

CEREBROSPINAL FLUID SAMPLING IN PHASE 1 CLINICAL TRIALS: MIND OVER MATTER?

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INTRODUCTION

Cerebrospinal fluid (CSF) is a clear watery liquid filtrate that is formed and secreted by the choroid plexus, special tissue that has many blood vessels and lines the small cavities or chambers (ventricles) in the brain. CSF flows around the brain and spinal cord, surrounding and protecting them. It is continuously produced, circulated, and then re-absorbed into the blood system. The normal (non-diseased) choroid plexus produces CSF at a continuous rate of 20 mL/h. On average, 500mL of CSF is produced daily. The total CSF volume at any given time is about 150 ml. So in normal conditions there is 3 to 4 times per day a complete wash-out of the CSF volume.

For drugs that directly act on targets in the central nervous system (CNS), sufficient drug delivery into the brain is a prerequisite for drug action. Systemically administered drugs can reach CNS either by direct passage across the choroid plexus or by indirect passage across the so-called blood-brain barrier (BBB). Once across these barriers, the drug will diffuse through the interstitial fluid towards the CSF. When the drug reaches the CSF, it will be transported throughout the CNS by convection through the ventricular system. Upon exiting the fourth ventricle the drug then flows through the cerebellomedullary cistern down the spinal cord and over the cerebral hemispheres. It is thought that CSF returns to the vascular system by

entering the dural venous sinuses via the arachnoid granulations/villi.^{1,2}

Evidence from preclinical and clinical studies suggests that drug concentration in cerebrospinal fluid (CSF) is reasonably accurate in predicting unbound drug concentration in the brain. Therefore, CSF can be used as a useful surrogate for in vivo assessment of CNS exposure and provides an important basis for the selection of drug candidates for entry into development.

Besides the pharmacokinetic (PK) driven rationale to perform CSF sampling, the CSF compartment is as close as we can get to the effector compartment of drugs acting on the CNS. The CSF can be

searched for many different biomarkers, either disease specific or drug specific. Investigating CSF for pharmacodynamic (PD) actions of the drug under study could give a clear view on the desired/undesired changes in CSF-specific biomarkers.²

CSF biomarkers provides an excellent rationale for taking a direct PK/PD assessment in the CNS compartment as this could potentially speed up the “go/no go” decision in early clinical development of CNS drugs. As a result of these developments, SGS has encountered an increasing demand from sponsors for cerebrospinal fluid sampling in Phase 1 clinical trials during the past few years.

SAMPLING RISK PREVENTION

It is clear that in an early phase setting, primarily dealing with healthy volunteers, SGS is very alert for possible risks and discomforts related to CSF sampling. Possible theoretical risks associated with lumbar punctures or spinal catheterizations include:

- Procedural pain during insertion of needle/catheter
- Spinal headache (also called postdural puncture headache (PDPH))
- Epidural infection
- Spinal cord trauma / nerve root trauma
- Spinal / epidural haematoma
- Cerebral herniation

To reduce these risks maximally several additional inclusion and exclusion criteria and other preventive actions are put in place.

The BMI is restricted to maximum 32 kg/m² for technical reasons. Bony landmarks are masked in overweight persons and the needles used are not suited for use in overweight persons. Medical or surgical conditions in which a lumbar puncture is contraindicated will be excluded.

The far most important complication, inherent to dural puncture is a post dural puncture headache (PDPH) due to persistent CSF leakage. Therefore the subjects are placed on bed rest in a supine position during sampling and up to 6 hours after single puncture or even 12 hours after removal of the spinal catheter.

Introducing an infection into the CSF, resulting in meningitis, is a rare complication. This is avoided by performing the lumbar puncture or inserting the spinal catheter under strict sterile conditions. Any topical infection or local dermatological condition at the puncture site is a possible risk and should be examined thoroughly prior to puncture. The CSF collections themselves also need to be performed under aseptic conditions.

The needle or the catheter can irritate nerve roots upon insertion. This irritation manifests itself by induction of paresthesia in the affected dermatome. This is usually benign and self-limiting; when the needle/catheter is withdrawn, the nerve root will relax and the symptoms fade. A second type of sensation occurs typically upon insertion of a spinal catheter: the subject senses short "electric shocks" when the catheter

touches a nerve root. This pain stops automatically when the catheter is in place.

Expertise in performing these procedures, together with the introduction of atraumatic needles and new catheter types, help to prevent permanent trauma and with these measures it is unlikely to occur.

As for additional serious side effects, as long as the normal puncture sites are respected (L3-L4 or L4-L5), spinal cord trauma in healthy volunteers and in adults without anatomical malformations will not occur.

Cerebral herniation (due to pre-existing intracranial hypertension) and a spinal epidural haematoma with compression of the cauda equina are very rare complications; nevertheless additional in- and exclusion criteria should be included in the protocol to reduce the risk. Coagulation tests at screening have to be within the laboratory's reference ranges; an eye exam with fundoscopy is always performed before the procedures to exclude intracranial hypertension.

