Review paper

Recommendations of the Polish Association of Neuropathologists on performing post-mortem examination of the brain and spinal cord

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Neuropathological central nervous system (CNS) post-mortem examination is a highly specialistic element of the autopsy procedure with methodological specificity. Herein we propose updated recommendations for CNS autopsy for pathologists and neuropathologists. The protocol includes the compendium of neuroanatomy with current nomenclature, consecutive steps of gross examination, as well as appropriate sampling algorithms in different clinical and pathological settings. The significance of pathoclinical cooperation in differential diagnosis is exposed. We believe it is essential to create and promote the guidelines to improve the quality of CNS post-mortem examination at the national level.

Key words: autopsy, brain, spinal cord, recommendations.

Introduction

Post-mortem neuropathological examination of the central nervous system (CNS) is a comprehensive medical examination performed to identify or confirm the morphological background of clinically diagnosed diseases and accompanying changes in the brain and/or spinal cord, verify clinical diagnoses, analyse pathological, radiological, and clinical correlations, and often to determine the cause of the patient's death [1, 2]. The significance of neuropathological post-mortem examination for physicians, other medical personnel, and hospital administration also lies in monitoring the quality of health services, detection of inherited, environmental, occupational, and infectious diseases, and the adopted diagnostic and therapeutic management [3–5]. Moreover, brain autopsy constitutes a permanent element conditioning the advancement of medical and neurobiological sciences [6].

Despite significant advancement in the field of neuroimaging and laboratory diagnostics, in many cases it is still not possible to make an equivocal diagnosis during hospitalization, for various reasons.

In addition, as demonstrated by concordance analyses of the clinical and post-mortem diagnoses, there are inconsistencies in as much as 25% of cases [7–9]. Therefore, post-mortem neuropathological examination remains in many cases the final method of verifying the clinical and radiological diagnosis [6, 9, 10].

The presented guidelines have been developed by members of the Polish Association of Neuropathologists – neuropathologists, neurologists, neurosurgeons, and pathologists dealing with this issue and experienced in CNS autopsies. The proposed standards are based on recommendations of scientific societies from the European Union and the United States, the available literature, and our own practice. The legal basis is the current Polish legislation. The main aim of these recommendations is to standardise procedures connected with CNS autopsy, the handling of tissue material, the scope of necessary additional examinations, and the method of formulating autopsy protocol. Moreover, the study aims to introduce a binding standard and quality control tool for neuropathological autopsy procedure.

Purpose and importance of post-mortem examination

The neuropathological autopsy is closely related to, and is part of, the general autopsy, including the external and the internal examination of the body organs with the collection of specimens for histopathological examination, and less frequently it is a separate examination performed in a pathomorphology facility or in a neuropathology unit. The necessity of a description of individual anatomical structures and knowledge of the sampling methodology make CNS autopsies requiring additional meritoric preparation [11, 12]. During the post-mortem neuropathological examination, recommended random and targeted samples are taken from the structures of the nervous system, depending on the gross findings and clinical diagnosis. Collecting sections from specific areas of the brain is necessary due to the complex anatomy, vasculature, and specific topography of functionally important structures. All specimens are subject to routine laboratory procedures connected with the processing of tissue material. In addition to the standard microscopic evaluation, examination may require special additional histochemical, immunohistochemical, and/or genetic tests [13–15]. Ultrastructural, microbiological, and/or toxicological analyses of the specimens and/or cerebrospinal fluid (CSF) may also be required [16]. Neuropathological post-mortem examinations should be performed carefully and their results processed within a specified timeframe in the context of the clinical data and neuroimaging [1, 17, 18].

General conditions for performing autopsies and necessary documentation

In legal terms, there are 3 main types of post-mortem examinations: forensic, medical (clinical, hospital), and rarely processed sanitary type (administrative). General necropsy should be carried out in a dissecting room equipped with suitable instruments. Each autopsy should be performed with the precautions and personal protective measures necessary when a high-risk infectious disease is suspected such as protective clothing that fully protects medical staff, i.e. disposable gowns, rubber or plastic aprons, special rubber autopsy gloves and, optionally, metal or Kevlar® gloves, surgical masks, FFP2 or FFP3 filter masks, protective glasses or face shields, disposable caps, and rubber work shoes [12, 18, 19]. A clinical post-mortem examination is ordered by a clinician and performed by a specialist in pathomorphology or a resident under the supervision of a specialist or – in the case of CNS autopsy – a specialist in neuropathology or pathologist with experience in neuropathological material. An autopsy technician performs preparatory cleaning and sanitary activities related to the body of the deceased and the autopsy room, and, during the autopsy, auxiliary activities related to the removal of the brain and/or spinal cord, under the supervision of the physician.

Depending on the clinical diagnosis, either the brain alone or the brain and spinal cord is removed from the cadaver. A comprehensive neuropathological examination may also require evaluation of the sensory organs, peripheral nerves, or muscles. In problematic cases, it is advisable to contact the appropriate reference centre for consultation prior to harvesting the organs.

The post-mortem referral card is an internal document issued in accordance with the unit's procedures and legal regulations. It should contain information specified in the Regulation of the Minister of Health of November the 9th 2015 on the types, scope, and templates of medical documentation and the method of its processing including, among others, the clinical data with the suspected diagnosis, the results of neuroimaging, and the medical case summary. The patient's medical documentation should be provided preferably before or at the latest for the general autopsy and be available for the neuropathological post-mortem examination. Some detailed information is included in the announcement of the Minister of Health from the 24th September 2021 concerning accreditation standards for the provision of health services and the functioning of pathological diagnostics units. The participation of the clinician in the post-mortem is advised, as well as a pathoclinical discussion before the autopsy.

Scope of post-mortem examination of the brain and spinal cord

The typical clinical post-mortem should be performed on 3 body cavities: the skull, the thorax, and the abdominal cavity; however, it is also possible to perform partial autopsies. It is permissible to perform only the brain autopsy in cases of patients with diseases of the central nervous system, when diagnostic tests performed *in vivo* did not reveal changes in other organs [4]. It is recommended that photographic documentation be made, as well as to mark and describe changes on schematic drawings of the brain. As far as technically possible, the autopsy can be recorded using a dictation device and/or video cameras or various modern integrated hospital systems [1, 12].

The stages of a neuropathological post-mortem examination during a general autopsy include external examination of the head and surrounding tissues of the spine, general evaluation of the body structure in terms of symptoms and consequences of nervous system diseases, *in situ* examination (evaluation of the dura mater, vessels, pituitary gland, examination of the cranial and spinal nerves), removal of the brain, possibly removal of the spinal cord, and macroscopic examination of the brain and spinal cord with collection of samples for histopathological analysis and other tests [4, 17].

