

**UNIVERSITY OF MEDICINE AND PHARMACY
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DOCTOR DEGREE

Clinical-Statistical, Histopathological and
Immunohistochemical Study of Ischemic Strokes

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Abstract

Key words: cerebral ischemia, cerebral vascular accident (stroke), atheromatosis, neurons, astrocytes, risk factors, ramollissement, edema, immunomarquage.

Considering the fact that brain softening (ramollissement) is the result of circulatory deficiencies occurring under the circumstances of vascular atheromatosis, atherotrombosis and arteriosclerosis, as well as the existence of ischemic injuries registering the same age and regular seat determined by the lack of vascular occlusions (for example global brain hypoperfusion generated by the hemorrhagic shock, arterial hypotension, arrhythmia), our purpose is to elaborate a clinical-statistical study meant to present different correlations according to age, sex, social environment, a microscopic and macroscopic histopathological study meant to emphasize brain vascular pathology through classical anatomo-pathological methods of prevailing injuries and an immunohistochemical study.

The paper points out cerebrovascular transformations and changes of the cerebral structures in a macroscopic perspective, correlated to the existence of determining factors of stroke, and tries to achieve a microscopic and immunohistochemical study considering the timing from the acute cerebrovascular event to death.

GENERAL DATA

Anatomy and Histophysiology of the Central Nervous System

Considered as the most complex system of the human body, the nervous system consists of organs composed of nervous tissue, blood vessels and connective tissue. The hematoencephalic barrier acts as a separation between the circulating blood and the nervous tissue and presents selective permeability. The correlation between structure and function is highly reflected at the nervous system level, providing the functional unit of the body and its adaptation to the environment (Mogoantă L, 2004).

Recent studies have shown that, after injury or insult, the neuronal circuit may reorganize itself by generating new synapses in order to compensate for the loss of other synapses. The brain's ability to form new neural connections after injury or insult defines *neuroplasticity*.

At the nervous system level we find a parenchyma and a stroma. The parenchyma of the central nervous system designates all the nerve cells. Stroma includes glial cells, fine connective framework and capillaries (Hickey WF, 2001). The parenchyma of the nervous system is organized on two tissular structures displaying distinct morphological and functional aspects: the grey matter and the white matter.

The white matter consists mostly of myelinated axons which are parallel in distribution, clustered into bundles and strands. The axons are enwrapped by layers of numerous glial cells, particularly oligodendroglial cells, which coils around axons as myelin sheaths. The white matter plays a fundamental role in conducting the nervous influx which demands a reduced energy consumption, determining thus, a reduced number of capillaries in the white matter as compared to that corresponding to the grey matter (Bogdan FI, 1989).

The grey matter – is distributed on columns (at the level of the spinal cord), on layers (in the depth of the cerebral cortex and of the diencephalon) or as a continuous pallium (at the surface of the cerebral and cerebellar hemispheres). Histologically, the grey matter is made up of neuronal cell bodies, dendrites, the initial unmyelinated extremity of the axons and neuroglial cells; it is also composed of numerous blood vessels, a complex network of capillaries allowing the development of an intense oxidative metabolism, specific to neurons.

Brain's Blood Supply

Arterial System. The brain, although it represents only 2% of the body weight, it receives 15% of the cardiac output and consumes 20% of the oxygen supply of the body, in the basal state. Cerebral irrigation is provided by four major vascular vessels (main blood vessels) symmetrically organized at cervical level: two carotid arteries and two vertebral arteries, linked together by an anastomotic circuit.

Carotid arteries provides 75% of the cerebral output. These arteries present a paired structure and follow the same course, except their origin, on the right, respectively, on the left side. The right carotid artery originates from the brachiocephalic arterial trunk – the most large branch of the aortic arch, which divides into the right common carotid artery and the right subclavian artery at the level of the superior extremity of the right sternoclavicular joint. On the left side, the common carotid artery originates close to the cranial region of the aortic arch, and travels backward and to the left side of the brachiocephalic arterial trunk.

