昭衍 JOINN

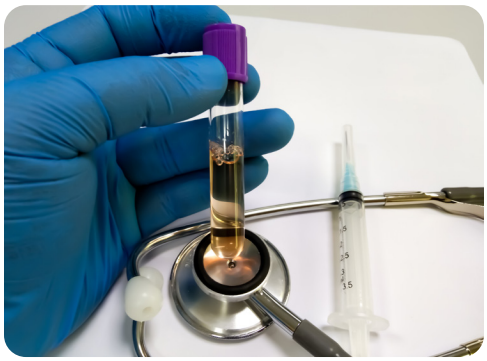
Biomere a JOINN company

YOUR GLOBAL PRECLINICAL CRO
MASSACHUSETTS • CALIFORNIA • CHINA

APPLICATION NOTES

APPLICATION NOTE: CSF SAMPLING TO AID DIAGNOSIS AND PRECLINICAL DRUG DISCOVERY

The Importance of CSF Sampling in Diagnostics

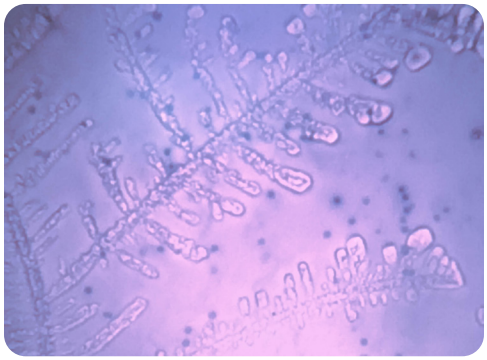


Cerebrospinal fluid (CSF) is a filtrate of plasma that surrounds the brain and spinal cord that provides nutrients, removes waste and cushions the central nervous system (CNS). The CSF can be accessed via a lumbar puncture where a needle is inserted between vertebrae into the space around the spinal cord. Due to the relatively noninvasive procedure, CSF is a high value biofluid for the diagnosis of various diseases including cancer, neurodegenerative diseases and infectious diseases[1]. CNS tumors have been reported to shed cells directly into CSF so these circulating tumor cells can be identified using sensitive methods like the FDA approved CellSearch® system[2]. Currently, the CellSearch® system is used to identify circulating tumor cells from breast, colorectal and prostate tumors, and has been shown to identify tumor cells in breast cancer related brain metastases. Newer technologies such as the Target Selector™ from Biocept can identify circulating tumor cells as well as cell free nucleic acids (DNA and RNA)[3]. Sequencing DNA or RNA from tumor cells provides valuable information on disease causing mutations, second hit mutations and other genetic abnormalities.

Neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, dementia etc. are diagnosed through a combination of tests that includes biomarker analysis and cognitive testing. Analysis of CSF for specific biomarkers is established for Alzheimer's disease where markers such as the beta-amyloid peptides (Aβ40 & Aβ42), phosphorylated and total Tau protein levels[4] as well as ratios of the different protein markers. Current efforts are focused on combining biomarker analysis from patient CSF samples with imaging studies to correlate disease progression with biomarker expression levels[5]. As of now, there are no clear Alzheimer's disease diagnostic markers but it is possible that future research may identify a specific combination of CSF markers, imaging and other diagnostic approaches to detect early-stage disease. Interestingly, research interest to identify CSF markers for Parkinson's disease has been limited but there are some ongoing studies to identify CSF biomarkers for Parkinson's disease. Disease agnostic markers for glial cell inflammation or cell damage can be identified in CSF samples such as YKL40, a microglia inflammation marker that is hypothesized to be involved in tissue remodeling due to inflammation. YKL40 is a secreted glycoprotein that has been reported to be upregulated in several diseases including cancer, diabetes and several neurological diseases[6].

Infectious diseases in the brain can range from relatively benign to life threatening. In most cases, early accurate diagnosis is critical for successful management and CSF analysis is a critical diagnostic tool to detect pathogen caused infections. The CSF is considered to be a mirror of CNS health so analysis of CSF samples can be used to diagnose acute and chronic infectious diseases using various physiological and molecular endpoints[7] including analytical chemistry, serology and PCR. Since CSF sampling is noninvasive and is commonly performed in a doctor's office, it is a useful method to monitor chronic infectious diseases such as AIDS and certain forms of meningitis and encephalitis.

