol neurolysis

Study two: Independent: alcohol injected at Thoracic 8-10 and intercostal nerve (11th) and fluoroscopy-guided thoracic epidural injection left T9-10 interlaminar space of 0.125% chirocaine 8ml & dexamethasone 5 mg
Dependent: bilateral motor response (neurological effects)</td><td>Study one
Setting: N/A
Subject: male with pancreatic cancer (50 yrs)

Study two:
Setting: Department of Anesthesiology and Pain Medicine, Konkuk University Medical Center, Seoul, Korea

Subject: woman with lung cancer (42 yrs old)</td><td>Study one
numerical rating scale (NRS) for pain rating.

Study two: Used an MRI to compare and contrast before and after the epidural injection and they also used a VAS (visual analogue scale) to assess pain at different time periods before the epidural injection.</td><td>Study one: the patient reported relief of his pain & seventh post-procedure day, he reported an 80% improvement and needed less opioids for pain management
Study two: paraplegia was caused by spinal cord ischemia, or possibly spinal cord neurolysis. significant motor weakness in the lower extremities (hip flexion 0/0, abduction 0/0, adduction 0/0, knee extension 0/0, flexion 0/0, dorsi-flexion 0/0) and a sensory decrease below the L2 level
Implications
Study one: abdominal pain related to pancreatic cancer was improved with RFA and alcohol neurolysis of bilateral splanchnic nerves

Study two: Motor movement should be checked out before the procedure. Alcohol should be injected distal from the spine with small amounts of alcohol for epidurals. The contrast solution should be used for correct placement.</td><td>Study one:
Methodological flaws: small/purposive sample size
Inconsistency: rating of pain afterwards
Indirectness: the results of the independent variables as an individual
Imprecision: small sample on participants for the treatment
Publication bias: none
Study two
Methodological flaws: small purposive sample size, didn’t state what kind of alcohol was used,
Inconsistency: no conclusiveness on what the cause of the paraplegia
Indirectness: no direct evidence of what caused the paraplegia
Imprecision: small sample size
Publication bias: none</td></tr>
</table>

<table>
<tr><td colspan="6">References
Leung, K. Y., Chan, W. K., Man, W. K., & Ko, C. H. (2021). Comparison of alcohol and phenol neurolysis in children with spasticity: A pilot matched controlled trial. HK J Paediatr (new series), 26, 141-8.
Kang, J., Liu, Y., Niu, L., Wang, M., Meng, C., & Zhou, H. (2020). Anesthesia upstream of the alcoholic lesion point alleviates the pain of alcohol neurolysis for intercostal neuralgia: a prospective randomized clinical trial. Clinics, 75.</td></tr>
<tr><td>Purpose</td><td>Variables</td><td>Setting/Subjects</td><td>Measurement and Instruments</td><td>Results</td><td>Evidence Quality</td></tr>
<tr><td>Study 1: To evaluate the effectiveness of phenol and alcohol neurolysis as treatment of spasticity
Study 2:
To find single point vs two point methods and result of alcohol neurolysis</td><td>Study 1
Independent: 5% phenol injection of 0.5l-1 ml & 50-70% alcohol 0.5-1 ml, musculocutaneous nerve & obturator nerve
Dependent: abolition of muscle contraction/spasticity
Study 2
Independent:
single point vs two- point method
Dependent: the degree of nerve injury/relief of pain and satisfaction</td><td>Study 1:
Setting: Pediatric rehabilitation unit in Caritas Medical Center in Hong Kong
Subjects: 6 patients less than 18 YOA suffering from chronic spasticity
Study 2: Setting: Hospitalized in the Fourth Affiliated Hospital of Harbin Medical University
Subjects: 33 patients (50-80 YOA) diagnosed with intercostal neuralgia</td><td>Study 1: Modified Ashworth Scale (MAS), Independent physiotherapist, Friedman test. Wilcoxon's sign rank test, Mann-Whitney-U test, Passive range of movement (PROM) of hip abduction / elbow extension
Study 2
10-point VAS, degrees of numbness, blood pressure, heart rate, chi-square test and Fisher’s exact test</td><td>Study 1: PROM statistical significance in alcohol at one month (p=0.024), three months (p=0.630) and six months (p=0.317) post injection. PROM statistical significance in phenol at one month (p=0.034), 3 months (p=0.059) and six months (p=0.317) post injection
Study 2: VAS in the two point evaluation was greater than the single point as well as level of numbness at one and three months postoperatively.</td><td rowspan="2">Study 1:
Methodological flaws: small sample size
Inconsistency: unstable availability of phenol, subjective data (pain and sensation) are difficult to assess in the subjects
Indirectness: none
Imprecision: small sample size
Publication bias: none

