Journal of the American Association for Laboratory Animal Science
Copyright 2024
by the American Association for Laboratory Animal Science

Vol 63, No 4
July 2024
Pages 357–358

Association of Primate Veterinarians Guidelines for Cerebrospinal Fluid Aspiration for Nonhuman Primates in Biomedical Research

Purpose

The Association of Primate Veterinarians (APV) recognizes that cerebrospinal fluid (CSF) collection may be required for both clinical and research purposes in nonhuman primates (NHP). Because there are inherent risks associated with the technique, the laboratory animal veterinarian should determine the need and utility of CSF collection for clinical evaluation. CSF collections for research purposes must be scientifically justified and approved by the institutional animal care and use committee (IACUC) or equivalent regulatory body hereafter referred to as IACUC. The following recommendations provide basic information for IACUCs, researchers using NHP models, and veterinary personnel to consider when developing policies and standard operating procedures for CSF collections.

DOI: 10.30802/AALAS-JAALAS-24-000029

Background

CSF analysis is a powerful tool that can augment detection of various processes occurring within the central nervous system (CNS). Examples include hemorrhage, inflammation, infection, neoplasia, and other clinical conditions. In addition, serial evaluation of compounds within the CSF, such as hormones, medications, neurotransmitters, biomarkers, metabolites, and viruses, may be extremely useful in both basic and translational research. CSF volume in the CNS is constantly being maintained by CSF formation and absorption.

Technique and Procedural Considerations

CSF is typically collected via puncture of the cisterna magna or lumbar vertebral space. Collection of CSF by lumbar puncture may involve less risk of complications because the lumbar site is well below the termination of the spinal cord in most NHPs. The animal should be appropriately anesthetized, and the procedure should be performed using an aseptic technique, including hair clipping, surgical preparation of the collection site, and use of sterile materials. At a minimum, gloves, syringes, and needles should be sterile. Sterile surgical drapes may also be considered. CSF collection is typically performed in lateral or sternal recumbency, or in a sitting position, with flexion of the neck or spine to improve access to the cisterna or widen the intervertebral space, respectively.[6,13,18] Needle size and length selected should be appropriate for the species and size of the NHP.[2,6,13,18] The techniques, supplies, and equipment used, as well as pre-, intra-, and postprocedural care, may vary depending on the NHP species, research aims, and preference of the clinician.

Some research protocols may require consecutive CSF collections over time, and/or require CSF samples from unanesthetized subjects. For these protocols, serial collection of CSF from conscious rhesus macaques may be accomplished through a chronically implanted cisternal catheter that is connected to a subcutaneous access port.[7,8,12]

In the creation of this document, every effort was made to find current references and literature regarding objective values for total CSF volume, limitations, or recommendations for the maximum number of dural punctures, frequency of collections, safe volume for withdrawal, and CSF replenishment rates. There is a paucity of literature in this area for most primate species. Furthermore, the techniques for collection vary as survey results collected from the APV membership indicate that facilities are successfully performing CSF collections using several different methods. We are therefore providing considerations and recommendations for CSF collection based on the best evidence currently available.

For reference, prior studies have determined the apparent CSF volume in adult male rhesus macaques (8.0 to 10.6 kg) was 10 ± 0.06 mL and the CSF flow rate was 0.018 ± 0.003 mL/min.[15] The CSF formation rates in rhesus macaques range from 28.6 to 44.2 µL/min.[4,11,14] Further research specific to NHPs is needed in this area to facilitate the development of more prescriptive recommendations.

Potential Adverse Effects

While few complications have been reported with CSF collections in NHPs, anatomic variation as well as inappropriate or poor procedural technique can result in a compromised sample and/or complications such as hemorrhage, herniation, infection, brain stem, spinal cord or nerve root damage, and animal discomfort. All these complications, while rare, can compromise animal welfare.