Internal carotid arteries (ACI) deliver blood to the anterior part of the brain and of the eye. 2/3 of the ACC flow is undertaken by the ACI. From an anatomical point of view, ACI are larger in diameter than the ACE (external carotid arteries) and present a posterolateral trajectory (Carpenter MB, 2001). Normally, ACI do not bifurcate at the cervical level of its trajectory. At the basal level of Sylvian fissure, ACI divide into four terminal branches: anterior cerebral artery, middle cerebral artery (sylvian), posterior communicating artery, anterior choroidal artery which supply blood to the largest part of the cerebral hemispheres. From a surgical point of view, Bouthillier divides AIC into 7 segments, following the course of the blood flow: cervical segment, petrous segment, lacerum segment, cavernous segment, clinoid segment, ophthalmic and communicating segment, and Dănăilă adds the choroidal segment (Dănăilă L, 2001).

Ophthalmic artery represents an important branch of the internal carotid artery, which enters the orbit together with the optic nerve through the optic canal, and bifurcates into numerous branches.

Of a great histopathological and clinical importance is the sylvian artery or the middle cerebral artery which arises close to the anterior cerebral artery. It can be classified into 4 segments: M1, horizontal; M2, insular; M3, opercular; M4, cortical.

The anterior cerebral artery presents three parts: the basal region which crosses the inferior portions of the head following the median, tangentially, to the interhemispheric fissure; the caudate region, so termed due to the angle formed by the two pair arteries through the anterior communicating artery, at the interhemispheric fissure level; the interhemispheric region at the interhemispheric fissure level which extends from forward to backward, goes beyond the knee of the corpus callosum to the splenium.

Posterior communicating artery

The posterior communicating artery indicates a variable length (7-25 mm), diameter (0,1-2 mm) and structure (often hypoplastic, remarkably absent), and divides into 3-5 thin collateral branches which provides blood to the hypothalamus and thalamus

Anterior choroidal artery

The anterior choroidal artery is the terminal branch of the internal carotid artery, registering the smallest diameter, its occlusion determines lacunar infarct.

Subclavian-vertebral artery

The right subclavian artery arises from the brachiocephalic trunk, to the level of the right sternoclavicular joint, and on the left side the subclavian artery comes directly from the arch of aorta. There are four branches of the subclavian artery. Vertebral arteries (AV) supplies blood to the posterior part of the brain (cerebral cortex, cerebellum and occipital lobe).

Posterior cerebral artery

The main branch supplies the lateral-basal temporal area, the occipital lobe, the lateral geniculate ganglion, the thalamus; its occlusion determines hemianopsia, and for injuries of the dominant hemisphere, persistent sensory aphasia.

Etiology of Cerebral Vascular Diseases

The cerebrovascular disease determines an increased morbidity degree, and, according to statistics, it occupies the third position after cardiac diseases and neoplasia (Manton si Baum -1996). One may notice an increasing number of cases of cerebrovascular circulatory disorders generated by the prevalence of atherosclerosis and arterial hypertension (the main causes of the cerebrovascular distress).

The gravity, prevalence and consequences of the strokes constitute the most important chapter in brain pathology. The stroke is defined as a sudden neurological loss caused by ischemia or bleeding into the brain.

Ischemic strokes represent almost 75% of the total amount of cerebrovascular accidents, following acute focal vascular occlusion which determines the lack of oxygen and glucose supply to the brain disturbing the metabolic reactions of the affected area. In parallel, there occurs a reduction of the blood flow, and, a series of aging-associated risk factors induce the existence of atheroma plaques, which represent the main cause of stenosis, occlusions and emboli within the cervicocerebral arterial system (Bonnet -1991). The loss of vasodilator properties which protect the injured endothelium is associated with a prevalence of the vasoconstrictor tonus, particularly of the noradrenergic tonus, and with a platelet aggregation.