Study 2:
Methodological flaws: small sample size
Inconsistency: subjective data
Indirectness: none
Imprecision: distribution of drug was not clear due to that the contrast agent was not used
Publication bias: none</td></tr>
<tr><td>Design</td><td colspan="3"></td><td>Implications</td></tr>
<tr><td>Study 1: Pilot Matched controlled trial

Study 2: Prospective randomized clinical trial</td><td colspan="3"></td><td>Study 1: Both phenol and alcohol in obturator and musculocutaneous neurolysis is an effective treatment to reduce spasticity. The two treatments have no significant difference.

Study 2: The nerve blocks decreased the pain derived from alcohol and there was an improvement of patient status</td></tr>
</table>

References

Amr, S. A., Reyad, R. M., Othman, A. H., Mohamad, M. F., Mostafa, M. M., Alieldin, N. H., & Hamed, F. A. (2018). Comparison between radiofrequency ablation and chemical neurolysis of thoracic splanchnic nerves for the management of abdominal cancer pain, randomized trial. *European journal of pain (London, England)*, *22*(10), 1782–1790. https://doi.org/10.1002/ejp.1274

Choi, E. J., Choi, Y. M., Jang, E. J., Kim, J. Y., Kim, T. K., & Kim, K. H. (2016). Neural Ablation and Regeneration in Pain Practice. *The Korean journal of pain*, *29*(1), 3–11. https://doi.org/10.3344/kjp.2016.29.1.3

Purpose	**Variables**	**Setting/Subjects**	**Measurement and Instruments**	**Results**	**Evidence Quality**
Study One: to compare treatment of Cancer pain in RFA vs Alcohol **Study Two:** the goal hear was to review current practice of neural ablation. In addition, to evaluate the subsequent regeneration of nerves.	**Study One:** RFA, Chemical alcohol ablation **Study Two:** alcohol, phenol, glycerol are all different alcohols considered in this study	**Study One setting:** South Egypt Cancer Institute (SECI) of Assuit University, Egypt. **Study One subjects:** 60 patients with abdominal cancer all of which were older than 18 years of age **Study Two setting:** not stated **Study Two Subject:** Systematic review of current literature	**Study One:** all analysis used SPSS **Study Two:** None	**Study One:** the time needed was shorter with the alcohol than with the RF, showing a P<0.001. In addition, blocking splanchnic bilateral nerves RFA was more effective in treating pain than alcohol **Study Two:** neural ablation can be done by physical and chemical methods. thermal conventional RFA is the most common and safest for patient use	**Study One:** **Methodological Flaws:** Convenience Sampling **Inconsistency:** none **Indirectness:** focus is on patient outcome and pain management **Imprecision:** none **Publication bias:** none **Study Two:** **Methodological flaws:** none **Inconsistency:** no discussion on which type of alcohol is used **Indirectness:** none **Publication bias:** yes
Design				**Implications**	
Study One: Prospective randomized clinical trial **Study Two:** Systematic Review				**Study One:** the patient effect of RFA and chemical neurolysis of bil thoracic splanchnic nerves was evaluated **Study Two:** The best measure of a neural ablative technique is adequate strength, duration of action, and lack of complications	

References

Goyal, M., Mishra, N. K., Sharma, A., Gaikwad, S. B., Mohanty, B. K., & Sharma, S. (1999). Alcohol ablation of symptomatic vertebral hemangiomas. *AJNR. American journal of neuroradiology*, *20*(6), 1091–1096.

Khawaja, S. N., & Scrivani, S. J. (2020). Utilization of neurolysis in management of refractory head and neck cancer-related pain in palliative patients: A retrospective review. *Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*, *49*(6), 484–489. https://doi.org/10.1111/jop.13058