Removal of the brain from the cadaver begins with an incision of the skin and the epicranial aponeurosis, which should be made from one mastoid process, upwards and towards the other mastoid process. The skin together with the epicranial muscle should then be pulled forward over the forehead as far as about 1 cm above the eyebrows. The posterior part of the integuments should be brought down to the level of the external occipital protuberance. Bilateral incision of the temporal fascia and the attachments of the temporal muscles allows the entire cranial vault to be exposed. The cranial vault is cut along a horizontal line connecting the glabella with points approximately 1–2 cm above the external auditory canals and further with the external occipital protuberance. The posterior half of the incision may be made higher but at the most obtuse angle to the anterior half, depending on the thickness of the cranial bones and the degree of muscle and integument development. The skull should be opened with an oscillating saw using methods limiting formation of bone dust aerosol, and sharp bone edges should be smoothed.

The dura mater should be incised along both sides of the superior sagittal sinus. Cutting off the attachment of the cerebral falx to the crista galli anteriorly allows the gradual severing of the superior cerebral veins flowing into the sinus and the extraction of the cerebral falx from between the hemispheres. If it is desirable to preserve the pineal region and the confluence of the great cerebral vein, the cerebral falx should be left in the longitudinal fissure and cut off with long scissors from the cerebellar tentorium on both sides of the sinus rectus. The frontal lobes of the brain should be gently pulled away from the base of the skull with one hand, and the olfactory nerves, optic nerves, internal carotid arteries, pituitary stalk, oculomotor nerves, and trochlear nerves should be cut successively. The attachment of the tentorium cerebelli should then be cut to the petrous part of the temporal bone on both sides and, using a long knife, the roots of the abducent and trigeminal nerves, complexes of the seventh and eighth nerves and lower cranial nerves, the vertebral arteries and finally the medulla oblongata/beginning of the cervical part of the spinal cord should be cut as far as possible, and then the whole brain should be carefully rolled backwards. If a thorough *in situ* assessment of the infratentorial structures, the cranial nerves, or the vertebrobasilar system is advisable, it is possible, after dissecting the structures of the parasellar region, first to cut the cerebral peduncles together with the posterior cerebral arteries and remove the brain, then to cut the tentorium, and assess and extract the infratentorial structures. To remove the pituitary gland, the sellar diaphragm must be carefully incised longitudinally and transversely. Once the dura has been inspected, it can be peeled from the internal surface of the cranial base to examine the bones, the trigeminal ganglion, the cavernous sinus, and the internal carotid artery. Assessment of the orbital structures and possible removal of the eyeball can be performed from above, after removal of the orbital roof with a chisel or an oscillating saw, or from the front through the palpebral fissure. Inspection of the middle ear is possible from above, after chiselling off the tegmental wall, which is the lateral part of the anterior surface of the petrous part of the temporal bone. Examination of the inner ear requires excision of the petrous part of the temporal bone with an oscillating saw and decalcification [1, 12].

The entire spinal cord is removed using a posterior or anterior approach (Fig. 1). In the posterior approach, after removal of the brain or before opening the skull, the body is placed prone on the table and the skin and soft tissues are incised over the spinous processes from the external occipital protuberance down to the sacrum. The vertebral arches and intervertebral joints should be exposed on both sides, then cut with an oscillating saw at the intervertebral joints, and the back wall of the spinal canal gradually lifted. After opening the spinal canal, the dural sac should be separated just below the foramen magnum together with the spinal cord, if it has not already been cut from the brain stem. The spinal cord in the dural sac is then removed by cutting bilaterally the spinal nerve roots together with the ganglia with

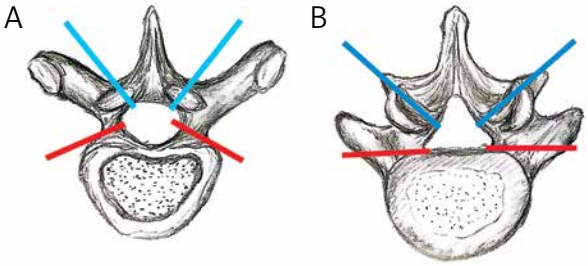


Fig. 1. Thoracic and lumbar vertebrae with cut lines to open spinal canal
Blue lines - posterior, red lines - anterior approach to spinal cord

a scalpel gradually from the top to bottom, while supporting the dura with forceps. In the anterior approach, after removal of the organs of the neck, thorax, and abdominal cavity and exposing the spine, the vertebral bodies should be sawn off from the bases of their arches: in the lumbar section the saw blade should be directed horizontally from the side (in the frontal plane), in the thoracic section obliquely backwards and medially, and in the cervical section vertically (in the sagittal plane). Cutting the intervertebral discs every few segments can facilitate removal of the anterior wall of the spinal canal. After opening the spinal canal, the procedure is the same as in the posterior approach [1, 12]. The anterior approach enables the assessment or collection of plexuses (cervical, brachial, lumbosacral), sympathetic trunk, and the extradural sections of the vertebral and cervical arteries for examination. After removal of the spinal cord, the dural sac should be cut lengthwise with scissors in a median line anteriorly and posteriorly. The spinal cord should be fixed laid flat or stretched out on a board with pins driven into the dura in a container with formalin. In the case of lesions located at the level of the craniovertebral junction (e.g. Chiari syndrome), it is possible to combine the above-described posterior opening of the spinal canal with the posterior opening of the posterior cranial fossa, by means of an oscillating saw cutting through the previously exposed squamous part of the occipital bone from the foramen magnum to the transverse sinuses.

Central nervous system macroscopic examination

Macroscopic examination of the brain should be performed in the dissecting room or in the macroscopic laboratory, in compliance with the applicable health and safety regulations and the rules of procedure. The brain and spinal cord can be evaluated fresh at the time of general autopsy or after fixation. There are 2 fixation methods: immersion and perfusion; the former, more popular method consists of immersing the entire brain in a fixative. The most commonly used fixative is 10% buffered formalin solution (pH 7.0–7.2) [20]. The amount of formalin for the entire brain should be 5–6 litres; it is recommended that it be replaced every week for about 4 weeks [20, 21]. The perfusion method, involves cannulation and injection of fixative into the cerebral vascular system and its distribution throughout all the brain structures [22]. In special situations, short fixation is possible (about 3 days), for which it is recommended to make several sections and fix them in a flat position, with individual sections separated with blotting paper. In this case, it is more common to use 20% neutral buffered formalin solution or, additionally, the perfusion method [23, 24].

The above procedures are used to accurately assess the topography of brain lesions and structures; significant pathological changes may become visible in the brain only after its fixation [21]. In these situations, without whole brain fixation there may be significant problems with proper macroscopic assessment [25].