At the cerebral level, the neuronal ischemia determines multiform symptomatology infarct: motor disorders, amnesic disorders, attention and awareness disorders, leading to what is presently termed as vascular dementia. At the neurosensorial level, the vertigo, acouphene, hypoacusis and retinopathies may seriously affect the daily activity of elderly (Calandre - 1985; Bonnet - 1991).

Hematoencephalic Barrier

According to a neurophysiological analysis, the hematoencephalic barrier plays an important well-determined role, constituting a complex structure which protects neurons against contingent toxic substances into the circulating blood. A proper permanent hematoencephalic barrier (BHE) is created between blood and wall of cerebral capillaries and a hemato-liquid barrier (BHL) between blood and cephalorachidian liquid (LCR).

The endothelial cells in the cerebral capillary are tightly joined through occlusive junctions (distinct structure differing from that of capillary cells of other tissues), thus, they lack fenestration. Another characteristic of endothelial cells in cerebral vessels refers to the presence of a large number of mitochondria, unlike those in systemic capillaries, a very important detail in the analysis of the active role of cerebral capillaries in the transfers of the hematoencephalic barrier, and simultaneously, of their ability to create a vulnerable area in different pathological stages.

Cerebral blood capillaries are directly connected to the nervous tissue and they are enveloped by a sheath of glial substance made up of astrocytes. This type of astrocytic sheath separates vascular from neuronal elements, maintaining, thus, the hematoencephalic barrier and allowing direct transfers between the two environments (McKay -1997). Astrocytes represents the most ample cell type in the central nervous system and, recently, the their function has been reconsidered, and they are now thought to play a of active roles in the brain activity.

MORPHOPHYSIOPATHOLOGY IN CEREBRAL VASCULAR ACCIDENTS

The cerebrovascular disease represent the third major cause of death in the world (after cardiovascular diseases and cancer). In Europe the mortality rate per year varies between 63.5 and 273.4/100,000; cerebrovascular diseases are the most frequent neurological disorders considering the morbidity and mortality rates. The term of cerebrovascular disease infers any injury to the brain able to determine a pathological process of blood vessels.

According to clinical observations, cerebrovascular diseases include three major categories: thrombosis, embolism, hemorrhage; this active classification is very constructive regarding the patient's therapeutic approach which differs from one group to another. Stroke is the clinical definition for all cases and, particularly, refers to the acute manifestation of symptoms. The stroke the major cause of mortality and long-term disability in Europe, as well as in other industrialized countries; the induction rate varies between different European countries and it is estimated to 100-200 recent cases per year reported to 100,000 inhabitants. From a physiopathological and anatomopathological point of view, cerebrovascular diseases include 2 processes:

- hypoxia, ischemia and infarct resulting from the impairment of the blood flow and of the oxygenation of the cerebral tissue (Lo EH, 2003);
- hemorrhage resulting from the rupture of an artery to the brain.

Certain forms of hypertensive cerebrovascular disease combine both aspects and constitute a distinct group. The most common cerebrovascular disorders are thrombosis following atherosclerosis, embolism, hypertensive intraparenchymal hemorrhage and aneurysm rupture (Victor M, 2000).

SPECIFIC DATA

Material and Method

NECROPTIC STUDY

STUDIED MATERIAL

A thorough examination of 265 deceased patients has been carried out to the Clinic of Neurology within No 4 University Clinical Hospital of Neuropsychiatry of Craiova city, during the period 2000-2004, after a detailed analysis of their observation sheets (neurological exam, clinical diagnosis, paraclinical tests); the necropsies were performed within 24 hours from death, the time of death being mentioned in the observation sheets

During the necropsy, each corpse was examined, considering the macroscopic anatomopathological examination of each organ of the body, focusing on the pathological aspects in order to determine the cause of death, as well as the evidence harvesting from organs (meant to confirm the microscopic histopathological diagnosis) followed by their operation and processing by means of different histological and immunohistochemical techniques.