Purpose	Variables	Setting/Subjects	Measurement and Instruments	Results	Evidence Quality
Study One: Evaluation of the therapeutic efficacy on alcohol ablation for treating vertebral hemangiomas. **Study Two:** chemical neurolysis procedures for management of refractory HNC-related pain was studied to evaluate and determine the effectiveness	**Study One:** steroid administration prior to the procedure, Location of vertebral hemangioma, **Study Two:** alcohol, phenol, glycerol are all different alcohols considered in this study	**Study One setting:** not stated **Study One subjects:** ages of 15 to 59 years, 10 are male & 4 are female **Study Two setting:** Medicine clinic, Shaukat Khanum Memorial Cancer Hospital patients were used as a retrospective chart review who underwent chemical neurolysis in regions of head and neck for HNC/orofacial pain **Study Two Subject:** 33 patients. All adult male and female	**Study One:** MR imaging was performed and then followed up at different time intervals.14 patients were seen 48 to 96 hours and then at 2 months; 6 patients at 9 to 15 months. Results were broken into excellent, good, and failure of treatment. Subjective information was categorized. The MRIs revealed improvement or deterioration. **Study Two:** Independent and dependent variables were divided with Chi- square and *t* tests. If they couldn't be divided into these methods then Fisher's exact test was used. Version 22.0 SPSS software was used. Variables were formulated as descriptive statistics.	**Study One:** All subjects showed temporary deterioration of neuro status after alcohol ablation. 5 were categorized as excellent and 8 as good. 1 patient had paravertebral abscess. Improvement was seen in 11 patients after the treatment for a time span of 5 to 31 months. **Study Two:** At 1-month follow-up, pain relief was 72.7% of participants . Adverse effects were in 9.1% after neurolysis. Neurolysis effectiveness and chronicity of pain showed a correlation.	**Study One:** **Methodological Flaws:** subjective **Inconsistency:** n/a **Indirectness:** focus is on patient and reduction of vertebral hemangiomas **Imprecision:** n/a **Publication bias:** n/a **Study Two:** **Methodological flaws:** n/a **Inconsistency:** n/a **Indirectness:** clinical pain outcome **Publication bias:** N/a
Design				**Implications**	
Study One: Controlled clinical trial **Study Two:** Retrospective meta-analysis				**Study One:** improvement was seen in 85% of patients with symptoms of vertebral hemangiomas that received alcohol ablation. **Study Two:** Chemical neurolysis when applied to head and neck nerve regions is effective in treatment of pain	

<table>
<tr><td colspan="6">References
Koyyalagunta, D., Engle, M. P., Yu, J., Feng, L., & Novy, D. M. (2016). The Effectiveness of Alcohol Versus Phenol Based Splanchnic Nerve Neurolysis for the Treatment of Intra-Abdominal Cancer Pain. Pain Physician, 19(4), 281-292. 10.36076/ppj/2019.19.281
Poddar, K., Dasgupta, S., & Gulati, R. (2016). Epidural Alcohol Neurolysis – A Good Option for Cancer Pain Management in Developing Countries. Journal of Anesthesia & Critical Care: Open Access, 6(5). https://doi.org/10.15406/jaccoa.2016.06.00244</td></tr>
<tr><td>Purpose</td><td>Variables</td><td>Setting/Subjects</td><td>Measurement and Instruments</td><td>Results</td><td>Evidence Quality</td></tr>
<tr><td>Study One: A retrospective chart review of patients that underwent SNN and incidence of alcohol induced complications.
Study Two: creating safety measures for epidural alcohol neurolysis for cancer pain.</td><td rowspan="2">Study One:
Primary Outcome: no complications were seen between agents. Agents are appropriate for use.
Secondary Outcome: The choice of neurolytic agent can be to clinical discernment.
Study Two:
Primary Outcome: Epidural alcohol neurolysis is an alternative for cancer pain
Secondary Outcome: Safety is linked to installation, daily assessment of pain and position, and dosing with of ethyl alcohol less than 5 ml</td><td rowspan="2">Study One:
Setting: new diagnostic and therapeutic interventions were absent. Direct patient contact did not occur.
Subjects: 93 patients spanning 3 years

Study Two:
Setting: the study did not disclose the setting
Subjects: 10 patients with proven cancer</td><td rowspan="2">Study one: Filemaker Pro version 9 database was created. Data was collected from clinical notes, BPI, and ESAS. Fisher’s exact test and Chi-square test were used. Wilcoxon rank sum test or Kruskal-Wallis test were also used. Statistical software SAS 9.1.3 (SAS, Cary, NC) was used for analyses.
Study Two: Microsoft excel and t-tests were used using SPSS v20.0. The values were presented as a number, percent, range, mean ± SD. At 95% the differences in confidence interval were significant at $p<0.05$.</td><td>Study one: No pain differences were found between alcohol and phenol.
Study Two:
Subarachnoid phenol injection is painless.</td><td rowspan="2">Methodological flaws:
Study One: The study did not completely assess burden of the biopsychosocial symptom
Study Two: large studies that are controlled and refined while using alternative radiological methods are needed to improve safety and efficiency

Inconsistency:
Study One: limited evidence showed no comparison between techniques observing side effects and efficiency.
Study Two: None