Brain dissection is most commonly performed using Spielmeyer's method [1, 25]:

1. The arachnoid of the basal cisterns should be dissected and the vessels of the circle of Willis and the vertebrobasilar system should be separated, with preservation of their arrangement and branches. In the case of subarachnoid haemorrhage, it is advisable to describe the distribution of the extravasated blood and at least partially wash it out before fixing the brain. In the case of whole brain fixation, vessel separation is recommended prior to fixation.

2. The brain stem with the cerebellum is separated from the brain at the level of the cerebral peduncles. For this purpose, the blade of the scalpel is inserted anteriorly into the space between the cerebellar hemisphere and the occipital lobe and, directed medially to the brain, the peduncles are severed first on one side and then on the other. The incision plane should be even and include a section of the substantia nigra and red nucleus.

3. The brain is laid with its base downwards. The pathologist, holding both hemispheres with one hand, makes parallel cuts at 1–1.5-cm intervals in the frontal plane, starting from the frontal pole, with the other hand. To facilitate the process, the pathologist can divide the brain into the anterior and posterior part by making a cut in the frontal plane through the mammillary bodies and sectioning it serially, as above. To prevent deformation and to obtain parallel cutting planes, a glass plate positioning may be useful, before the next section.

4. The cerebellum is separated from the brain stem by cutting through the cerebellar peduncles on both sides, allowing assessment of the floor of the fourth ventricle. Then the entire cerebellum is cut parallel to its upper surface, along the horizontal fissure. Alternatively, the cerebellar vermis can be dissected with a median incision and, after assessing the section of the arbor vitae, the hemispheres of the cerebellum should be separately cut parallel to its upper surface.

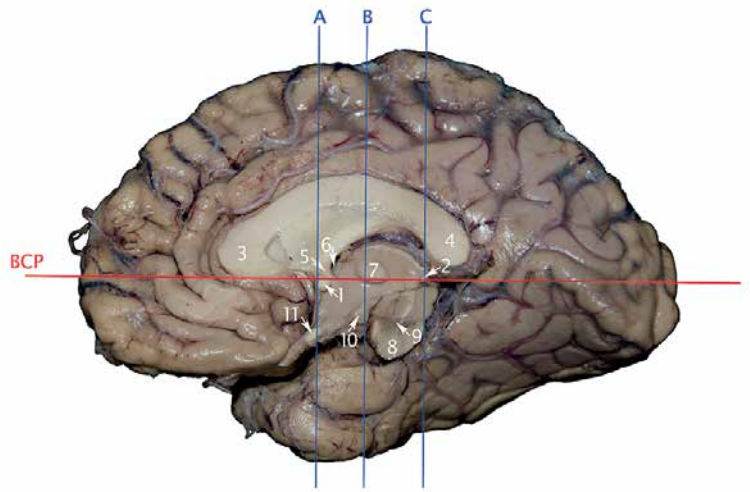


Fig. 2. Median section of the cerebrum

BCP – bicommissural horizontal reference plane passing through the anterior (1) and posterior (2) commissures BCP is usually used in anatomical atlases and to present standard MRI scans. Notice that section planes perpendicular to the BCP need to be tilted posteriorly if the specimen is lying flat on the lower surface of the temporal and occipital lobes: 3 – genu of corpus callosum, 4 – splenium of corpus callosum, 5 – column of fornix, 6 – interventricular foramen, 7 – interthalamic adhesion, 8 – cerebral crus, 9 – substantia nigra, 10 – mamillary body, 11– optic chiasm

6. The midbrain, pons, and medulla oblongata should be cut in a plane perpendicular to the long axis of the brain stem into slices approximately 0.5-cm thick.

7. Sections of the spinal cord are made transversely at 1-cm intervals.

8. All sections should be placed on an even flat surface, so that all right sides are on the right side of the pathologist, who should then proceed with description, macroscopic evaluation, photographic documentation, and collection of the sections.

The macroscopic description should include the mass of the brain, general appearance of the brain (symmetry, presence of herniations and oedema, relation of gyri to sulci, cortical atrophy), dura mater (including the dural sinuses and assessment of their patency), arachnoid and subarachnoid space with emphasis on haemorrhage, pia mater (transparency, presence of exudates), cranial nerves (their roots and places where they pass through the dura mater), vessels (anatomical variants, malformations, stage of atherosclerosis), in frontal sections the appearance of cortex, white matter, and the border between them, evaluation of corpus callosum, hippocampus, basal ganglia, thalamus, insular cortex, in brain stem sections the appearance of midbrain (special assessment of substantia nigra), pons, medulla oblongata (with attention to the olives), examination of cerebellum (assessment of the cortex, the vermis and hemispheres, dentate nucleus), spinal cord, and description of the ventricular system (size, symmetry, contents) and the choroid plexus [1, 12].

Basics of neuroanatomy necessary for performing central nervous system autopsy

An essential feature of the brain is its hierarchical structure resulting from the embryological development of the cerebral vesicles and their smaller segments, from which the various structures of the brain are formed. The most anterior is the forebrain, from which the telencephalon and diencephalon develop. Next is the midbrain, caudally joined with the hindbrain. The hindbrain gives rise to the pons and medulla oblongata, which, together with the cerebellum, lie in the infratentorial space of the cranial cavity [26]. The largest part of the brain comprises the cerebral hemispheres together with the connecting structures of the telencephalon medium. The cerebral hemispheres are divided into lobes (Fig. 2) [13, 26–29].

The frontal lobe is limited posteriorly by the central sulcus, and at the bottom by the lateral sulcus. Within it, on the superolateral surface, the superior (F1), middle (F2), and inferior (F3) frontal gyri can be distinguished, as well the precentral gyrus located posteriorly between them and the central sulcus. Cortex of the precentral gyrus acts as the primary motor centre. On the lower surface of the frontal lobe, the medially located straight gyrus and the orbital gyri are separated by the olfactory sulcus, within which is the olfactory bulb with the olfactory tract. The parietal lobe lies above the lateral sulcus, posterior to the central sulcus, and is separated from the occipital lobe by a deep parieto-occipital sulcus, clearly visible on the medial surface. Within the parietal lobe there is a postcentral gyrus parallel to the central sulcus, formed by the somatosensory projection cortex. The postcentral sulcus separates it from the superior (P1) and inferior (P2) parietal lobules. The temporal lobe is located below the lateral sulcus, and it is separated from the occipital lobe by a conventional line drawn through the parieto-occipital sulcus and the preoccipital notch located on the upper edge of the petrous part of the temporal bone. On the superolateral surface the superior (T1), middle (T2), and inferior (T3) temporal gyrus are distinguished. The transverse temporal gyri, which are visible on