RESULTS AND CONCLUSIONS OF CLINICAL AND STATISTICAL ANALYSIS

Previous occurrences of cerebrovascular disorders, either transient ischemic attacks or strokes (if any data are provided) were also mentioned in the observation sheet, their number including numerous variables; for this reason the risk factors submitted to the analysis were: high blood pressure, dyslipidemia, atrial fibrillation, type 2 diabetes mellitus, alcohol consumption and cigarette smoking; in addition, in order to indicate the ischemic stroke prevalence to the deceased subjects submitted to the study, we have considered the following aspects: social class, age and sex.

Most of the deceased patients submitted to the study were inhabitants of rural areas. Men are more likely to suffer strokes, their presence indicating a rate of 52.21%. The most affected age interval ranges between 60-69 years in rural areas and 70-79 years in urban areas.

Personal results concerning the the presence of risk factors recorded in the case of the deceased patients submitted to the study have indicated the fact that, high blood pressure is the most frequent risk factor involved in the etiopathogenesis of ischemic strokes, followed by dyslipidemia, atrial fibrillation, alcohol consumption, then, type 2 diabetes mellitus and cigarette smoking.

Risk, sex and social environment factors prevalence:

- high blood pressure, the leading cause of stroke, highly frequent in women than in men and in rural areas rather than in urban areas;
- dyslipidemia more common in men than in women and in rural environment than in urban environment;
- atrial fibrillation more common in men than in women and in rural environment than in urban environment;
- type 2 diabetes mellitus usually prevalent in urban environment than in rural environment, evenly recorded by men and women;
- alcohol consumption manifested by men rather than by women, more common in rural area than in urban area,
- cigarette smoking more common in men than in women and in rural environment than in urban environment.

Considering the number of risk factors associated to one patient who suffered of acute stroke, one may notice an increased number of states of ramollissement, thus, of a total number of 265 diseased patients submitted to the study, there were 106 patients registering one risk factor, 134 patients registered two or more risk factors and in 25 cases there was no risk factor.

The most frequent association indicated the involvement of two risk factors, namely, high blood pressure with dyslipidemia, followed by dyslipidemia with alcohol consumption and high blood pressure with atrial fibrillation; for three risk factors involved, the most frequent association was high blood pressure, dyslipidemia and atrial fibrillation, followed by another association registering high blood pressure, dyslipidemia and alcohol consumption.

RESULTS OF HISTOPATHOLOGIC AND IMMUNOHISTOCHEMISTRY ANALYSIS

Statistical analysis of cerebral ramollissement (softening). Results and conclusions

Topographical remarks regarding the terminal area of cerebral arteries, mainly achieved through correlation of neurological indications and injury of certain nervous centers with a degenerate and faulty condition of blood vessels, allowed the identification of distinct anatomo-clinical forms of cerebral softening.

According to the necropsy performed in this study, only 80 cases provided evidence necessary for the macroscopic, microscopic and immunohistochemical analysis. All 265 patients were submitted to the statistical analysis. For an accurate localization of the cerebral softening, we have used terms specific to the necropsy protocol and avoided carotid vascular and vertebrobasilar areas.

Forms of cerebral softening encountered to the deceased patients within the study:

- Recent cerebral softening, localized to the right and left hemisphere level, a cortical subcortical and deep form of brain softening;
- Recent cerebral softening, identified in the cortex and cerebellum;

- Old cerebral softening- yellow softening and cystic softening – identified during the rehabilitation process, generated by liquefaction of the cerebral substance;
- Old cerebral softening determined by hemorrhagic changes.

This study emphasizes the causality relationship between the existence of risk factors and the number of ramollissement conditions deriving from the necropsy of the 265 deceased patients submitted to the analysis.

Results and conclusions of the macroscopic analysis

The macroscopic aspect analysis of cerebral softening depends on changes registered after the heart attack occurrence and it is related to the time elapsed between the moment of acute neurological signs installation and death, and performed within 24 hours from the necropsy.

For the cases where death occurs in 48 hours from the acute moment, the necropsy reveals a pale smooth edematous cerebral tissue and an unclear corticomedullary junction.