Indirectness:
Study One: None
Study Two: None

Imprecision:
Study One: None
Study Two: None

Publication bias:
Study One: None
Study Two: None</td></tr>
<tr><td>Design</td><td>Implications</td></tr>
<tr><td>Study One:
Retrospective Evaluation

Study Two:
Statistical Analysis</td><td>Study one: no difference in complications was seen between the agents. Choice of neurolytic agent can appropriately be left to the clinical judgment
Study Two: Alcohol neurolysis can increase quality of life. No adverse effects were observed.</td></tr>
</table>

References

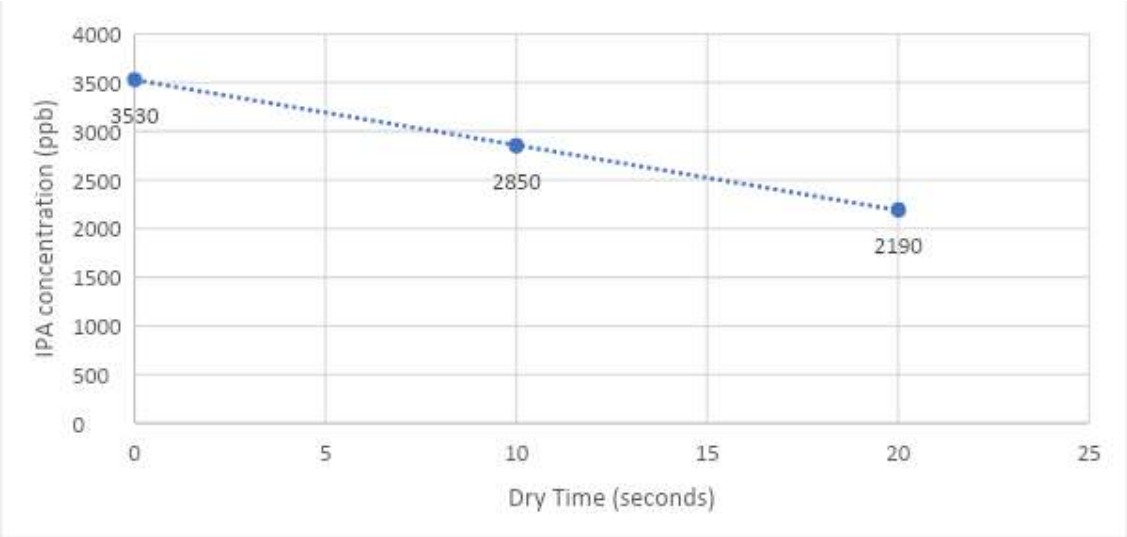
Campbell, J. P., Plaat, F., Checketts, M. R., Bogod, D., Tighe, S., Moriarty, A., & Koerner, R. (2014). Safety guideline: skin antisepsis for central neuraxial blockade : Association of Anaesthetists of Great Britain and Ireland Obstetric Anaesthetists' Association Regional Anaesthesia UK Association of Paediatric Anaesthetists of Great Britain and Ireland. *Anaesthesia, 69*(11), 1279. 10.1111/anae.12844

Doan, L., Piskoun, B., Rosenberg, A. D., Blanck, T. J. J., Phillips, M. S., & Xu, F. (2012). In Vitro Antiseptic Effects on Viability of Neuronal and Schwann Cells. *Regional Anesthesia and Pain Medicine, 37*(2), 131-138. 10.1097/AAP.0b013e31823cdd96

Purpose	**Variables**	**Setting/Subjects**	**Measurement and Instruments**	**Results**	**Evidence Quality**
Study One: To create a document that considers which agent to use for skin antisepsis and how to apply it. **Study Two:** To evaluate the cellular damage of chlorhexidine and povidone-iodine in human and rat cells.	**Study One:** Primary Outcome: Precautions to prevent alcohol from reaching CSF Secondary Outcome: Careful attention to antiseptic application are important factors to reduce neurotoxicity risks and infection rather than the choice of concentration of agent. **Study Two:** Primary Outcome: Numerous anesthesiology associations recommend chlorhexidine for antisepsis before regional anesthesia. However this is a concern the agent may be damaging to cells. Secondary Outcome: Chlorhexidine gluconate and povidone-iodine were toxic neuronal cells.	**Study One:** **Setting:** The setting was not described **Subjects:** the study was conducted by collecting previously established guidelines, lab studies, and clinical reports **Study Two:** **Setting:** the setting was not described **Subjects:** Human neuroblastoma and rat schwann cells	**Study One:** The Royal College of Anesthetists, American Society of Anesthesiologists, and the American Society of Regional Anesthesia published criteria **Study Two:** Human neuroblastoma cells and rat Schwann cells were incubated with 2% chlorhexidine gluconate and 10% povidone-iodine. Viability was assessed with the MTT colorimetry assay and the fluorescent assay using calcein and ethidium.	**Study One:** Information was limited on the risk of neurotoxicity with chlorhexidine. None of the guidelines specify concentrations, nor do they have guidelines for it. **Study Two:** In the tested concentrations of chlorhexidine, there were decreased viability of nerves, and proved to be more toxic than povidone-iodine for all cells with a $P < 0.001$.	**Methodological flaws:** **Study One:** N/a **Study Two**: N/a **Inconsistency:** **Study One:** n/a **Study Two**: N/a **Indirectness**: **Study One:** N/a **Study Two**: N/a **Imprecision:** **Study One:** n/a **Study Two**: n/a **Publication bias**: **Study One**: n/a **Study Two**: N/a