the upper surface of the superior temporal gyrus, are formed by the primary auditory cortex. On the inferior surface, successively medially from the inferior temporal gyrus, the occipitotemporal sulcus, fusiform (T4) gyrus, collateral sulcus, and parahippocampal (T5) gyrus are visible. The occipital lobe has a rather variable pattern of gyri on the superolateral surface and a characteristic cuneus between the parieto-occipital sulcus and the calcarine sulcus on the medial surface. Below the calcarine sulcus is the lingual gyrus. The area surrounding the calcarine sulcus constitutes the primary visual cortex. The insular lobe is a lobe hidden in the depths of the lateral sulcus. The insular cortex is separated by a thin layer of white matter – the extreme capsule – from an equally thin layer of grey matter – the claustrum. Together they form the claustro-insular complex. The parts of the frontal, parietal, and temporal lobes that cover the insular cortex are called the frontal, parietal, and temporal opercula, respectively. In the frontal operculum of the dominant (usually left) hemisphere is the motor speech centre, and in the posterior part of the temporal operculum – the sensory speech centre. The limbic lobe, which includes the elements surrounding the corpus callosum and the choroidal fissure, is arranged in 2 rings. The outer ring, i.e. gyrus limbicus, consists of the subcallosal area, cingulate gyrus, parahippocampal gyrus, and uncus. The anterior part of the parahippocampal gyrus and the uncus are formed by the entorhinal cortex. The inner ring is the hippocampal formation, which includes the hippocampus proper (also called Ammon's horn), the alveus and fimbria of the hippocampus, the fornix and hippocampal commissure, the dentate gyrus, and the subiculum.

The surface of the cerebral hemispheres is covered with 2–4-mm-thick grey matter. About 90% of the cerebral cortex area consists of a 6-layered cortex (also called isocortex or neocortex). The cortex differs in the details of the development of individual layers in different regions, which is the basis of cytoarchitectonic divisions. In general, the sensory and association cortical areas have the granular layers in varying degrees of development (granular isocortex), while in the motor areas the granular layers are poorly developed (isocortex agranularis). The remaining 10% of the cortex has fewer layers – the heterogenic cortex (allocortex), within which the paleocortex and the archicortex are distinguished. The paleocortex is limited to small areas connected with the sense of smell: the olfactory bulb with the retrobulbar region and the piriform cortex. The archicortex forms the hippocampus, the dentate gyrus and the subiculum, the entorhinal cortex, and the cingulate cortex belt. Between the areas of the archicortex and the neocortex are transitional areas of the mesocortex [26, 30].

The basal ganglia comprises the largest group of subcortical grey matter clusters; other subcortical structures include the amygdaloid body and the basal forebrain proper. Most of the grey matter of the telencephalon located deep in the hemispheres of the brain is involved in the connection loops between the thalamus, cortex, and other centres of the diencephalon (subthalamic nucleus) and midbrain (substantia nigra). This is why these structures are often described together with the subcortical nuclei. After development, the nuclei usually cross the conventional and externally distinguishable boundaries between the organizational levels of the CNS [28, 29, 31, 32]. The basal ganglia, or the corpus striatum, include the circumferentially located caudate nucleus and the deeper lenticular nucleus, which are separated from each other by the fibres of the internal capsule. In the lenticular nucleus there is a putamen, located laterally, and a medially located globus pallidus. The caudate nucleus and putamen fuse in the vicinity of the septum forming the nucleus accumbens. These 3 parts are known as the striatum. The substantia nigra is a cluster of neuromelanin-stained dopaminergic neurons separating the cerebral crus from the mesencephalic tegmentum. Within the substantia nigra, a hypercellular pars compacta and, located ventrally in relation to it, pars reticularis are visible [28–30]. The amygdaloid body, bordered posteriorly by the temporal horn of the lateral ventricle, is medially adjacent to the uncus, frontally to the piriform cortex, and laterally to the temporal lobe peduncle [26, 33].

Modern research has changed the view on the course of the forebrain axis and revealed its originally segmental structure. According to this model, the pretectum, thalamus and epithalamus, prethalamus, and prerubral tegmentum are now classified as parts of the diencephalon. The nuclei of the cranial nerves from III to XII are located in the brain stem. The midbrain contains the cerebral crus, the mesencephalic tegmentum, and the mesencephalic tectum. In the interpeduncular fossa, the oculomotor nerves exit the brain stem. There is a cluster of grey matter around the cerebral aqueduct – the periaqueductal grey substance. The quadrigeminal plate (tectal plate) consists mainly of 2 superior and 2 inferior colliculi. The pons is the widest part of the brain stem. The root of the trigeminal nerve marks the border between the pons and the middle cerebellar peduncle. On the ventral side of the medulla oblongata there are distinct medullary pyramids with corticospinal fibres and laterally located olivary bodies (Fig. 3, 4).

The cerebellum is connected with the brain stem by the cerebellar peduncles: superior – reaching the midbrain; middle – reaching the pons; and inferior – reaching the medulla oblongata [13, 27]. The border between the centrally located cerebel-

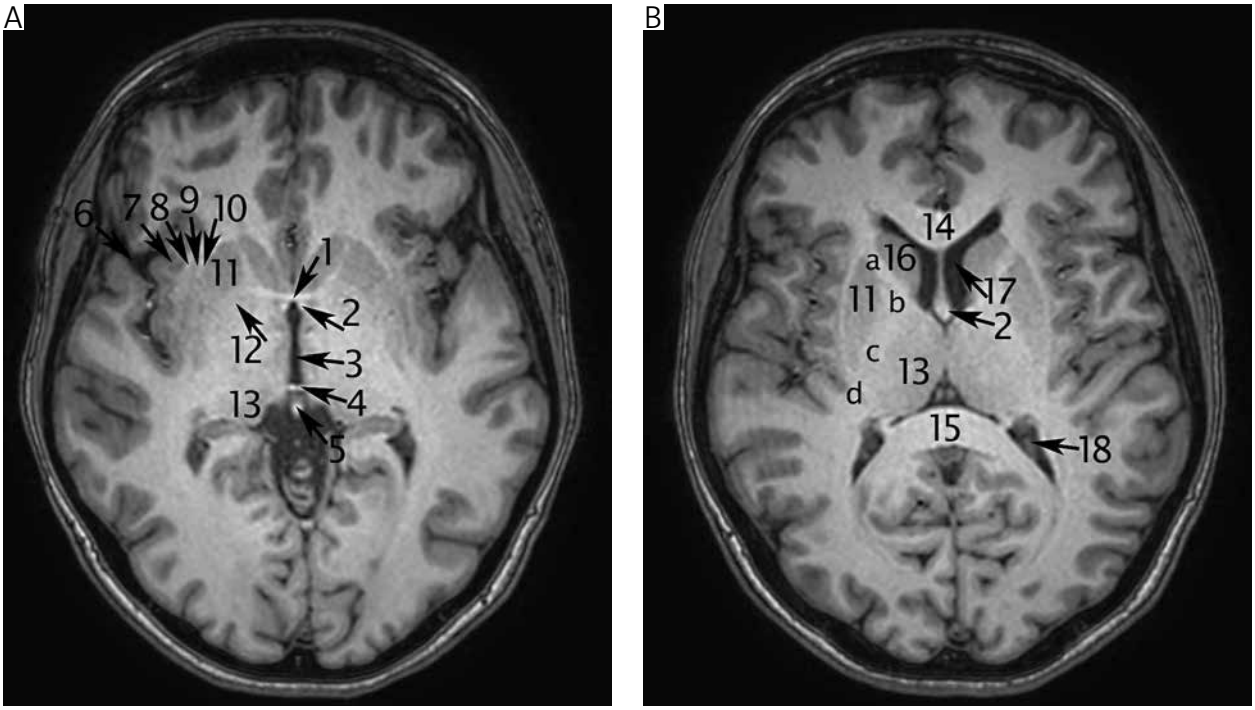


Fig. 3. Magnetic resonance scans (T1)