For the cases where death occurs in two to ten days from the acute stroke, after the necropsy, in 24 hours the cerebral tissue acquires a jelly-like consistent structure, the range between the normal nervous tissue and the injured nervous tissue becomes more distinct and the edema is subtly immersed by the contiguous surviving tissue.

If the necropsy is performed in an interval of ten to three weeks from the acute stroke occurrence, the injured cerebral tissue presents a liquefaction state, and there may be found a cavity filled with fluid, located along the grey matter, presenting a gradual extension to the damaged cerebral tissue. The most frequent forms of ramollissement were the recent ones, followed by the recent softening generated by hemorrhagic strokes, then yellow softening and finally the less frequent, the cystic softening.

Recent cortical-subcortical deep ramollissement is more often localized in the left cerebral hemisphere, rather than in the right hemisphere; recent ramollissement is commonly encountered in the cortex, particularly in the pontine area of the brain, followed by bulbar and bulbopontine areas; cerebellar recent ramollissement forms are detected more often in the vermis of the cerebellum rather than in cerebellar hemispheres.

The individual number of ramollissement cases depends on the presence of one or more risk factors.

Results and conclusions of the microscopic analysis

Our research includes a macroscopic and a microscopic analysis of the blood vessels comprised into the circle of Willis, which revealed flexuous, firm blood vessels with thick walls and accumulation of a yellowish material on the plaques, providing a moniliform configuration of the arteries; the cross-sectional analysis infers the fact that blood vessels still present a circular opening and they do not bend when they are in vertical position (pasta-like aspect); moreover, beside the atheromatous injuries within Willis polygon, we have also noticed atheromatous injuries within the aortic arch.

We have performed a microscopic analysis of aortic atheromatous injuries found in the context of generalized atheromatosis.

Supporting evidence gathered from different injured cerebral areas, with cerebral forms of ramollissement, in different stages of evolution, have been used for processing microscopic formulations, concluding that the most frequent cases are those registering two-week ramollissement.

A histopathological study of atheromatous injuries of the brain blood vessels (Willis polygon) has revealed the fact that these injuries were 80% present in the cases submitted to

the analysis, and cerebral injuries were accompanied by aortic atheromatosis within the process of generalized atheromatosis.

There were serious arteriosclerosis type vascular injuries at the level of brain and meningeal blood vessels associated with serious vascular hyalinization

In the areas of total ischemia, most of the cerebral ischemic injuries were accompanied by severe perineuronal and perivascular edema.

A late occurrence of death, considering the acute event, has determined the liquefaction necrosis and the phagocytosis process, and astrocytes have been found in the microscopic formulations, their structure depending on the age of injuries, thus, old injuries revealed split astrocytes of increased volume developing an extended protoplasmatic network.

In the areas of acute ischemia (recent ramollissement) red neurons pathognomonic to acute injuries have been found in the joint area of penumbra.

We have identified the Goldner Szeckeli coloration in microhemorrhages at the outer limits of the focal point of ramollissement, which were generated by cerebral hypoxia.

Using the PAS coloration we have analyzed vascular injuries, focusing on the lack of continuity and the amount of thickness diminution of the basal membrane at the level of brain blood vessels and determining vascular extravasations with red cells, PMN and lymphocytes.

An over ten-day cerebral ramollissement was accompanied by serious macrophage infiltrations and by hemosiderin deposits into the ramollissement area and at the level of macrophage cytoplasm.

In the ischemic penumbra area, the neuronal blasting changes were reduced in intensity, as compared to areas of total ischemia, allowing, thus, the reperfusion, the restoration of damaged neuronal functions.

Results and conclusions of the immunohistochemical analysis

Concerning the GFAP immunomarkage, we have noticed its presence in astrocytes surrounding the focal point of recent ramollissement, variable as number; the astrocytosis being joint with severe perineuronal and perivascular edema. The immunohistochemical analysis revealed a profuse astrocytic proliferation, particularly, after two weeks from the acute event, a spread of hypertrophic astrocytes and abundant cytoplasm and hypertrophic and hyperchromic nuclei, confirming an intense metabolic and biochemical activity.