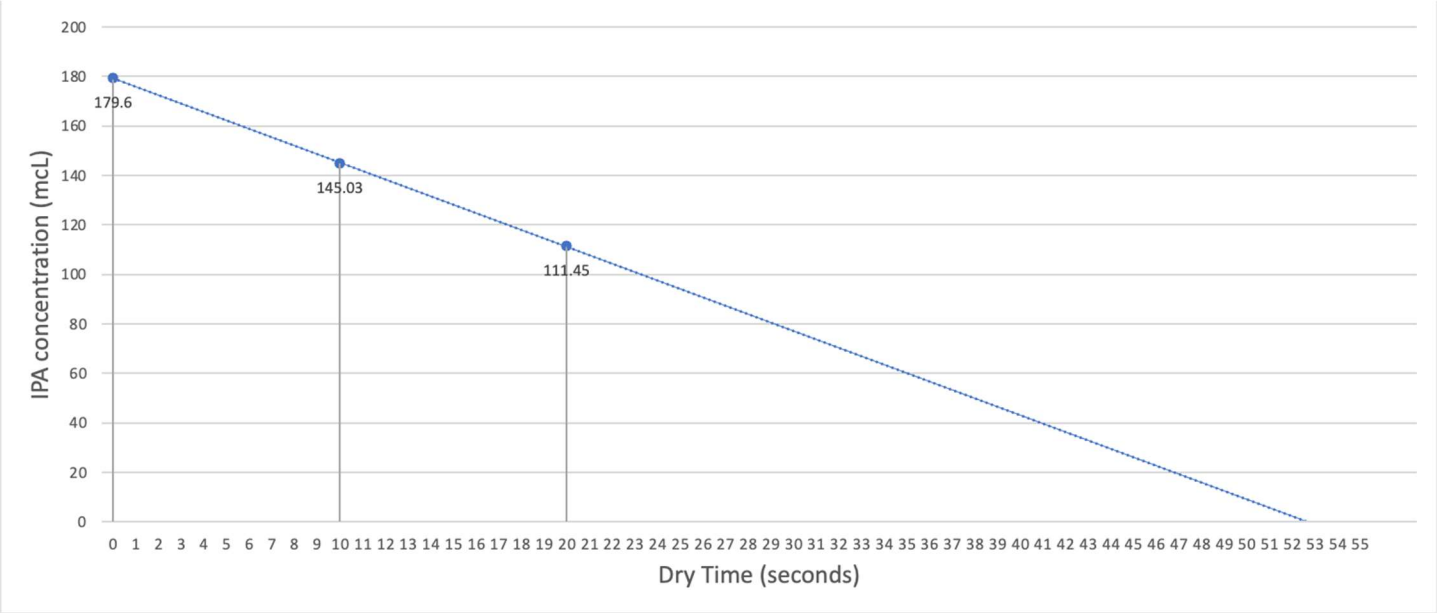
Appendix B

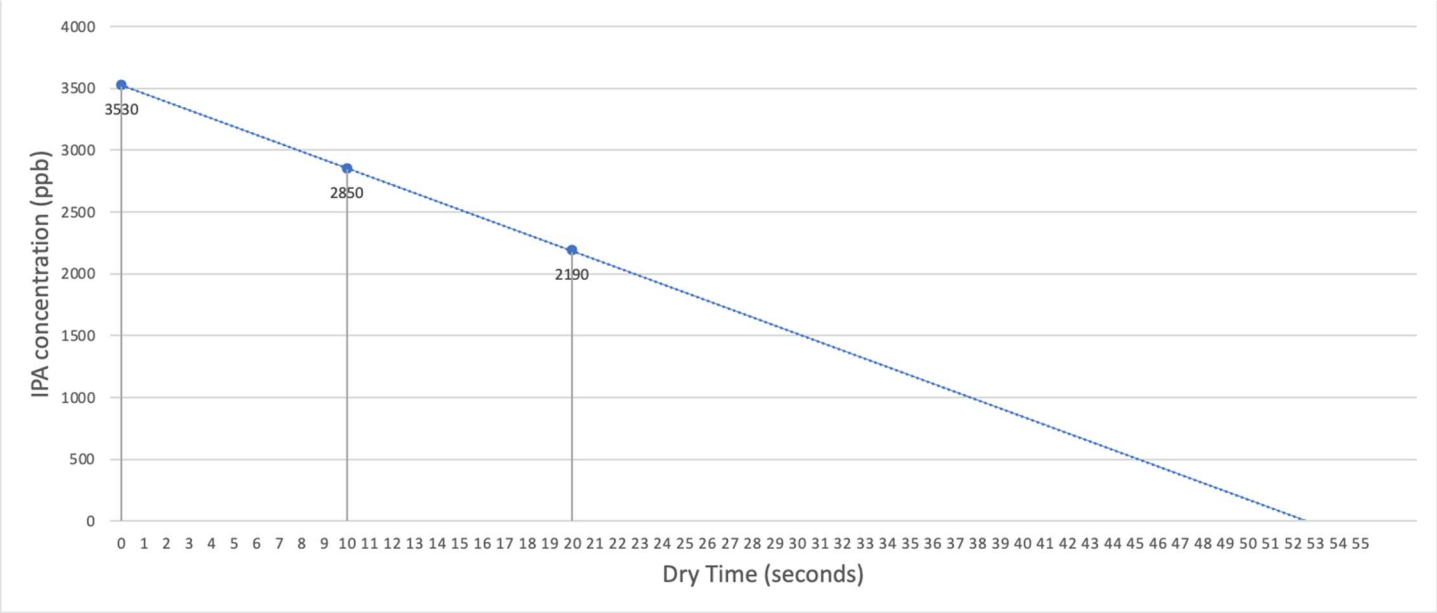
Figure E1:Concentration in parts per billion and Dry time Response Curve



Appendix C

Figure E2: Concentration in microliters and Dry Time Response forecast curve





Appendix D

Lab Results

Analytical Results

Client Sample ID: **S40 A,B,C** | Lab Sample ID: **2309037-001** | Date Collected: **09/25/2023 12:00** | Collected By: **Sebastian Farrell** | Matrix ID : **AQUEOUS-Other**

VOC BY GC/MS (8260D)

Analyte Name (Analyte ID)	Results/Qual	Units	DF	MDL	PQL	Method	Analyzed Date	By	Batch	Notes
Isopropyl Alcohol (67630)	*128*	µg/L	1	6.29	50	8260D	10/03/23 13:46	ALS	R172022	-

Client Sample ID: **S80 A,B,C** | Lab Sample ID: **2309037-002** | Date Collected: **09/25/23 12:05** | Collected By: **Sebastian Farrell** | Matrix ID : **AQUEOUS-Other**

VOC BY GC/MS (8260D)

Analyte Name (Analyte ID)	Results/Qual	Units	DF	MDL	PQL	Method	Analyzed Date	By	Batch	Notes