TECHNIQUES

SGS uses two techniques depending on the requirements of the study: A single lumbar puncture is used for one single CSF sampling) and spinal catheterization is used for serial CSF sampling over 12 to 36 hours.

Single Lumbar Puncture

A single CSF sample is obtained by performing a classic lumbar puncture in a sitting position at level L3-L4 or L4-L5 under local anaesthesia. For this procedure SGS uses a 22 gauge 'pencil-tipped' atraumatic needle (Pencan Needle B Braun) to reduce the risk of a CSF leakage as much as possible (See Figure 1).

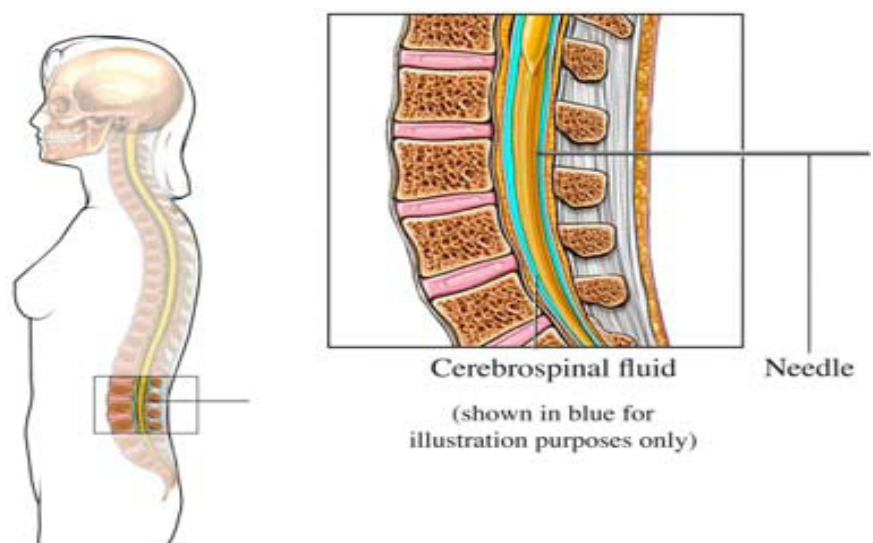


FIGURE 1: SINGLE LUMBAR PUNCTURE

CONTINUOUS/SERIAL CSF SAMPLING

For continuous CSF sampling up to 36 hours a spinal catheter is placed in the subarachnoid space. First an introducer needle (Tuohy) is placed; the stylet is removed and the peridural space is entered using the loss of resistance (LOR) technique. Subsequently a Spinocath (B Braun) catheter is introduced through the Tuohy needle and perforates the dura ('dural click'). At that time the spinal needle

is withdrawn while moving up the spinal catheter into the subarachnoid space. The Tuohy needle is subsequently removed.

The Spinocath® is rather unique as it is a catheter-over-needle system (instead of the classic catheter-through-needle systems). In case of a catheter-over-needle system the catheter (22 G) is larger than the spinal needle (27 G) and

so dilating and sealing the dural opening. With a catheter-through-needle system the catheter is smaller than the dural opening and a considerable CSF leakage after removal of the puncture needle is to be expected.

So we use the Spinocath® system to reduce the occurrence of a CSF leakage maximally (See Figure 2).³



FIGURE 2: CONTINUOUS/SERIAL CSF SAMPLING

OVERVIEW OF SAFETY: SGS'S EXPERIENCE

Single Lumbar Punctures

In SGS's clinical pharmacology unit two multiple dose studies have been conducted involving single lumbar punctures (SLP).

In one study SGS performed a SLP at steady state on the 14th day of dosing, in total 29 procedures were performed during the study. The second study (ongoing) uses a SLP pre-dose as a

baseline reference sample. 1 cohort of 8 subjects has been dosed so far and 8 SLPs have been performed. An overview of the population and incidence of PDPH is given in table 1.

In total 37 healthy volunteers (males and females) underwent a SLP in the phase I centre. Withdrawn volumes of CSF ranged between 5 and 8 mL per sample. Three of these subjects developed PDPH despite the initial bed rest of 6 hours

after the procedure. The mean interval between the end of procedure and occurrence of PDPH was 18.5 hours. The mean duration of these cases of PDPH was 3.46 days (83 hours). All cases of PDPH were treated conservatively with additional bed rest and regular pain killers (acetaminophen PRN). None of the subjects needed an epidural blood patch to treat the PDPH.