Astrocytosis has been emphasized through GFAP and the immunomarkage has been more intense in the ischemic penumbra area as opposed to the outer limit of the acute ramollissement area, hypertrophic astrocytic nuclei indicate an intense metabolic activity, and the severity of perineuronal edema is reduced in the penumbra area.

The type IV collagen and laminin immunomarker determines the lack of continuity and the amount of thickness diminution of the basal membrane at the level of brain blood vessels, and the CD 31 and CD 34 immunomarker indicates negative values at the level of brain blood vessels determined by the presence of severe cerebral edema.

The NSE immunomarker shows a variable activity in marking the products responsible for neuronal wounding; considering the acute event, it is associated to timing, thus, it becomes more intense in the case of an over two-week ramollissement.

The immunomarkage with neurofilaments indicates a reduced immunoexpression as compared to that registered by the NSE immunomarker; the products responsible for neuronal wounding are marked with neurofilaments and associated with severe astrocytosis.

The CD 68, CD 44 immunomarker is an important feature in achieving a precise identification of macrophages met at the outer limits of a six-day ramollissement in the attempt of acquiring phagocytized products of neuronal impairment.

Final Conclusions

- The macroscopic aspect analysis of cerebral softening depends on changes registered after the heart attack occurrence and it is related to the time elapsed between the moment of acute neurological signs installation and death.
- For the cases where death occurs in 48 hours from the acute moment, the necropsy reveals a pale smooth edematous cerebral tissue and an unclear corticomedullary junction.
- For the cases where death occurs in two to ten days from the acute stroke, after the necropsy, in 24 hours the cerebral tissue acquires a jelly-like consistent structure, the range between the normal nervous tissue and the injured nervous tissue becomes more distinct and the edema is subtly immersed by the contiguous surviving tissue.
- The individual number of ramollissement cases depends on the presence of one or more risk factors.
- Our research includes a macroscopic and a microscopic analysis of the blood vessels comprised into the circle of Willis, which revealed flexuous, firm blood vessels with thick walls and accumulation of a yellowish material on the plaques, providing a moniliform configuration of the arteries.
- Supporting evidence gathered from different injured cerebral areas, with cerebral forms of ramollissement, in different stages of evolution, have been used for processing microscopic formulations, concluding that the most frequent cases are those registering two-week ramollissement.
- In the areas of total ischemia, most of the cerebral ischemic injuries were accompanied by severe perineuronal and perivascular edema.
- An over ten-day cerebral ramollissement was accompanied by serious macrophage infiltrations and by hemosiderin deposits into the ramollissement area and at the level of macrophage cytoplasm.
- Astrocytosis has been emphasized through GFAP and the immunomarkage has been more intense in the ischemic penumbra area as opposed to the outer limit of the acute ramollissement area, hypertrophic astrocytic nuclei indicate an intense metabolic activity, and the severity of perineuronal edema is reduced in the penumbra area.
- The type IV collagen and laminin immunomarker determines the lack of continuity and the amount of thickness diminution of the basal membrane at the level of brain blood vessels, and the CD 31 and CD 34 immunomarker indicates negative values at the level of brain blood vessels determined by the presence of severe cerebral edema.
- The NSE immunomarker shows a variable activity in marking the products responsible for neuronal wounding; considering the acute event, it is associated to timing, thus, it becomes more intense in the case of an over two-week ramollissement.
- The immunomarkage with neurofilaments indicates a reduced immunoexpression as compared to that registered by the NSE immunomarker; the products responsible for neuronal wounding are marked with neurofilaments and associated with severe astrocytosis.
- The CD 68, CD 44 immunomarker is an important feature in achieving a precise identification of macrophages met at the outer limits of a six-day ramollissement in the attempt of acquiring phagocytized products of neuronal impairment.

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