PH. D. THESIS

ABSTRACT

CLINICAL AND HISTOLOGICAL CORRELATIONS IN ISCHEMIC STROKES

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A. GENERAL PART

INTRODUCTION

Stroke constitutes a major urgency and an important problem of public health, characterized by very high morbidity and mortality, disabling sequelae, representing exorbitant costs to the health insurance systems.

World wide these kill five million people annually, and leave five million more with severe disabilities.

In Europe the incidence of stroke varies from one country to another, and is estimated at between 100 and 200 new cerebral vascular strokes per 100,000 inhabitants annually, representing an immense economical burden. High differences of incidence, prevalence and mortality between Eastern and Western Europe were reported. This was attributed to the differences between risk factors, resulting in a more severe form of stroke in Eastern Europe [2].

From an epidemiological point of view, strokes in Romania represent the first cause of death in vascular pathologies, meaning cerebro-vascular diseases and cardio-vascular diseases combined, an approximately 300 new cerebral vascular strokes being recorded per one hundred thousand inhabitants, compared to the European average of below 200.

Of the patients that survive a stroke, a third present an improvement of the general status in the first week of evolution, 40% present a slow evolution, with permanent disabilities, while 20% suffer an aggravation of the initial symptomatology during the first week of evolution [3].

CHAPTER I

ANATOMY AND HISTO-PHYSIOLOGY OF THE CENTRAL NERVOUS SYSTEM

The nervous system is made up of over 100 billion neurons which achieve the integration of the organism in the external environment and the coordonation of the function of internal organs. Considered the most complex system of the human organism, the nervous system is constituted of an ensemble of organs, made up of nervous tissue, blood vessels and connective tissue [4].

The human nervous system is divided in the central and peripheric nervous system. The brain and brain stem make up the central nervous system (CNS), and the cranial, spinal, autonomous and their ganglions make up of the peripheral nervous system (PNS). The nervous system is made up of the parenchyma and the stroma. The parenchyma is constituted of the totality of nervous cells. The stroma is made up of glial cells, fine connective tissue and
cappillaries [5]. The parenchyma is divided into two morphologically and functionally distinct tissue structures - the grey matter and white matter.

The grey matter is formed of the neuronal bodies, dendrites, the initial, non- myelinated of axons and neuroglial cells; moreover, there is a rich network of blood vessels, especially capillaries, within the grey matter, which allow an intense oxidative metabolism, specific of neurons.

The white matter is made up mainly of parallel myelinated axons, grouped in fascicles and cords.

CHAPTER II

THE ANATOMY OF THE CEREBRAL VASCULAR SYSTEM

Cerebral arterial vascularization is provided by an anastomotic system made up of the vertebral and the internal carotid arteries, a system situated at the base of the brain in the subarachnoid space.

Carotid arteries make up 75 % of the cerebral blood flow.

Internal carotid arteries (ICA) ensure the vascularization of the anterior portion of the brain and the eye. It is considered that 2/3 of the common carotid artery's flow is taken by the ICA. In its trajectory the internal carotid artery presents numerous collateral and terminal off-shoots [6].

Terminal branches are represented by: anterior cerebral artery, the middle (sylvian) cerebral artery, the anterior choroidal artery and the posterior communicating artery.

The most important ICA branch from a histopathological and clinical point of view is the sylvian or middle cerebral artery (MCA).

Vertebral arteries (VA) supply the posterior region of the brain (brainstem, cerebellum and occipital lobe). The two VAs unite on the median line forming the basilar system, from which the pontine, labyrinthine, cerebellum antero-inferior and superior, and posterior cerebral arteries emerge.

CHAPTER III

ISCHEMIC STROKE

DEFINITION

The most recent definition considers the symptomatology to be a stroke either if the clinical symptoms have lasted for more than 24 hours, or if the symptoms remit below this threshold but imagistic show an acute ischemic lesion in concordance with the clinical picture of
the ictus [7]. Ischemic stroke is characterized by definitive ischemic suffering of the cerebral parenchyma in an area where regional blood flow drops below 10ml/100g of tissue/min, secondary to partial or total occlusion of an artery or cerebral vein.

