TECHNIQUES FOR COLLECTING CEREBROSPINAL FLUID DURING AUTOPSY

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TECHNIQUES FOR COLLECTING CEREBROSPINAL FLUID DURING AUTOPSY (Abstract) : The cerebrospinal fluid (CSF) is collected and analyzed post-mortem, and can often contribute to the identification and diagnosis of the cause that led to death. Collecting CSF can be considered a routine investigation to rule out possible meningitis or even certain cerebral hemorrhages. The CSF samples can be collected by different techniques represented by the peripheral puncture techniques - lumbar or central puncture techniques - with CSF aspiration from the level of the cistern, the third ventricle or the lateral ventricles. In order to perform the CSF analysis, it must first be correctly collected. The CSF collecting technique involves certain steps that must be followed, so that the integrity of the CSF is not affected / altered. When errors occur in performing the procedure, local vascular lesions can be performed, with consecutive bleeding, which can lead to false-positive results. In order for these steps to be able to be correctly performed, it is essential to know the anatomical notions of the elements that are addressed while performing the CSF puncture technique. This article aims to bring to the attention of forensic scientists and anatomists, the imperiousness of obtaining the correct anatomy information when making a point aimed at collecting and analyzing CSF. **Key-words :** CEREBROSPINAL FLUID, AUTOPSY, VENTRICULAR SYSTEM, TECHNIQUE, BIOCHEMISTRY

INTRODUCTION

The cerebrospinal fluid (CSF) is collected and analyzed post-mortem, and can often contribute to the identification and diagnosis of the cause that led to death. In certain circumstances, such as sudden death in children and adolescents, collecting CSF can be considered a routine investigation to rule out possible meningitis or even certain cerebral hemorrhages (1). The CSF samples can be collected by different techniques represented by the peripheral puncture technoque - lumbar or central puncture techniques - with CSF aspiration from the level of the cistern, the third ventricle or the lateral ventricles. In order to perform the CSF analysis, it must first be correctly collected. The CSF collecing technique involves certain steps that must be followed, so that the integrity of the CSF is not affected / altered. When errors occur in performing the procedure, local vascular lesions can be performed, with consecutive bleeding, which can lead to false-positive results (1). In order for these steps to be able to be correctly performed, it is essential to know the anatomical notions of the elements that are addressed while performing the CSF puncture technique. This article aims to bring to the attention of forensic scientists and anatomists, the imperiousness of obtaining the correct anatomy information when making a point aimed at collecting and analyzing CSF, so as to minimize technical errors such as CSF contamination - issues that may lead to real errors in the results of the analysis of the CSF components.

PRODUCTION AND CIRCULATION OF CEREBROSPINAL FLUID

The cerebrospinal fluid is a fluid that in physiological conditions is colorless and clear as rock water, which fills the ventricular system and the subarachnoid space of the brain, having a low level of cellular constituents and proteins (2-4). This fluid comes from the blood plasma so that any of its constituents can be found in the plasma, but in different concentrations. For example, in the CSF, the following elements are found in lower concentrations than in plasma : potassium, calcium, glucose, proteins, urea, uric acid but also pH (2-4). There are constituents that can be found in the same quantities in both fluids, namely sodium and water. In contrast, magnesium and chloride concentrations in CSF are higher than plasma (2-4).

As early as 1865, Luschka showed that most of the CSF is actively secreted at the level of the choroid plexuses (about 60-70%), especially those of the lateral ventricles (5). Subsequently, different researchers have shown that CSF can be produced also at the subarachnoid, periencephalic and spinal level, so the extraplexal origin can vary from 20-50% of the total secretion (5,6).

CSF circulation occurs from the ventricular compartment to the subarachnoid compartment. In the Ventricular System, CSF it flows from the lateral ventricles to the IV ventricle, successively crossing the foramina interventricularis (foramen of Monro), the third ventricle and the mesencephalic aqueduct. The communication of the ventricular system with the subarachnoid space takes place at the level of the fourth ventricle, when the CSF penetrates into the cisterna magna (part of subarachnoid space) through 3 apertures : 1 median – foramina of Magendie and 2 lateral – foraminaes of Luschka. Lazorthes denied the existence of the last two communications, stating that they would rather be "2 weak points" of the ependymal membrane (5,6). Arrived in the subarachnoid compartment, the CSF follows two directions : one in the spinal subarachnoid space and one in the pericerebral subarachnoid space. In the subarachnoid spinal space, the CSF reaches the bottom of the dural sac, which is arranged between the second lumbar vertebra and the first sacral vertebra, a space with CSF in which the nerves of the so called ”ponytail” float. In the pericerebral subarachnoid space, the CSF circulates from the cisterna magna to the other cisterns : basal, prepontine, interpeduncular, supraselaris, ambiens, the cerebral magna vein, the superior cerebellar, the pericalosal and the terminal blade.