Isopropyl Alcohol (67630)	*186*	µg/L	1	6.29	50	8260D	10/03/23 14:11	ALS	R172022	-

Client Sample ID: **DT0 A,B,C** | Lab Sample ID: **2309037-003** | Date Collected: **09/25/23 12:10** | Collected By: **Sebastian Farrell** | Matrix ID : **AQUEOUS-Other**

VOC BY GC/MS (8260D)

Analyte Name (Analyte ID)	Results/Qual	Units	DF	MDL	PQL	Method	Analyzed Date	By	Batch	Notes
Isopropyl Alcohol (67630)	*3530*	µg/L	10	62.9	500	8260D	10/03/23 12:30	ALS	R172022	- D10

Client Sample ID: **DT10 A,B,C** | Lab Sample ID: **2309037-004** | Date Collected: **09/25/23 12:15** | Collected By: **Sebastian Farrell** | Matrix ID : **AQUEOUS-Other**

VOC BY GC/MS (8260D)

Analyte Name (Analyte ID)	Results/Qual	Units	DF	MDL	PQL	Method	Analyzed Date	By	Batch	Notes
Isopropyl Alcohol (67630)	*2850*	µg/L	10	62.9	500	8260D	10/03/23 12:56	ALS	R172022	- D10

Client Sample ID: **DT20 A,B,C** | Lab Sample ID: **2309037-005** | Date Collected: **09/25/23 12:20** | Collected By: **Sebastian Farrell** | Matrix ID : **AQUEOUS-Other**

