Szent István University
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Anatomical relations, volume and 3D reconstruction of the canine cerebrospinal fluid compartments

Brief summary of PhD thesis

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1. Introduction

The brain and the spinal (together: Central Nervous System; CNS) cord are covered by a number of layers, the meninges, and the space between these layers (subarachnoid space, SA space) is filled with the cerebrospinal fluid (CSF). The CSF is mostly produced inside the brain ventricles and flows to the outside surface of the CNS through openings near the medulla oblongata– exact location and number may vary depending on the species. Reabsorption takes place in venous plexuses, therefore the venal pressure has a direct effect on the physical specifics of the CSF circulation. Its density is nearly as much as the water's, it contains glucose, metabolites and ions. Its most important physiological function is the protection of the CNS against physical impacts, but it participates in the supply of the brain cells, with the removal of metabolites and in the transport of the synaptic transmission materials.

Accordingly, it has multiple clinical relevancies. CSF samples are often used for diagnostic purposes, but abnormalities of the volume and distribution of the CSF are also known.

Myelography is one of the most common diagnostic procedures, and hydrocephalus is the most important

volumetric disease. Myelography has lost ground in the human medicine for the Magnetic Resonance Imaging (MRI), but – due to the market-orientated structure of the veterinary healthcare- it is still commonly used in dogs. The dosage of the contrast material in this procedure is debated because the background of the well-documented relation between the body size and the post-myelography neurological symptoms is unknown.

Hydrocephalus is often found as a developmental malformation in pups, but it may also occur in adults. In the latter case, the cause of the problem remains hidden in most cases, although the presence of a pathologic structure obstructing the pathway of the CSF may be diagnosed occasionally. The lack of data for the CSF quantity and distribution in dogs makes the in vivo diagnosis more challenging. Additionally, no reliable method exists to measure the CSF quantity in clinical circumstances in a living dog.

The aim of my PhD study was to develop a reliable, accurate method for the in vivo measurement of the dog's CSF and to use this method in a group of healthy subjects to gain basic data about the overall quantity and distribution. We also aimed to compare this data with the physical measures of the subject to help defining the

adequate dosage protocol for injecting material (contrast or medicine) into the SA space and the to lessen the complications of the diagnosis of the hydrocephalus and other, similar diseases. A major objective was to create high quality images, 3D models and animations of the SA space for teaching purposes.

2. MATERIALS AND METHODS

Upon the findings of the literature review we decided not to use *post mortem* measurements at all but to proceed solely with *in vivo* MRI examinations. As no such was carried out in dogs so far, we used the existing human methods to develop the basics.

MRI was performed using a 1.5 T MRI system (Siemens Magnetom Avanto, Siemens AG, Erlangen, Germany) with the subjects lying in dorsal recumbency at the Institute of Diagnostic Imaging and Radiation Oncology, Kaposvár University, Hungary (KU).

The preliminary studies were carried out on two dogs, which arrived into the KU for routine, clinical MRI examination. Both were free of neurological signs and their respective owners provided a written consent to participate in the study.

Anesthesia was induced with intravenous usage of Propofol and was maintained with isoflurane. The MRI sequence designed for CSF detection was run on both animal in the entire length of the CSF. During the evaluation of the clinical sequences, disc hernia was diagnosed in the extracranial (EC) region of one subject, thus, the dog was excluded from the further EC measurements.

The distance between the dorsal and the ventral laminas of the dura mater was measured in the cranial edge of every second vertebra starting from the second cervical vertebra until the sixth lumbar vertebra of the other subject. The diameter of the spinal cord was also measured in the same locations. The phantom of the SA space was built based on this data. The phantom was filled with water and was examined with the same MRI sequence.

The reconstruction and volume measurement of the fluid compartment inside the phantom was carried out with the 3D Slicer 4.1. The adequate tools and settings were identified during a test on a series of digitalized immunhistochemical slices of a mouse's brain.

The preliminary studies were followed by the MRI examination and 3D reconstruction based CSF measurement of 12 healthy, male, mongrel dogs between 2-5 years old.

The research was approved by the Institutional Animal Care and Use Committee of the Veterinary Faculty of the Szent István University. Each dog was privately owned, and their owners received detailed, written information about the planned procedure and gave their written consent to carry out the CSF measurement.

The EC and intracranial (IC) SA space and the ventricles were measured on the MRI series. The overall volume and the volume of each anatomical recess of every compartment were also measured.

Linear regression was used to verify the association between the physical measurements and the volumetric data. R software was used for statistical calculations.

3. RESULTS

Results of the validation processes

The result of the phantom's measurement matched the real volume with 99.96% accuracy. A second validation was performed parallel with the EC measurement as water containers with known quantity of water was placed next to the backbone of each subject. The measurement of the containers provided a result between 99.8± 3.1%.