ETHYOPATHOGENY OF ISCHEMIC STROKE

The etiology of the cerebrovascular pathology is represented by three large categories: thrombosis, embolism, critical deterioration of cerebral hemodynamic "low flow infarct"

The most frequent causes for cerebral infarct are atherothrombosis (atherosclerosis with thromboembolism) and cardiogenic embolism. Non-rheumatic atrial fibrillation is the most frequent cause of cerebral embolism.

The factors which increase the risk of an ischemic cerebral vascular accident are numerous. They are divided into factors which can be modified by treatment and a change in lifestyle and factors which cannot be modified [7]. Factors which can be modified are represented by: hypertension, diabetes mellitus, high blood cholesterol, smoking, excessive consumption of alcohol, obesity, sedentarism, atrial fibrillation, acute myocardial infarction, cardiomyopathies, cardiac valvulopathies, hypercoagulation, the use of contraceptives pills. Factors which cannot be modified are: age, sex, race, family history of cerebrovascular events, personal history of cerebrovascular events.

PHYSIOPATHOLOGY OF ISCHEMIC STROKE

PHYSIOPATHOLOGICAL STADIALIZATION

- Acute phase of ischemic necrosis (debut - day three)
- Subacute resorption phase (day four - six weeks)
- Chronic disabling phase (organization phase) (beyond six weeks)

MECHANISMS OF NEURONAL INJURY

The decrease of cerebral blood flow below 10 ml/100g tissue/min determines the infarction of cerebral parenchyma, the size of the ischemic necrosis area being dependent on the caliber of the obstructed area and the efficiency of collateral circulation (communicant arteries of the Willis circle, extra - intracranial anastomosis, leptomeningeal arteries). In the first 24 hours around the central nucleus of the ischemic necrosis an area of "ischemic penumbra" persists, in which only a functional or metabolic alteration of structures is present, due to the persistence of a 15 - 25 ml/100g tissue/min perfusion ("misery perfusion"). In the peripheral hypoperfusion area cerebral function is maintained, with a regional perfusion of 25 - 80 ml/100g tissue/min [8].

The intracellular influx of water determines the cytotoxic edema, which predominates in the grey matter. Its histopathological basis is the perivascular astrocytes and endothelial cells
edema. The collapse of the hematoencephalic barrier with the flow of water and protein macromolecules from the intravascular to the interstitial space determines the vasogenic edema, which includes both grey and white matter.

The two processes which result in neuronal death are liquefactive necrosis and apoptosis. Liquefactive necrosis is based on the denaturation of proteins, both structural and enzymatic. The process is the basis of some phenomena through which cells die when their function or role is achieved.

**CLINICAL MANIFESTATIONS OF ISHEMIC STROKES**

The occlusion of a cerebral artery leads to the emergence of a specific clinical picture, depending on the affected cerebral area (topographic vascular syndromes).

**PARACLINICAL INVESTIGATIONS IN ISCHEMIC STROKES**

The patients with ischemic stroke must have priority access to cerebral imagistic, because time is crucial. Cerebral CT scan, which is generally available, can identify most pathologies which mimic stroke, can distinguish between ischemic and hemorrhagic stroke in the first 5 - 7 days [9-11]. Cerebral CT is highly specific for early identification of ischemic cerebral lesions [12-14].

**B. PERSONAL CONTRIBUTIONS**

**CHAPTER IV**

**OBJECTIVES**

This thesis aims at achieving:

1. A retrospective **clinical statistical study** regarding ischemic stroke in a representative hospital, which would highlight the following aspects of the pathology: establishing the incidence of ischemic cerebral vascular accidents on a seven year period, distributed by sex, background, age groups; evaluating the modifiable vascular risk factors; determining evolutive and prognostic particularities of the ischemic stroke and specifying neurologic factors and co-morbidities that lead to an unfavorable evolutions; specifying the role of early cerebral imagistic in the emergency diagnosis and the evolution of the acute ischemic stroke; correlating clinical and imagistic aspects to the evolution of patients with ischemic stroke.

2. A **histological study** on the human brain, originating in persons deceased from ischemic stroke, to highlight: the modifications of the cerebral parenchyma at the level of the lesion and perilesional, the modifications of the meningo-cerebral arteries; the modifications of the small intraparenchymal vessels; the modifications of the blood - brain barrier.
3. An immunohistochemical study, to complete the histopathological study in showing:
The reaction of the monocyte - macrophage system; the reaction of the glial system; the process
of neuronal apoptosis; the modifications of the vascular endothelium as an essential element of
the blood - brain barrier.