Transverse section on level of marked bicommissural reference plane (left) and 1 cm above (right): 1 – anterior commissure, 2 – left column of fornix, 3 – third ventricle, 4 – posterior commissure, 5 – pineal body, 6 – right lateral sulcus, 7 – cortex of insula, 8 – extreme capsule, 9 – claustrum, 10 – external capsule, 11 – putamen, 12 – globus pallidus, 13 – thalamus, 14 – genu of corpus callosum, 15 – splenium of corpus callosum, 16 – head of caudate nucleus, 17 – frontal horn of left lateral ventricle, 18 – choroid plexus in atrium of lateral ventricle, a – anterior limb, b – genu, c – posterior limb, d – retrolentiform part of internal capsule

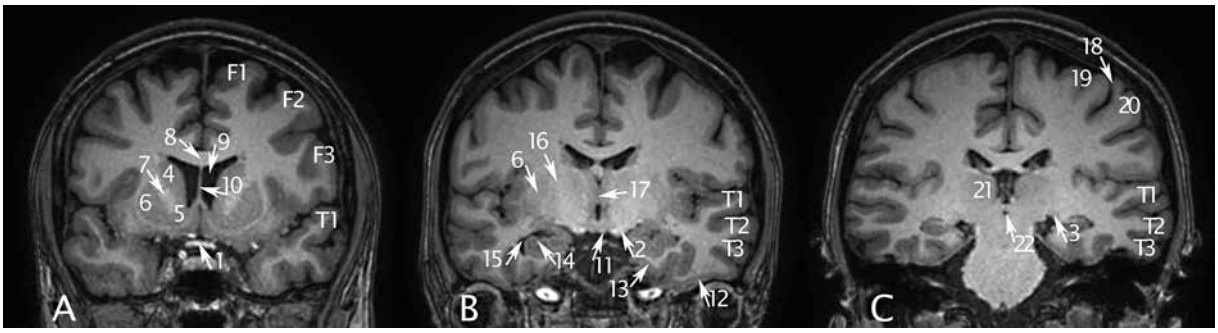


Fig. 4. Magnetic resonance scans (T1) Coronal sections marked on Fig. 2 as through optic chiasm (A), through mammillary bodies (B), and through posterior commissure (C)

1 – optic chiasm, 2 – optic tract, 3 – lateral geniculate body, 4 – head of caudate nucleus, 5 – nucleus accumbens, 6 – putamen, 7 – anterior limb of internal capsule, 8 – trunk of corpus callosum, 9 – frontal horn of left lateral ventricle, 10 – septum pellucidum, 11 – left mammillary body, 12 – fusiform gyrus, 13 – parahippocampal gyrus, 14 – pes hippocampi, 15 – temporal horn of lateral ventricle, 16 – posterior limb of internal capsule, 17 – interthalamic adhesion, 18 – central sulcus, 19 – precentral gyrus, 20 – postcentral gyrus, 21 – thalamus, 22 – cerebral aqueduct, F1-3 – superior, middle, and inferior frontal gyrus, T1-3 – superior, middle, and inferior temporal gyrus

lar vermis and the cerebellar hemispheres is visible on the lower surface above the median aperture of the fourth ventricle. The lowest lobuli of the cerebellar hemispheres – the tonsils of cerebellum – adjoin the medulla oblongata and may reach the plane of the foramen magnum.

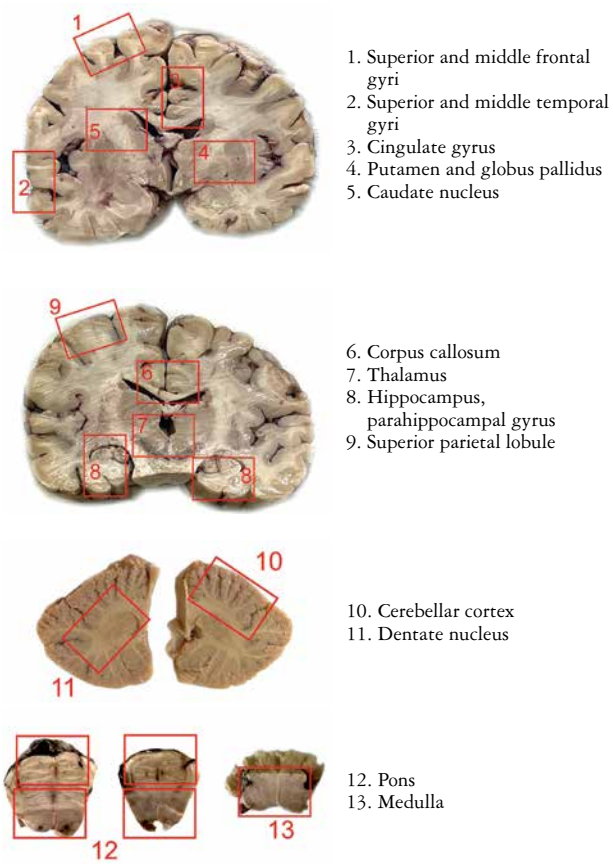
In each of the brain hemispheres there is a lateral ventricle, which consists of the anterior (frontal) horn located anterior to the interventricular foramen, the body, i.e. the middle part, located above the thalamus, the atrium, i.e. the trigone of the ventricle, which branches into the posterior (occipital) horn, and inferior (temporal) horn. The lateral ventricle connects through the interventricular foramen with the third ventricle, which communicates through the mesencephalic aqueduct with the fourth ventricle, leading into the central canal of the medulla oblongata and then the spinal cord. The fourth ventricle communicates with the subarachnoid space through paired lateral apertures of Luschka and the median aperture (foramen of Magendie). All ventricles contain the choroid plexus, which produces the CSF [13, 27, 33].