Due to the risks involved, CSF is usually collected by veterinarians or trained veterinary staff. Practices may vary with regard to unsuccessful CSF collection attempts. A common practice is the allowance of up to 3 unsuccessful attempts, or "sticks" by one individual. After the third failed attempt, another qualified individual can be allowed to attempt the CSF tap. If the second individual is unable to collect CSF after 3 attempts, the collection attempts are discontinued, and the animal should be provided appropriate analgesia while recovering from anesthesia. Changing collection sites (from cisterna to lumbar, for example) after failed attempts often results in a successful collection. Fluoroscopy may be used to assist in needle placement if attempts without imaging are unsuccessful. In addition to improving success rates, image guidance may reduce the incidence of traumatic puncture.[5]

Postprocedural monitoring should be provided continuously until the patient is returned to its home enclosure and able to remain consistently in an upright position without support. Patient monitoring should be done regularly for a minimum of 24 h or as indicated in the IACUC-approved protocol. Monitoring should include evaluation of basic NHP physiologic, behavioral, and locomotor parameters. In particular, animals

Submitted: 11 Mar 2024. Accepted: 27 Mar 2024.

should be assessed for signs of neurologic impairment including but not limited to lethargy, depression, obtundation, and head-pressing. In human medicine, spinal headache is not uncommon following CSF aspiration and resulting intracranial hypotension. In consideration of these potential sequelae in NHPs, analgesia should be provided at the time of the procedure and extended 2 to 3 d, or as needed based on postprocedural observations for behavior associated with headache or spinal pain. The most effective analgesia is likely of the multimodal variety administered preprocedurally.

IACUC Considerations and Recordkeeping

IACUCs should be aware of the CSF collection logistics and all possible complications and review them at least annually. All relevant information such as drug dosages, preprocedural preparation, CSF volume collected, frequency of collections, and postprocedural short- and long-term recovery events should be described in the IACUC protocol and entered in the medical record or laboratory logs where appropriate.

CSF can be a critical resource in a research protocol, but its collection can have consequences that impact animal welfare if the technique is not performed appropriately. Scientific justification should be provided for the number and frequency of collections performed. Specifically, the IACUC should also carefully evaluate each proposal involving CSF collection and consider the following issues:

1) Are CSF collections essential to address the scientific objectives stated in the protocol?
2) Are alternatives and less invasive methods of evaluating the CNS (such as imaging modalities) available and, if so, have they been considered?
3) Are the personnel performing the procedures appropriately trained and do they have adequate skills?
4) Will appropriate periprocedural anesthesia and analgesia be provided? The laboratory animal veterinarian should be consulted regarding the anesthesia and analgesia plan.
5) Is the volume of CSF to be collected appropriate for the age and size of the animal?
6) Are the number and frequency of meningeal punctures appropriate for the animal and have the risks of complications been minimized? If multiple CSF collections are performed as part of research investigations, the maximum number of collections per animal should be specified.
7) Is the plan for postprocedural monitoring adequate?
8) Have all possible postprocedural complications been considered and all reasonable interventional methods, inclusive of euthanasia, listed?

Disclaimer

The position statements and/or guidelines produced by the APV are intended to be recommendations and guidance and are not a regulatory requirement. The Scientific Advisory Committee (SAC) within APV is tasked with the generation and revision of guidance documents for use by the membership and primate specialists worldwide. A subcommittee of current APV members and subject matter experts who have expertise in the area of interest are recruited to draft a document that is then sent out for comment and input from the SAC committee, the APV Board of Directors, and the APV membership. The final version was approved by the Board of Directors before being published on the APV website. We would like to extend special thanks to the committee members who worked on and contributed to this document: Theodore R Hobbs, DVM (Oregon National Primate Research Center), Kristine E Killoran, PhD, DVM, DACLAM (Frederick National Laboratory for Cancer Research), and Rachelle L Stammen, DVM, DACLAM (Emory National Primate Research Center).