CHAPTER V

CLINICAL STATISTICAL STUDY OF ISCHEMIC CVA BETWEEN 2005 - 2011

We conducted a clinical - statistical epidemiological retrospective study on a 7-year interval (2005-2011), taking into account all the cases of ischemic stroke (10,908) hospitalized in the Clinical Hospital of Neuropsychiatry Craiova. Both clinical observation charts and statistical data provided by the Bureau of Statistics of the Hospital were analyzed.

In regards to ischemic stroke distribution by sex, it was noticed that cerebral infarctions occurred more frequently in men - 51% and less frequently among women - 49%.

The study of cerebral ischemia distribution by background allowed us to observe that there are major differences between urban and rural areas - 56% of cases originated from rural areas and only 44% from urban areas, raising special issues regarding rural healthcare, diet and preventive measures.

As to the distribution by age groups, the highest incidence was recorded in the interval 65 - 74 years - 38%. Annual distribution of ischemic stroke was very close from one year to the next.

Mortality in patients hospitalized with ischemic stroke during 2005-2011 in the Clinical Hospital of Neuropsychiatry Craiova, was of 3.6%, the lowest mortality being recorded in 2006 of only 2.3% of all patients admitted with ischemic stroke and the highest mortality being recorded 2010 when the rate of death reached 4.14%. When compared with other international statistics, according to which the cumulative monthly mortality after ischemic stroke is 5% [15], the mortality level recorded at the Clinical Hospital of Neuropsychiatry Craiova is extremely low, which does not correspond to the severity of the disease, as most stroke patients with unfavorable prognosis are discharged at the request of the family and their death is recorded at the patient's home. De Jong and others have observed a monthly mortality rate of 10% from 998 patients at their first stroke [16].

Of the etiopathogenic factors analyzed, ischemic stroke is correlated in a very high percentage with hypertension (65.87%), dyslipidemia (58%), ischemic cardiopathy (48%),
history of TIA (41%), alcohol abuse (37%), smoking (32%), diabetes mellitus (25%), atrial fibrillation (23%), personal history of myocardial infarction (18%).

Prevalent neurological dysfunctions on admission were motor dysfunctions - 81%, language and speech disorders - 68%, sensory - 37%, field of vision - 24%, disorders suggesting damage to the posterior area - 21%, impaired state of consciousness was recorded at admission in a relatively small number of patients (8.61%), which is explainable by the relatively rapid emergency presentation and hospitalization.

Basic brain imaging, both during emergency and hospitalization, was the computed tomography, conducted on all 10908 patients. As to the vascular topography of tomographic lesions, the most common were within MCA territory - 72% of all cerebral infarctions.

The evolution of patients with ischemic stroke during the hospitalization period showed a favorable trend in 82% of cases, a worsening in 14.4% of cases, and death in 3.6% of cases.

The main factors for negative prognosis within the study group were: age and major comorbidities (diabetes mellitus, uncontrolled hypertension); "strategic" stroke locations (infarcts in the vertebral basilar territory, affecting the ventro-lateral spinal cord, large cerebellar infarcts, large carotid ischemic strokes (complete ACM or complete ICA) and onset with coma; major neurological complications of ischemic stroke (hemorrhagic transformation, mass effect and recurrent stroke); cardiac complications (acute coronary syndrome and heart failure).

CHAPTER VI
HISTOLOGICAL STUDY OF THE CEREBRAL VASCULAR ISCHEMIC ACCIDENT

For the histopathological study brain fragments recolted during necropsy on 83 patients were used, clinically and imagistically diagnosed with ischemic stroke, admitted to the Neuropsychiatry Hospital of Craiova between 2005 and 2011. Histological dishes were colored with hematoxilin - eosin, the most used method of tissue staining and with trichromic on blue toluidine base after the Masson method.

Starting from the idea that the modifications of nervous tissue are extremely different from one area to the next, depending on the size of the ischemic focus, the vessels affected by the ischemic physiopathological process, collateral circulation and possible anastomosis, as well as the general status of the cerebral vascular system, possible comorbidities, the status of nervous cells within the ischemic focus, the modifications of meningo - cerebral arteries, of the small intraparenchymal vessels and of the blood - brain barrier were analyzed, in correlation with the
time passed since the onset of the symptoms (of clinical signs which correspond to the debut of cerebral ischemia) and the patient's death.