Within the spinal cord, 2 enlargements can be distinguished: the cervical at the level of the brachial plexus and the lumbosacral at the level of the lumbosacral plexus, tapering into the final section – conus medullaris. The spinal cord is divided into symmetrical halves by the anterior median fissure and the shallow-

er posterior median sulcus. The rootlets of the spinal nerves produce additional sulci: anterolateral sulcus and posterolateral sulcus. Thus, the peripheral white matter of the spinal cord is divided into the anterior, lateral, and posterior funiculi. In the posterior funiculus of the cervical part of the spinal cord and the medulla oblongata, there is a division into the gracile fasciculus and the cuneate fasciculus, which contain fibres of the proprioceptive and sensory pathway from the lower and upper limb, respectively. The fibres of pain and temperature sensation pathways are located on the border between the anterior and lateral funiculi. The descending motor fibres are located in the lateral funiculus. In the grey matter lying deeper, on both sides, the anterior (motor) horn, the posterior (sensory) horn, and the central intermediate substance are located, as well as the lateral intermediate substance, in which the lateral horn is distinguished in segments C8-L2, containing neurons of the autonomic system. The horns visible in the cross-sections form columns [26, 33].

General principles of taking specimens for histopathological examination

During a neuropathological autopsy, the brain is classified as macroscopically normal or abnormal.



1. Superior and middle frontal gyri
2. Superior and middle temporal gyri
3. Cingulate gyrus
4. Putamen and globus pallidus
5. Caudate nucleus
6. Corpus callosum
7. Thalamus
8. Hippocampus, parahippocampal gyrus
9. Superior parietal lobule
10. Cerebellar cortex
11. Dentate nucleus
12. Pons
13. Medulla

Fig. 5. Topography of the routine sampling for the histopathological central nervous system exam

A "normal brain" is defined as without macroscopic pathological changes in patients with a negative clinical neurological history and in the absence of, or normal, *in vivo* neuroimaging. The specimens are taken from the frontal lobe with cingulate gyrus, superior and middle temporal gyri, parietal and occipital lobe along with the pia mater, globus pallidus with putamen and claustrum, hippocampus, thalamus, periventricular white matter, midbrain, pons, and cerebellar cortex with dentate nucleus (minimum 8 from the above-mentioned). Moreover, it is possible to take additional samples from the available section of the spinal cord, spinal ganglia, mamillary bodies, medulla oblongata, and basal ganglia. A normal brain can be sectioned without prior fixation.

An "abnormal brain" is a macroscopically altered brain from patients with described neurological symptoms or symptoms related to CNS damage, with changes found in neuroimaging, from oncological patients, and in cases with no established cause of death. In the case of an abnormal brain, it is recommended that an autopsy of the brain and spinal cord be performed after fixation [21]. Before performing the examination of the previously fixed brain, one should remember to gently rinse the brain under running water for a minimum of 1–2 hours. From each abnormal brain, samples should be taken from pathological changes and, additionally, from macroscopically unchanged regions, similarly to the autopsy of the normal brain. Moreover, depending on the disease entity, clinical symptoms and the results of neuroimaging diagnostics, samples are taken in accordance with the algorithm of neuropathological diagnostics of a given disease group/entity. The most common ones include vascular damage, toxic and metabolic encephalopathies, encephalitis, neurodegenerative diseases, leukodystrophies, and epilepsy [17, 34, 35]. The basic sampling approach, minimum sample number and their localisation in particular groups of CNS diseases are showed in Figure 5 and Table I. Each collected sample should be topographically described and placed in separate labelled cassettes, with markings designating left or right side and the surface from which to start cutting with a microtome. There are many methods of sampling in neuropathological autopsy, from extended multi-sectioning to simplified systems [17, 34, 36]. Usually, typical pathological cassettes are used, but in referential centres, especially in brain banks, even hemispheric sections are possible.

Foetal and newborn brain autopsy

Foetal and neonatal brain dissection is a special type of neuropathological examination. The provided medical documentation should include information on the course of pregnancy, complications in the perinatal period, maternal diseases, medications, family medical history, as well as imaging results [37].

Table I. Recommended sample collection in common central nervus system disorders

Disease	Samples
CNS hypoxia/ischaemic stroke	Areas sensitive to hypoperfusion (hippocampus, cerebellar cortex, thalamus, midbrain) Watershed areas (superior and middle frontal gyrus, superior and middle temporal gyrus, cingulate cortex, occipital gyri, putamen with globus pallidus, T4 level of spinal cord) Minimum 11 samples
Encephalitis, infectious conditions	Cortex of both hemispheres (including those from the medial areas of both temporal lobes with hippocampi), deep white matter, periventricular white matter, several levels of the brainstem, cerebellum, basal ganglia, spinal cord, paravertebral ganglia From 11 to over 40 samples
Neurodegenerative diseases – the sampling approach depends on the clinical diagnosis/suspicion and dedicated disease-specific recommendations (shortened or distended forms)	Middle frontal gyrus, cingulate cortex, superior and middle temporal gyrus, hippocampus with hippocampal gyrus and entorhinal cortex, superior parietal lobule, putamen with globus pallidus, midbrain, substantia nigra, pons, caudate nucleus, cerebellar vermis, cerebellar cortex with dentate nucleus, medulla oblongata Spinal cord and paravertebral ganglia (in specific conditions) Minimum 13 samples
Epilepsy	Hippocampus with entorhinal cortex, cingulate cortex, parahippocampal gyrus, middle temporal gyrus, middle frontal gyrus, caudate nucleus, putamen, globus pallidus, thalamus, cerebellar vermis, cerebellar hemispheres with dentate nucleus Minimum 12 samples
Endogenous and exogenous encephalopathies (nutrient, post-alcoholic encephalopathy)	Corpus callosum, cerebellar hemispheres with cerebellar vermis (its upper and lower parts), mammillary bodies, periaqueductal grey matter, periventricular region, pons, medulla oblongata, spinal cord (in specific conditions) Minimum 8 samples
Creutzfeldt-Jakob disease	Frontal, parietal, occipital and temporal cortex, hippocampus, basal ganglia, thalamus, midbrain, cerebellum, medulla oblongata Minimum 10 samples

Scheme mentioned below is as a supplement to sample collection for "normal brain".