Results of the EC measurements

The 3D reconstruction models are anatomically accurate with a volume between 20.21-44.06 ml. The proportion of the cervical region is 41.03±3.33%, the proportion of the thoracic region is 35.93±2.13%; the proportion of the lumbar region is 23.06±1.92% volt.

The statistical analysis revealed the following correlation between the bodyweight in kg (BW) and the volume of the EC CSF compartment in ml ($V_{EC\ CSF}$) (p=0,0002; adjusted r^2=0,74):

$V_{EC\ CSF}$=BW*0.93+11.8 (Eq. 1.)

Adding either the length of the spinal cord or the shoulder height did not improve the model due to their collinearity and these body measures did not show correlation with

the $V_{EC\ CSF}$ when examined independently. There was no significant correlation between the body weight and the proportional quantity of the cervical CSF (p=0.907), the proportional quantity of the thoracic CSF (p=0.42) and the proportional quantity of the lumbosacral CSF (p=0.50).

The results of the ventricular compartment

The 3D reconstruction models of the ventricles do not always follow the anatomical descriptions exactly as several recesses are missing on some models, but this – due the size of the missing parts – did not interfere with our results. The total volume of the ventricles were measured between 0.97-2.94 ml with 62.12±11.7% in the lateral ventricles, 17.58±4.92% in the third ventricle, 4.85±1.57% in the *aqueductus mesencephali* and 15.45±6.61% in the fourth ventricle. The left and right lateral ventricles of the same subject showed significant differences in 11 cases (91.7%); in 6 cases (50%) the right sided in 5 cases (41.7%) the left ventricle was larger. There were no significant correlation between the registered body measures and the ventricle sizes.

The results of the IC SA compartment

The CSF layer lacks continuality on the temporal region of each 3D reconstruction model, but the grooves of the brain surface and the cisterns of the SA space are always well identifiable. The compartment was measured between 8.44-22.62 ml. The statistical analysis revealed the following correlation between the BW and the volume of the IC CSF compartment in ml ($V_{IC\ CSF}$) (p=0.0085; adjusted r^2=0.468):

$V_{IC\ CSF}$=BW*0.62+0.12 (Eq. 2a)

The proportional IC SA volume values of subject no. 12 were edge values. After excluding this subject from this statistical analysis the correlation is modified as follows (p=8,3*10-5; adjusted r^2=0,817):

$V_{IC\ CSF}$=BW*0.48+3.44 (Eq. 2b)

Combining the results

The overall volume of the CSF volume was between 29.62-67.74 ml, with 28.57%±4.91% in the IC SA space, 4.45% ±1.96% in the ventricles and 66.99%±5.15% in the EC SA space. The following correlation was found between the BW and the volume of the total CSF volume in ml ($V_{Full\ CSF;}$ p=1.94*10-5; adjusted r^2=0.836):

$V_{Full\ CSF}$=BW*1.39+17.5 (Eq. 3)

4. DISCUSSION

The aim of this study was to perform accurate measurements on dog's CSF. Because of the continuous production and the physiological role of the CSF, the full volume cannot be removed from a living dog. Post mortem measurement with the removal of the CSF through the atlanto-occipital space and/or the lumbo-sacral space and through the opening of the skull may seem like good alternative for volume measurement. However there are multiple factors that would make such an attempt impossible with reliable accuracy. These include: the direct relation between CSF's direction, production and reabsorption, and the blood circulation, the anatomy of the ventricles, the size and location of the connections between the ventricles, and the osmotic relation between the CSF and the interstitium of the brain.

Measurement through corrosion casting may also be considered. In this case, the technical difficulties of creating such casts, the shrinking of the casting material when it solidifies, along with the effect of the materials on the nearby tissues would hinder the efforts. Additionally, it is not possible to maintain the original IC pressure

relations while the pressure of the material when injecting would compress the brain tissues.

Because of these effects we decided not to use post-mortem methods for even preliminary studies but to proceed with in vivo imaging procedure.

Out of these the MRI is capable to highlight the CSF, while the Computer Tomography (CT) requires injection of contrast material to achieve that. Such would interfere with our results because of its own volume, and its effect on the production and reabsorption of the CSF. As we did not find any study that would satisfy every requirement, we developed our methods based on the existing experience. All measurements were carried out in clinical conditions and there were no artifacts on the images. The accuracy of the method was validated in two different ways. As every such measurement was at least 99% accurate we can safely assume that the volumetric data is accurate enough and refers to the actual CSF volume of the subject.

The requirements of the selection in the subject group served to exclude any external factor (age, breed, sex, health condition) that may interfere with our results. These however also limit the validity of our results for a given age and sex of animals.

The models are anatomically accurate. The high significance level of Eq. 3., that describes the relation between the total CSF volume and the BW indicates the volume of the missing regions in the EC region and in the ventricular compartment is not significant.

Our results and findings in the ventricular compartment did not differ significantly from independent studies.