Of the total 83 patients taken into study: 57 % died within three days from the onset of ischemic stroke, 15 % in days 4 and 7 from the onset of ischemic stroke and 8 % seven days after the from the onset of ischemic stroke.

**Three days after the onset of cerebral ischemia**, in the area of the lesion the vast majority of both neurons and glial cells had undergone a process of necrosis. Another part of the neurons, especially the larger neurons, had undergone a ballooning process. In the areas at the periphery of the core infarction focus (the ischemic penumbra), the presence of characteristic neurons - "red neurons" or ischemic neurons - was highlighted, condensed neurons and neural ghosts. Hemorrhagic infiltrates within the brain parenchyma were present in both the lesion area as well as around the ischemic focus and farther away. The presence of immune cells in the cerebral ischemia focus and the penumbra area was reduced. Cerebral parenchymal lesions decreased from the ischemic outbreak center towards the periphery, to the areas of ischemic penumbra. Perineuronal and perivascular edema was noted as early as the first 3 days after the onset of disease. Within the white matter, demyelinating nerve fibers with a spongy appearance away from the focus of ischemia were noted, which advocates the idea that this demyelination is secondary to the death of the neuronal bodies.

**Four to seven days from the onset of cerebral infarction**, the modifications of the cerebral parenchyma intensified as time passed. The reduction in neuronal population was noticeable not only around the ischemic focus, but at distance as well. Both within the focus and the penumbra area the presence of numerous large macrophages was noticed.

**After seven days from the onset of cerebral infarction**, the damage to the cerebral parenchyma seems to continue as time passes, both in the ischemic focus, the penumbra area and the rest of the central nervous system. A massive agglomeration of macrophages were observed within the cerebral ischemic focus, with the role of eliminating necrotic tissue.

The entire cerebral microcirculation presented microscopic changes in the ischemic stroke, regardless of the duration of time since the lesion was produced and until the histological examination. At the level of the large vessels, atherosclerosis lesions were highlighted, especially at the level of the meningeal arteries; frequently, intraparietal lesions were noticeable, with the disorganization of the arterial wall. In the ischemic focus, small caliber vessels, arterioles, capillaries and venules, were completely destructured. Vessels within the ischemic penumbra partially maintained their morphologic integrity, their aspect being most often congested, with deformed walls, hematic extravagated or perivascular edema.
CHAPTER VII
IMMUNOHISTOCHEMICAL STUDY OF THE ISCHEMIC STROKES

To complete the microscopic data, an immunohistochemical study was conducted, utilizing the four immunomarkers: GFAP - to highlight glial cells reaction; CD68 to highlight macrophages; caspase 3 to highlight neuronal apoptosis process; CD31 to highlight changes in the vascular endothelium.

The immunohistochemical study showed an intense reaction of the monocyte - macrophage system cells and astrocyte cells after 3 - 5 days, both perilesional and perivascular. What was remarked was not the numerical increase of glial cells, but rather the increase in number, length and volume of astrocyte extensions.

To evaluate the neuronal apoptosis process, the cerebral areas close or distant to the ischemic focus area were chosen for study. In patients deceased after 3 and 7 days respectively from the onset of cerebral ischemia, the number of caspase - positive neurons found was lower than that found in patients deceased within the first 3 days, which demonstrates that most neurons adjacent to the ischemic focus area die within the first days through both necrosis and apoptosis.

CHAPTER VIII
CONCLUSIONS

By corroborating the results of the three studies, the following clinical and histological correlations were established:

- liquefaction necrosis, astrocyte gliosis, phagocytosis phenomena are the more intense the later the death of the patient;
- apoptosis phenomena are the more intense the faster the death of the patient;
- the entire cerebral microcirculation presented microscopic modifications following the ischemic strokes, regardless of the time spent since the lesion occurred and the histological examination was made;
- the major neurological complications of the ischemic stroke - the hemorrhagic transformation phenomena, cerebral edema (histologically observed as perivascular and perineuronal edema) were microscopically objectified, regardless of the time spent since the lesion occurred and the histological examination was made.
BIBLIOGRAPHY