The removal of the brain begins with a cut across the skull, starting from the ear area, through the occiput to the other ear area, running the incision laterally to the neck area and towards the spine. Such an incision allows a better assessment of the cervical spine and the presence of hernias. The fontanelles should be measured, their tension should be assessed, and the bones of the calvaria examined. The cranial cavity is opened by making incisions along the suture lines of the skull, starting from the anterior fontanelle area forward and backward, and then ovally along the base of the skull so that the cerebral falx and the cerebellar tentorium are visible and available for *in situ* examination. Next, an incision is made in the cerebral falx along the transverse sinus of the dura mater from front to back. Both hemispheres of the brain are removed by gently separating the nerve tissue from the bones of the skull base by hand. Removal of the cerebellum takes place after cutting the cerebellar tentorium along the petrous part of the temporal bone towards the squamous part (base) from the peripheral edges of the cerebellum. The cervical spine needs to be cut off through the foramen magnum at the deepest possible point [12, 38, 39]. The entire brain needs to be weighed and placed in a fixative solution for about 10–20 days (7–10 days is sufficient for a pregnancy of less than 24 weeks), which is important in the case of suspected hydrocephalus, intracerebral bleeding, structural malformation, birth defects, perinatal injuries, and complications of prematurity. In a situation where early post-mortem changes are suspected, it might be possible to fix the brain inside the skull *(in situ* fixation) by administering a formalin solution with a syringe into the subdural space and a small amount into the ventricular system [40]. Brain hemispheres, the brainstem, and the cerebellum are required to be serially cut in the frontal plane into slices with a diameter of no more than 1 cm. The removal of the spinal cord and its assessment, if necessary, is performed according to the same principles as for adults [1, 38]. Samples from brain during paediatric autopsy are required to taken at least from the cere-

bral cortex, periventricular white matter, thalamus, basal ganglia, hippocampus, midbrain (inferior colliculi), pons, and cerebellum with dentate nucleus. In cases of malformation and specific diseases, appropriate extensive sampling is advised [41].

Brain dissection when prion disease is suspected

A brain autopsy in the case of suspected prion disease should be performed with special care because the CNS has the highest number of infectious agents in such diseases [42]. Autopsies and processing of non-decontaminated tissues should be performed in a laboratory with biosafety level 2 (BSL-2) or higher [43]. Tools should be disposable, and if this is not possible, they should be decontaminated [44]. Before fixing the brain, at least one fragment of it should be collected from the frontal cortex and the cerebellum in order to obtain fresh frozen material for biochemical and genetic tests [42].

Ancillary tests

The preservation and preparation of the material for microbiological, ultrastructural, and toxicological testing should be consulted with the appropriate microbiology/toxicology/forensic laboratory to avoid preanalytical errors. Ultrastructure tests require the use of a different fixative, usually 2–5% glutaraldehyde [1]. If collecting CSF is required, this procedure should be performed prior to removing the brain and/or spinal cord from the body. For molecular tests, usually a representative paraffin block is sufficient; in some cases it is necessary to have fresh frozen material preserved at an appropriate temperature [16, 45].

The most frequently used histochemical methods in CNS neuropathology include the following: Masson's trichrome, silvering by Gomori's method and Spielmeyer's method, staining with cresyl violet, toluidine blue, Congo red, Bielschowsky's method, Bodian's method, periodic acid-Schiff, and Groccott [13, 16, 46].

The neuropathological immunohistochemistry panel includes, *inter alia*, glial fibrillary acidic protein, neuronal markers (synaptophysin, S100, neurofilaments), epithelial membrane antigen, LCA, CD68, CD20, and CD3 [16, 46, 47]. Many other antibodies are used in neurodegenerative and inflammatory disorders, e.g. antibodies against β-amyloid, tau, α-synuclein, ubiquitin, PrPsc, and viral markers. Many more markers are available only in reference scientific centres [48].

Post-mortem neuropathological examination protocol

The brain and spinal cord post-mortem examination result is included in the autopsy report [18]. The elements of the protocol, specimen preparation and microscopic evaluation, and the final report should be performed in accordance with the general guidelines and internal regulations. The pathoclinical correlation discussion with neurologists, neurosurgeons, or other referring clinicians after the microscopical analysis in some cases is necessary to make the final consensus. The results might also be discussed by the panel on hospital death review committee. The final autopsy report is adviced to be available for up to 60 days in complex cases [19]. Tissue material collected during the dissection and the brain fixed in formalin should be stored for a minimum of 3 months after issuing the protocol (in units without a formalin specimen brain bank). The storage period for paraffin blocks and microscope slides, in accordance with the relevant Regulation of the Minister of Health regarding the storage of medical records (Journal of Laws of 2015, item 2069), is 20 years.

Quality control

As part of maintaining high-quality neuropathological services, it is recommended, in pathomorphology/neuropathology units/laboratories, to follow internal procedures as well as the recommendations of the National Consultant, the Polish Society of Pathologists, and the Polish Association of Neuropathologists. It is advised that every major pathomorphology facility, if it does not employ a neuropathologist, should train or enable training of a selected physician in neuropathological issues. In difficult and/or doubtful cases, it is recommended that material be sent for consultation to neuropathology specialists from other centres.

Conclusions

The recommendation for CNS autopsy includes a detailed description of autopsy techniques, macroscopic evaluation, and the basics of sample collection for histopathological examination. We emphasise the necessity of cooperation between different specialists and believe the guidelines might be applied in medical centres in Poland as a standard procedure for neuropathological post-mortem examinations.

The authors declare no conflict of interest.

References

1. Powers JM. Practice guidelines for autopsy pathology. Autopsy procedures for brain, spinal cord, and neuromuscular system. Arch Pathol Lab Med 1995; 119: 777-783.
2. Kotabagi RB, Charati SC, Jayachandar D. Clinical autopsy vs. medicolegal autopsy. Med J Armed Forces India 2011; 61: 258-263.
3. Javier Meana J, Callado LF, Morentin B. Do post-mortem brain studies provide useful information for Psychiatry? Revista De Psiquiatria Y Salud Mental 2014; 7: 101-103.