CSF Sampling in Preclinical Animal Models



It is clear that CSF sampling is a critical method to facilitate diagnosis of CNS diseases. Another critical application of CSF sampling is the monitoring of drug concentrations in the CNS after local or systemic administration. Drug levels in the CNS are a good indicator of bioavailability in the brain, so it is important to evaluate drug biodistribution in the CNS in preclinical animal models. The most common animal models are rodents but CSF sampling in mice is very challenging as only a few microliters of CSF can be accessed through a complicated dissection into the cisterna magna. Additionally, there is a significant probability of contamination with blood cells due to the proximity of the CSF

access site to major blood vessels[8]. However, some groups are working on improved methods to increase the volume of CSF collection[9] but surgical methods to access usable volumes of CSF are complex.

Large animal models such as minipigs, nonhuman primates etc. have been developed to evaluate drug pharmacokinetics and study neurotoxicity. Nonhuman primates are used in neurological studies but present ethical and budget issues. Minipigs are useful to study specific modes of drug delivery such as epidural or intrathecal while canine models are used to evaluate efficacy and systemic toxicity[10]. It is important to note that there are differences in CSF characteristics across the commonly used large animal models and analysis of CSF samples from NHPs, minipigs and canines showed differences in lymphocytes and monocytoid B cells and ion concentrations as well as differences in diffusion of test articles[10]. This finding suggests that it is necessary to establish baseline CSF characteristics for the large anima model of choice prior to evaluating drug pharmacokinetics.

Nonhuman primates (NHPs) are physiologically relevant models to study human neurological diseases due to similarities in brain structure, architecture, physiology and function. NHPs have larger CNS compared to rodents, so CSF sampling is easier and is typically performed either through lumbar punctures or through the cisterna magna. Both methods have been shown to collect sufficient volumes for downstream analysis such as disease associated biomarkers. A recent study compared biomarker levels across 2 different species of primates (rhesus and cynomolgus), and found higher levels of beta-amyloid peptide levels and neurofilament light in rhesus macaques compared to cynomolgus while tau levels were not significantly different[11].

Several neurodegenerative diseases are associated with aging, so the longer lifespan of NHPs compared to rodent models allows for longitudinal studies to study spontaneous and induced disease development[12]. Due to the duration of the studies, it is essential to develop models that allow repeated CSF sampling. A study published in February 2022 describes the development of an NHP model with a cisterna magna port (CMP) that supports repeated sampling. The insertion of a port allows CSF sampling from conscious animals with minimal distress for 18 years[13]. The other advantage of a repeated sampling model is the need for fewer animals over the duration of the study while generating multiple data points from the same animal. However, repeated sampling models are complicated to develop and maintain and the research group that developed the rhesus model were not successful in developing other repeat sampling models[13].

The growing body of work suggests that CSF sampling in large animal models is a useful method to evaluate drug biodistribution and drug induced biomarker changes. However, it is clear that these methods are complicated and require significant expertise and budget so the development of humane repeated sampling models will help reduce and refine animal use while generating physiologically relevant data.

References:

1. https://jcmtjournal.com/article/view/1321
2. https://academic.oup.com/clinchem/article/68/10/1311/6661459
3. https://ir.biocept.com/news-releases/news-release-details/study-shows-biocepts-target-selectortm-may-provide-more-robust
4. https://www.futuremedicine.com/doi/10.2217/fnl-2018-0029
5. https://onlinelibrary.wiley.com/doi/full/10.1111/joim.13332
6. https://molecularneurodegeneration.biomedcentral.com/articles/10.1186/s13024-017-0226-4
7. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7150022/
8. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5931779/
9. https://www.biorxiv.org/content/10.1101/2022.09.30.510413v1.full
10. https://journals.sagepub.com/doi/pdf/10.1177/1091581820905092
11. https://www.biorxiv.org/content/10.1101/2021.03.01.433384v1.full
12. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7999604/
13. https://pubmed.ncbi.nlm.nih.gov/34903315/