SINGLE LUMBAR PUNCTURE	
Number of Subjects	37 (10 Females and 27 Males)
Mean Age	44 years
Total CSF volume	5 mL – 8 mL
PDPH cases	3 (8,1%)
Mean Interval between removal needle and onset PDPH	18,5 hours
Mean Duration PDPH	83 hours
Subjects needing Epidural bloodpatch	0 (0%)

TABLE 1: OVERVIEW SLP CHARACTERISTICS

CONTINUOUS/SERIAL CSF SAMPLING

Since September 2007, SGS has performed five phase I studies using continuous/serial CSF sampling. All these studies incorporated CSF sampling as part of the PK/PD modelling in order to quantify possible effects on several biomarkers.

An overview of the population and incidence of PDPH is given in table 2.

In total, 71 healthy volunteers of both sexes have been included. The duration of serial CSF sampling ranged between 12 hours and 36 hours.

The volume of cerebrospinal fluid prelevated during the sampling period ranged between 15 mL and 156 mL (26 sampling timepoints @ 6 mL/sample). In total 24 cases of PDPH have been reported despite the initial bed rest of 12 hours after the procedure. The mean interval between removal of the spinal catheter and occurrence of PDPH was 16 hours. The mean duration of these cases of PDPH was 5.0 days (120 hours). All cases of PDPH were initially treated conservatively with additional bed rest for 24 hours and regular pain killers (acetaminophen PRN). Thirteen of the

subjects needed at least one epidural blood patch to treat the PDPH. One female subject underwent 2 epidural blood patches and one female subject needed even a third epidural blood patch to treat the PDPH, which is quite uncommon.

TABLE 2: OVERVIEW SERIAL CSF SAMPLING CHARACTERISTICS

SERIAL CSF SAMPLING	
Number of Subjects	71 (25 Females and 46 Males)
Mean Age	45 years
Total CSF volume	15 mL – 156 mL
PDPH cases	24 (33,8%)
Mean Interval between removal needle and onset PDPH	16 hours
Mean Duration PDPH	120 hours
Subjects needing Epidural bloodpatch	13 (18,3%)

Table 3 summarizes the occurrence of PDPH per sampling regimen as well as per sex. Overall occurrence of PDPH was 25.0% (27 cases in 108 subjects).

TABLE 3: DIFFERENCES IN OCCURENCE OF PDPH ACCORDING TO SAMPLING REGIMEN:

	SLP	12H REGIMEN	24H REGIMEN	36H REGIMEN	MALES	FEMALES
TOTALS	37	6	43	22	73	35
CASES OF PDPH	3 (8,3%)	0 (0%)	16 (37,2%)	8 (36,4%)	13 (17,8%)	14 (40,0%)

Other adverse events related to the procedure of SLP or spinal catheterization have been reported in 15 subjects. Those adverse events were all related to nerve root irritation. Paresthesia was noted in 2 subjects in the SLP regimen. Sciatic

pain, paresthesia and muscle cramping were noted in 13 subjects where a spinal catheter was placed. All those events were rated as being of mild intensity and resolved completely within hours after removal of the catheter.

DISCUSSION AND CONCLUSION

Despite all the preventive measures a CSF leakage can occur and persist resulting in a post dural puncture headache. Whenever PDPH occurred, we advised the volunteers to stay in a supine position for at least 24h. Spinal headache is a specific type of headache for which the anamnesis is quite typical: it is absent or minimal in supine position, and aggravated upon ambulation. Lying down immediately reduces the headache. The ethiology is still not completely understood, but probably a continuous leakage of CSF to the epidural space is one of the main contributors to this headache.

The risk for CSF leakage is apparently higher after removal of a spinal catheter that was in place for more than 12 hours compared to a single lumbar puncture. Out of SGS's experience in more than 100 procedures presented here, we can conclude that the risk for developing PDPH is about 8% after a single lumbar puncture and almost 30 % in the

continuous/serial CSF sampling group. The incidence of PDPH in female volunteers was twice as high as the incidence in males.

Those findings are comparable with the known literature on the occurrence of PDPH.^[4,5,6]

Of the 27 cases presented, 14 recovered under conservative treatment (bedrest and regular pain killers PRN). The mean duration in this group was 137 hours. 13 subjects came back to the clinical centre to receive an epidural blood patch. Mean duration of PDPH was reduced to 93 hours. Rather exceptionally we had 1 subject needing 2 blood patches and even 1 subject needing 3 to treat the PDPH.

The risk of developing PDPH after a CSF sampling is real, 1/4 subjects will develop such complication. Half of the affected individuals will need a blood patch to resolve the iatrogenic PDPH.

As a conclusion, generally speaking, CSF sampling in phase 1 clinical trials is an overall safe procedure in experienced hands even so risks never can't be ignored. Lastly, it is clear and obvious that informing the subjects in advance is of utmost importance. The subject volunteering to be submitted to such procedure needs to thoroughly understand the nature and the possible discomforts of the procedure.

The CSF sampling is a powerful CNS surrogate marker for in vivo assessment and definitely highly supports the Go No Go decision for a faster and safer drug candidate development.

With innovative study designs, optimal facilities and strong regulatory intelligence, SGS can favorably impact client's drug development timelines and decision-making process.

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