The total amount of CSF in adults is 140 ml ± 30 ml, in children 60-100 ml, and in infants 40-60 ml. In the Ventricular System, only 25 ml of CSF are found in the adult, the rest being present in the subarachnoid space (5-9). It is stated that in 24 hours approximately 400 ml of CSF is produced, thus deducing that the renewal of the liquid (the "turn over") takes place about 6 hours, so about 4 times / day (7,8). Under physiological conditions, the amount of CSF remains constant, so that this fluid is absorbed mainly by the venous tract and by other secondary elements. The CSF is drained into the venous system through some arachnoid invaginations that herniates into the thickness of the hard cranial and spinal mater, which is called the arachnoid villi. These velocities, first described in 1923, have valves that allow the flow to be unidirectional and global, from the subarachnoid space to the venous blood (9).

FUNCTIONS OF THE CEREBROSPINAL FLUID

CSF has three major functions : mechanics, nutrition and excretory. The mechanical function of CSF is translated by the capacity of suspension and protection of the brain, being considered a shock absorber against external shocks and pressure fluctuations in the cranial cavity. CSF fills the subarachnoid pericerebral space, thus allowing the brain to float, as in a bath. The second important function of the CSF, nutrition function, is given by ensuring exchanges with the extracellular cerebral fluid, maintaining a constant concentration of the perineural environment. CSF is also considered a transporter of neurohormones, neuromodulators and immunoglobulins. The third function, the excretory one, ensures the elimination of catabolism products of the brain, some protein substances and even certain drugs (9).

LATERAL VENTRICLE PUNCTURE TECHNIQUE

This technique aims to collect CSF from the two lateral ventricles that are part of the Ventricular System of the brain. The two ventricles are even, symmetrical cavities and are found in the cerebral hemispheres, each in the form of a horseshoe, in whose concavity are located the caudate nucleus and the posterior pole of the thalamus. The convexity of the two horseshoes comes in relation to the corpus callosum. During the autopsy, after the opening of the cranial cavity, the brain is removed from the cranial box and the Virchow section is performed, which is the classic method during the autopsy of highlighting the cavities of the two lateral ventricles. Thus, by sectioning corpus callosum, the CSF is aspirated with a syringe from the lateral ventricles, through a needle, avoiding contamination of the fluid with blood as much as possible (10). The fluid is harvested through the aspiration process, introducing a 22-gauge needle attached to a 10 ml syringe. According to other authors, fluid collection can be done directly, by inserting the needle through the corpus callosum, without cutting it in advance (10, 11). Although this technique is quite simple to perform, it is not among the favorites, being widely disputed by some authors, the reason being the high risk of contamination of the sample (10,11).

THIRD VENTRICLE PUNCTURE TECHNIQUE

This technique aims to collect CSF from the third ventricle, which is also part of the Ventricular System of the brain. The third ventricle, also called the diencephalic ventricle, is an odd, median cavity, located between the two thalamus nuclei, above the hypothalamus and below the fornix and upper choroidal velum. The diencephalic ventricle communicates with the lateral ventricles through the interventricular foramina of Monro and with the fourth ventricle through the mesencephalic aqueduct of Sylvius. During the autopsy, after opening the skull box, the brain is removed from the skull and placed on the autopsy table, with the base up. The fluid is collected through a blind aspiration, inserting a 22-gauge needle attached to a 10 ml syringe. This needle is inserted upright, about 10-20 mm posterior to the mammilary bodies, so 2-3 ml of clear CSF can be aspirated (12,13).

CISTERNAL PUNCTURE TECHNIQUE

This technique is considered to be the ideal one for collection of CSF from the central level, but also quite simple to perform (11). The method involves collecting CSF directly from cisterna magna. Cisterna magna also called lower cerebellar cistern or cerebello-medullary cistern occupies the space between the inferior face of the cerebellum and the posterior face of the medulla oblongata of the brainstem. In the great cistern the liquid from the fourth ventricle drains, through the median opening – foramen of Magendie, into the subarachnoid space. The advantage of this cistern, compared to the other cisterns of the subarachnoid space, is that it is not crossed by large brain vessels, making it a suitable site for collecting CSF.