4. Lee JA. Guidelines on autopsy practice. Report of a working group of The Royal College of Pathologists. The Royal College of Pathologist, London 2002.
5. Mukerji SS, Solomon IH. What can we learn from brain autopsies in COVID-19? Neurosci Lett 2021; 742.
6. Lee KH, Seo SW, Lim TS, et al. Proposal Guidelines for Standardized Operating Procedures of Brain Autopsy: Brain Bank in South Korea. Yonsei Med J 2017; 58: 1055-1060.
7. Roulson J, Benbow EW, Hasleton PS. Discrepancies between clinical and autopsy diagnosis and the value of post mortem histology; a meta-analysis and review. Histopathology 2005; 47: 551-559.
8. Pastores SM, Dulu A, Voigt L, et al. Premortem clinical diagnoses and postmortem autopsy findings: discrepancies in critically ill cancer patients. Critical Care 2007; 11: R48.
9. Shojania KG, Burton EC, McDonald KM, et al. Changes in rates of autopsy-detected diagnostic errors over time: a systematic review. JAMA 2003; 289: 2849-2856.
10. Marshall HS, Milikowski C. Comparison of Clinical Diagnoses and Autopsy Findings Six-Year Retrospective Study. Arch Pathol Lab Med 2017; 141: 1262-1266.
11. Love S. Neuropathological investigation of dementia: a guide for neurologists. J Neurol Neurosurg Psychiatry 2005; 76: V8-V14.
12. Fyfe B, Miller DV. Diagnostic Pathology: hospital autopsy. 1st ed. Elsevier 2015.
13. Liberski P, Papież W. Mossakowski's neuropathology [Neuropatologia Mossakowskiego.] 1st ed. Czelej, Lublin 2005.
14. Gray F, Duyckaerts C, Girolami U. Escourolle and Poirier's manual of basic neuropathology. 6th ed. Oxford University Press Inc., Oxford 2019.
15. Mossakowski M, Dymecki J, Wender M. Basics of neuropathology. [Podstawy neuropatologii.]. PZWL, Warsaw 1981.
16. Alafuzoff I. Techniques in neuropathology. Handb Clin Neurol 2017; 145: 3-7.
17. Sejda A, Wierzba-Bobrowicz T, Adamek D, et al. Central nervous system autopsy – a neuropathological procedure based on multidisciplinary pathoclinical cooperation. Neurol Neurochir Pol 2022; 56: 118-130.
18. Alfsen GC, Gulczyński J, Kholova I, et al. Code of practice for medical autopsies: a minimum standard position paper for pathology departments performing medical (hospital) autopsies in adults. Virchows Arch 2022; 480: 509-517.
19. Polish Society of Pathologists. Organizational standards and standards of conduct in pathomorphology. Guidelines for pathology departments/laboratories. [Polskie Towarzystwo Patologów. Standardy organizacyjne oraz standardy postępowania w patomorfologii. Wytyczne dla zakładów/pracowni patomorfologii.]. Warsaw 2020.
20. Fix AS, Garman RH. Practical aspects of neuropathology: a technical guide for working with the nervous system. Toxicol Pathol 2000; 28: 122-131.
21. Katelaris A, Kencian J, Duflou J, et al. Brains at necropsy: to fix or not to fix? J Clin Pathol 1994; 47: 718-720.
22. McFadden WC, Walsh H, Richer F, et al. Perfusion fixation in brain banking: a systematic review. Acta Neuropathol Commun 2019; 5; 7: 146.
23. Sharma M, Grieve JHK. Rapid fixation of brains: a viable alternative? J Clin Pathol 2006; 59: 393-395.
24. McKenzie A, Woodoff-Leith E, Dangoor D. Ex situ perfusion fixation for brain banking: a technical report. Free Neuropathology 2022; 3.
25. Iacono D, Geraci-Erck M, Peng H, et al. Symmetric Bihemispheric Postmortem Brain Cutting to Study Healthy and Pathological Brain Conditions in Humans. J Vis Exp 2016; 18: 54602.
26. Moryś J, Narkiewicz O. Functional and clinical neuroanatomy. Handbook for students and doctors. [Neuroanatomia czynnościowa i kliniczna. Podręcznik dla studentów i lekarzy.]. PZWL 2022.
27. Bochenek A, Reicher M. Human anatomy. Volume 4–5. [Anatomia człowieka. Tom 4–5.]. PZWL, Warsaw 2010–2014.
28. Ciołkowski M. Neuroanatomical nomenclature. Polish version of the international neuroanatomical nomenclature. [Mianownictwo neuroanatomiczne. Wersja polska międzynarodowego mianownictwa neuroanatomicznego.] Warsow 2019. Department of Clinical Anatomy, Medical University of Warsaw [Zakład Anatomii Prawidłowej i Klinicznej Warszawskiego Uniwersytetu Medycznego].
29. The Federative International Programme for Anatomical Terminology. A programme of the International Federation of Associations of Anatomists. Terminologia Neuroanatomica. 2017.
30. Ten Donkelaar HJ, Kachlík D, Tubbs RS. An Illustrated Terminologia Neuroanatomica. A Concise Encyclopedia of Human Neuroanatomy. Springer International Publishing 2018.
31. Bostan AC, Dum RP, Strick PL. Functional anatomy of basal ganglia circuits with the cerebral cortex and the cerebellum. Prog Neurol Surg 2018; 33: 50-61.
32. Lanciego JL, Luquin N, Obeso JA. Functional neuroanatomy of the basal ganglia. Cold Spring Harb Perspect Med 2012; 2: a009621.
33. Stelmasiak M. Atlas of the anatomy of the brain and spinal cord. [Atlas anatomii mózgowia i rdzenia kręgowego.] 1st ed. PZWL 1953.
34. Love S. Post mortem sampling of the brain and other tissues in neurodegenerative disease. Histopathology 2004; 44: 309-317.
35. Love S. Autopsy approach to stroke. Histopathology 2011; 58: 333-351.
36. Ravid R, Ikemoto K. Pitfalls and practicalities in collecting and banking human brain tissues for research on psychiatric and neulogical disorders. Fukushima J Med Sci 2012; 58: 82-87.
37. Putman MA. Perinatal perimortem and postmortem examination: obligations and considerations for perinatal, neonatal, and pediatric clinicians. Adv Neonatal Care 2007; 7: 281-288.
38. Thom M, Allinson K. Guidelines on autopsy practice: deaths in patients with epilepsy including sudden deaths. Royal College of Pathologists, London 2019.
39. Jaiman S. Performing a perinatal autopsy. J Fetal Med 2015; 2: 101-111.
40. Bass T, Bergevin MA, Werner AL, et al. *In situ* fixation of the neonatal brain and spinal cord. Pediatr Pathol 1993; 13: 699-705.
41. Jansen AC, Robitaille Y, Honavaret M, et al. The histopathology of polymicrogyria: a series of 71 brain autopsy studies. Dev Med Child Neurol 2016; 58: 39-48.
42. Love J, Ince P, Revesz T. Neuropathology autopsy practice: Post-mortem examination in dementia. The Royal College of Pathologists, London 2014.
43. Meechan PJ, Potts J. Biosafety in microbiological and biomedical laboratories. 6th ed. Centers for Disease Control and Prevention National Institutes of Health 2009.
44. Rutala WA, Weber DJ. Creutzfeldt-Jakob disease: recommendations for disinfection and sterilization. Clin Infect Dis 2001; 32: 1348-1356.
45. Watanabe N, Umezo T, Kyushiki M, et al. Optimal DNA quality preservation process for comprehensive genomic testing of pediatric clinical autopsy formalin-fixed, paraffin-embedded tissues. Pol J Pathol 2022; 73: 255-263.
46. Perry A, Brat DJ. Practical surgical neuropathology: a diagnostic approach a volume in the pattern recognition series. 2nd ed. Elsevier 2017.
47. Ellison D, Love S. Reference Text of CNS Pathology, Neuropathology. 3rd ed. Elsevier 2013.
48. Mendel TA, Wierzba-Bobrowicz T, Lewandowska E, et al. The development of cerebral amyloid angiopathy in cerebral vessels. A review with illustrations based upon own investigated post mortem cases. Pol J Pathol 2013; 64: 260-267.

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