VOC BY GC/MS (8260D)

Analyte Name (Analyte ID)	Results/Qual	Units	DF	MDL	PQL	Method	Analyzed Date	By	Batch	Notes
Isopropyl Alcohol (67630)	*2190*	µg/L	10	62.9	500	8260D	10/03/23 13:22	ALS	R172022	- D10

Detection Summary :

Client Sample ID: **S40 A,B,C** | Lab Sample ID: **2309037-001** | Date Collected: **09/25/2023 12:00** | Collected By: **Sebastian Farrell** | Matrix ID : **AQUEOUS-Other**

Analyte Name (Analyte ID)	Results/Qualifier	Units	DF	MDL	PQL	Date Analyzed	By	Batch ID	Method
Isopropyl Alcohol (67630)	**128**	µg/L	1	6.29	50	10/03/23 13:46	ALS	R172022	8260D

Client Sample ID: **S80 A,B,C** | Lab Sample ID: **2309037-002** | Date Collected: **09/25/23 12:05** | Collected By: **Sebastian Farrell** | Matrix ID : **AQUEOUS-Other**

Analyte Name (Analyte ID)	Results/Qualifier	Units	DF	MDL	PQL	Date Analyzed	By	Batch ID	Method
Isopropyl Alcohol (67630)	**186**	µg/L	1	6.29	50	10/03/23 14:11	ALS	R172022	8260D

Client Sample ID: **DT0 A,B,C** | Lab Sample ID: **2309037-003** | Date Collected: **09/25/23 12:10** | Collected By: **Sebastian Farrell** | Matrix ID : **AQUEOUS-Other**

Analyte Name (Analyte ID)	Results/Qualifier	Units	DF	MDL	PQL	Date Analyzed	By	Batch ID	Method
Isopropyl Alcohol (67630)	**3530**	µg/L	10	62.9	500	10/03/23 12:30	ALS	R172022	8260D

Client Sample ID: **DT10 A,B,C** | Lab Sample ID: **2309037-004** | Date Collected: **09/25/23 12:15** | Collected By: **Sebastian Farrell** | Matrix ID : **AQUEOUS-Other**

Analyte Name (Analyte ID)	Results/Qualifier	Units	DF	MDL	PQL	Date Analyzed	By	Batch ID	Method
Isopropyl Alcohol (67630)	**2850**	µg/L	10	62.9	500	10/03/23 12:56	ALS	R172022	8260D

Client Sample ID: **DT20 A,B,C** | Lab Sample ID: **2309037-005** | Date Collected: **09/25/23 12:20** | Collected By: **Sebastian Farrell** | Matrix ID : **AQUEOUS-Other**

Analyte Name (Analyte ID)	Results/Qualifier	Units	DF	MDL	PQL	Date Analyzed	By	Batch ID	Method
Isopropyl Alcohol (67630)	**2190**	µg/L	10	62.9	500	10/03/23 13:22	ALS	R172022	8260D