References

1. **Bistner SI, Ford RB.** 1995. Kirk and Bistner's handbook of veterinary procedures and emergency treatment. Philadelphia (PA): Saunders.
2. **Brady AG.** 2000. Research techniques for the squirrel monkey (*Saimiri* sp). ILAR J **41**:10–18.
3. **Bennett BT, Fortman J, Hewett T.** 2002. The laboratory nonhuman primate, p 174–177. Boca Raton (FL): CRC Press.
4. **Curran RE, Mosher MB, Owens ES, Fenstermacher JD.** 1970. Cerebrospinal fluid production rates determined by simultaneous albumin and inulin perfusion. Exp Neurol **29**:546–553. 10.1016/0014-4886(70)90079-8.
5. **Eskey CJ, Ogilvy CS.** 2001. Fluoroscopy-guided lumbar puncture: Decreased frequency of traumatic tap and implications for the assessment of CT-negative acute subarachnoid hemorrhage. AJNR Am J Neuroradiol **22**:571–576.
6. **Geretschlager E, Russ H, Mihatsch W, Przuntek H.** 1987. Suboccipital puncture for cerebrospinal fluid in the common marmoset (*Callithrix jacchus*). Lab Anim **21**:91–94.
7. **Gilberto DB, Zeoli AH, Szczerba PJ, Gehret JR, Holahan MA, Sitko CR, Johnson CA, Cook JJ, Motzel SL.** 2003. An alternative method of chronic cerebrospinal fluid collection via the cisterna magna in conscious rhesus monkeys. Contemp Top Lab Anim Sci **42**:53–59.
8. **Gilberto DB, Michener MS, Smith BE, Szczerba PJ, Holahan MA, Gray TL, Motzel SL.** 2022. Chronic collection of cerebrospinal fluid from rhesus macaques (*Macaca mulatta*) with cisterna magna ports: Update on refinements. Comp Med **72**:45–49.
9. **Li X, Han P, Guo Y, Sun H, Xiao Y, Kang YJ.** 2015. An improved technique for cerebrospinal fluid collection of cisterna magna in rhesus monkeys. J Neurosci Methods **249**:59–65.
10. **Lipman B, Palmer D, Noble J, Haughton V, Collier D.** 1988. Effect of lumbar puncture on flow of cerebrospinal fluid. Invest Radiol **23**:359–360.
11. **Lux WE Jr, Fenstermacher JD.** 1975. Cerebrospinal fluid formation in ventricles and spinal subarachnoid space of the rhesus monkey. J Neurosurg **42**:674–678.
12. **MacAllister RP, Lester McCully CM, Bacher J, Thomas ML III, Cruz R, Wangari S, Warren KE.** 2016. Minimally invasive lumbar port system for the collection of cerebrospinal fluid from rhesus macaques (*Macaca mulatta*). Comp Med **66**:349–352.
13. **Magden ER, Mansfield KG, Simmons JH, Abee CR.** 2015. Nonhuman primates, p 838–839. In: Anderson LC, Fox JG, Otto G, Pritchett-Coming KR, Whary MT, editors.Laboratory animal medicine, 3rd ed. San Diego (CA): Academic Press (Elsevier).
14. **Martins AN, Newby N, Doyle TF.** 1977. Sources of error in measuring cerebrospinal fluid formation by ventriculocisternal perfusion. J Neurol Neurosurg Psychiatry **40**:645–650. 10.1136/jnnp.40.7.645.
15. **Lester McCully CM, Rodgers LT, Garica RC, Thomas ML, Peer CJ, Figg WD, Barnard DE, Warren KE.** 2020. Flow rate and apparent volume of cerebrospinal fluid in rhesus macaques (*Macaca mulatta*) based on the pharmacokinetics of intrathecally administered inulin. Comp Med **70**:526–531.
16. **Smith MO, Lackner AA.** 1993. Effects of sex, age, puncture site, and blood contamination on the clinical chemistry of cerebrospinal fluid in rhesus macaques (*Macaca mulatta*). Am J Vet Res **54**:1845–1850.
17. **Ternes JW, Norton W, Leak D, Silverman D, Ehrman RN, O'brien CP.** 1986. Catheterization of the lumbar subarachnoid space for long-term CSF collection in unanesthetized nonhuman primates. Behav Res Meth Instrument Comp **18**:377–381.
18. **Wolf RF, White GL.** 2012. Clinical techniques used for non-human primates, p 323–336. In: Abee C, Mansfield K, Tardif S, Morris T, editors. Nonhuman primates in biomedical research: Biology and management, 2nd ed. San Diego (CA): Academic Press (Elsevier).