While more studies introduce myelography contrast dosage calculation that is not directly proportional with the BW, only Arany-Tóth explains his with direct research data. The other proposals were developed based on anecdotal clinical evidence. When comparing his results with ours, they show an almost identical regression model (adjusted r^2=0.9774) within the 7.5-35.0 BW range and even outside that (5.0-80 kg; adjusted r^2=0.9923). Base on this, our results may be utilized outside the measured BW range and suggest that the difference in the pressure-curve measured by Arany-Tóth is solely because of the volumetric difference of the SA space.

Due to the small amount of subjects, our results cannot be evaluated as the defined physiological values for the canine CSF volume but they provide a good basis for further studies that may include bitches or other age groups as well.

Our findings indicate that the current standard method of using body weight to calculate dosages of myelography contrast agents in dogs may need to be revised. The provided values for the CSF distribution may help the diagnosis of syringomyelia and hydrocephalus.

5. NEW SCIENTIFIC RESULTS

1. We developed a method for CSF measurement in clinical circumstances. The method allows the accurate measurement of both the total volume and the volume of selected compartments. The accuracy of the method was validated in two different ways at 99.96% and 99.8 ± 3.1% accurate. As the method is transferable to other species as well it may be used in studies of the SA space and the CSF as well as in clinical diagnosis of the hydrocephalus.

2. Our results on the total canine CSF volume and are the first to be published.

3. We defined the distribution of the canine CSF among the major compartments and within the compartments as well.

4. We found correlation between the volume of the EC CSF compartment and the BW.

5. We defied the total CSF volume values for male dogs between 7.5-35.0 BW. The relations with the body

measures and the distribution among compartments were also defined. Our results prove that those dosage protocols of administering contrast material or medicine into the SA space that are based on a direct proportional relation between the BW and CSF volume should be revised.

6. We concluded that the asymmetry of the canine lateral ventricles is a physiological, common phenomenon, with the larger ventricle may be up to 1.5 times larger. However, symmetrical ventricles may also be normal in around 10% of all cases.

7, Our results on the overall volume of the CSF confirm the validity of the myelography dosage protocol based on pressure-curve changes published earlier.

8. We created specimens, 3D models, images and animations of the CSF compartments for educational purposes.

6. OWN SCIENTIFIC PUBLICATIONS RELATED TO THE TOPIC OF THE PRESENT THESIS

full text papers in peer-reviewed journals

Reinitz L., Petneházy Ö., Bajzik G., Biró G., Garamvölgyi R., Benedek B., Sótonyi P.: Módszer a kutya (Canis familiaris) agykamráinak in vivo térfogatmérésére MRI-vel. Magyar Állatorvosok Lapja 135. évf. 2013/8 pp. 451-460 (IF.: 0,185)

Reinitz L., Bajzik G., Garamvölgyi R., Petneházy Ö., Lassó A., Abonyi-Tóth Zs., Lőrincz B., Sótonyi P.: Comparison between magnetic resonance imaging estimates of extracranial CSF volume and physical measurements in healthy dogs. Veterinary Radiology and Ultrasound Vol. 56, No. 6, 2015, pp 658–665. (IF.: 1,453) (IF.: 1,453)

Reinitz L., Szőke B., Várkonyi E., Sótonyi P., Jancsik V.: Three-dimensional visualization of the distribution of melanin-concentrating hormone producing neurons in the mouse hypothalamus. Journal of Chemical Neuroanatomy. Vol. 71, pp. 20-25. (IF.: 1,500)

Conference abstracts

Reinitz L., Petneházy Ö., Bajzik G., Biró G., Garamvölgyi R., Benedek B., Sótonyi P.: **Módszer a kutya (Canis familiaris) agykamráinak in vivo térfogatmérésére MRI-vel.** *Magyar Anatómus Társaság XVIII. Kongresszusa*; Budapest; 2013. 06.13-15

Reinitz L., Szőke B., Gerics B., Sótonyi P., Jancsik V.: **3-D reconstruction of the distribution of melanin-concentrating hormone producing neurons in the mouse hypothalamus.** *International Brain Research Organization Workshop 2014; Debrecen, 2014.01.16-17*

Reinitz L., Bajzik G., Garamvölgyi R., Petneházy Ö., Lassó A., Abonyi-Tóth Zs., Lőrincz B., Benedek B., Sótonyi P.: **Volumetric measurements of the canine cerebrospinal fluid using magnetic resonance imaging.** *The XXXth Congress of the European Association of Veterinary Anatomists*; Cluj-Napoca; 2014.07. 23-26

Reinitz L., Bajzik G., Garamvölgyi R., Benedek B., Petneházy Ö., Lassó A., Abonyi-Tóth Z., Lőrincz B., Sótonyi P.: **Volume and distribution of the cerebrospinal fluid in dogs.** *A Magyar Anatómus Társaság XIX. Kongresszusa. Szeged; 2015.06.11-13.*

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