During the necropsy, the corpse is placed in prone position, with the head support placed under the cervical and thoracic region, so that a flexion of the cervical spine is performed. The external occipital protruberance, atlanto-occipital membrane, spinal process of the atlas, mastoid processes and nasi craniometric point are identified and then located by palpation. Cisterna magna is located approximately under a drawn line that unites the postero-upper cervical portion with the edges of the mastoid processes, at a depth below the tegument of about 5 cm. A 22-gauge 90 mm spinal needle is used for this type of technique. The needle is advanced by puncturing the skin on the midline of the posterior cephalic region, just above the spinal process of the first cervical vertebra, in a slightly ascending direction to the nasion (14).The needle advancement is performed at a slow rate, until an increased resistance is felt, usually at a depth of about 2.5-3.5 cm, at which point the atlanto-occipital membrane is penetrated (11,14). Immediately after this moment is exceeded, there is a sharp decrease in resistance or a distinct pop, given by the needle entering the cistern. Sometimes a sensation of failure may be encountered when the needle enters the epidural space, in which case no fluid is evident but blood from an epidural vein can be externalized. In this circumstance it is recommended to forward the spinal needle up to a distance of 0.5 cm, in order to enter the reservoir of the great cistern. Other times, the spinal needle can hit a bone surface, which should be assumed to be the occipital bone. In this case, the needle will retract slightly, it will then be sloped lower and then the progression will continue (14).

LATERAL C1-C2 SPACE PUNCTURE TECHNIQUE

Percutaneous cordotomy by lateral puncture at the C1-C2 level was first described in the 1960s (15,16). Most often, the dorsal subarachnoid space is better represented at the C1-C2 level, the spinal cord in the cervical region becoming larger below C2, so that CSF will be much harder to collect (17,18). The thoracic spinal canal is narrow, its access being limited also by the presence of the costal grid, the CSF collecting being not preferred at this level (19).

During the necropsy, the corpse is placed in a supine position, preferring to perform a slight hyperextension of the cervical region because it is assumed that by extension, the dorsal subarachnoid space at the C1-C2 level increases in width (20,21). The puncture site is placed approximately 1 cm lower and 1 cm posterior to the mastoid process, and the puncture point can even be marked with a colored pen. Data from the literature that included fluoroscopic guidance say that the puncture site is 2-5 mm anterior to the spinolaminary line, 5 mm lower than the posterior arch C1 and posterior of C1 posterior arch flare point (17,22). Collection of CSF is done with a 20 or 22-gauge spinal needle. When the needle reaches several millimeters from the medial edge of the lateral mass C1, the CSF should stand out. And in this case it may leak from the needle venous blood, in which situation it is recommended the easy advancement of the needle with another 1-2 mm (17).

LUMBAR PUNCTURE TECHNIQUE

This technique is the most experienced and most common method of collecting CSF, especially in living persons but also in post-mortem examinations. The correct level of spinal needle entry is easily identified by palpation. Thus, the spinous processes of L3, L4 and L5, as well as the intervertebral spaces can be easily found. Also, it is known that, by drawing a line joining the two upper edges of the iliac ridges, the point on this line that intersects the posterior median line corresponds to the body of the fourth lumbar vertebrae (17). There are authors who claim that this line can intersect the spine at levels ranging from L1-L2 to L4-L5, as well as the fact that there is a tendency to show a higher spinal level among obese people and women (17,23,24). In cases with ankylosing spondylitis, the so-called Taylor approach can be applied by the paramedian needle insertion in space L5-S1 (25).

In lumbar puncture for collecting the CSF, during the necropsy it is preferred the lateral position of the corpse, but when difficulties are encountered in manipulating the corpse (large waist, obesity), in our experience, we affirm that this technique can be carried out when the body's position is in pronation. Once the insertion point of the needle has been identified, its advancement is made slowly, under an angle with cranial direction, towards the ombilicus. The approximate distance from the tegument to the epidural space is 45-50 mm, which can be greater in obese people (24,26,27). The stylet is then removed and the CSF will progress through the spinal needle.

PRESERVATION OF CEREBROSPINAL FLUID

After we have made sure that the collection steps have been followed correctly and we have managed to obtain some clear CSF troops, the sample must be transported in proper conditions and stored in the freezer, at a temperature of - 20 degrees, until it will be analyzed. Sodium chloride should be added to preserve the sample if it is desired to determine toxic substances such as alcohol, cocaine, cyanide or carbon monoxide. To determine glucose and protein, some authors recommend the use of sodium fluoride / EDTA when subarachnoid haemorrhage is suspected. If any xanthochromia is suspected, take 1 ml of the sample which will be stored in a container through which the light cannot enter, for spectrophotometric analysis (28,29).

CONCLUSIONS

We consider that every technique presented regarding the post-mortem puncture of the CSF must be known by any forensic doctor and not least by the anatomist. This knowledge must be a complete one and based on some constantly updated information from the specialized literature, which provides retrospective data but also a perspective capable of helping to carry out a more accurate analysis of the CSF. In order to be able to perform these puncture techniques as efficiently as possible regarding CSF collection, it is necessary to know and understand the anatomical elements that we approach when we want to collect this fluid, so that we are aware of the quality of the sample but also the possibility of contamination, these being very important aspects in the correct diagnosis of the cause of death. Moreover, the enterprise of knowledge regarding these CSF puncture techniques, is transposed over the quality of the post-mortem study of the biochemical markers present in this fluid.

CONFLICT OF INTEREST

None declared

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