Appendix E

Budget

Third-Party lab= $0

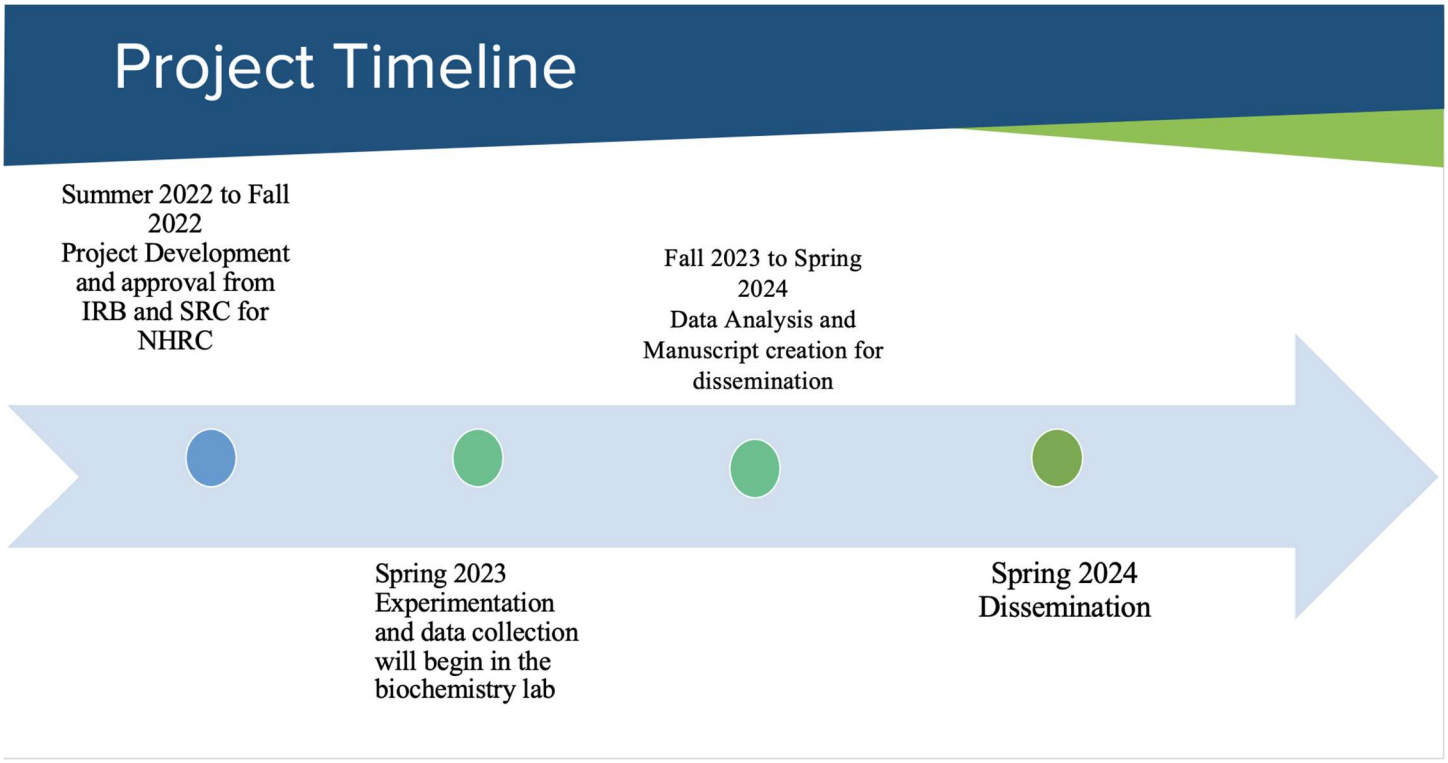
AHU Biochemistry lab supplies and use = $0

Total: $0

Appendix F

Timeline

Our timeline starts with the Summer of 2022 to Fall 2022 and project development and approval from IRB and SRC for non-human research committee (NHRC). In the spring of 2023, experimentation and data collection began in the biochemistry lab. Data collection extended to the Fall of 2023. Dissemination is to be reported in the Spring of 2024. Our initial proposed timeline was without deviation.



Appendix G

Conversion and math interpretation

Units – mcg/L is equivalent to ppb
1mcg = 1 x 10^-6gm = 1ppb

Using the formula volume = Mass/Density, we converted the results measured in mcg into mcL to relay the results in a more practical manner.

The density of IPA is 0.786gm/mL. For each detectable IPA level of the 3 dry times, we divided it by 1 liter which yielded us a result in grams per mL. We then divided that result by 40mL, the volume of the sample vial, which yielded us a result in grams per mL. We took that value and divided it by the density of IPA, which yielded us a result in mL. Finally, we took that value and converted to mcL to yield a final result measured in mcL.

Dry time	**Difference**	**Expressing in mcL because we measure in volumes in medical practicality (also emphasizes how small the vol is)**
0 seconds	37.9% difference between 0 and 20 dry times	3530mcg / 1000mL = 3.53mL = 0.00000353 grams/mL 0.00000353gm/ml x 40ml = 0.0001412gm/mL (in 40mL vial) 0.0001412gm/mL / 0.786gm/mL = 0.00017964mL (in 40mL vial) mL convert to mcL= _ x 1,000,000 = 179.6mcL (in 40mL vial)
10 seconds	19.3% difference between 0 and 10 dry times	2850mcg / 1000mL = 2.85 = 0.00000285gm/mL 0.00000285gm/mL x 40mL = 0.000114gm/mL (in 40mL vial) 0.000114gm/mL / 0.786gm/mL = 0.00014504mL (in 40mL vial) mL convert to mcL = 145.03mcL (in 40mL vial)
20 seconds	23% difference between 10 and 20 dry times	2190mcg / 1000mL = 2.19mL = 0.00000219 0.00000219gm/mL x 40mL = 0.0000876gm/mL (in 40mL vial) 0.0000876gm/mL / 0.786gm/mL = 0.00011145mL (in 40mL vial) mL convert to mcL = 111.45mcL (in 40mL vial)

There was breakthrough found with all the samples but the findings were significantly small.