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PH. D. THESIS

ABSTRACT

Cerebrovascular accidents in Arges County – clinical, histological and immunohistochemical study

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Introduction

Cerebrovascular accidents (CVA) imply a rapid loss of cerebral functions as consequence of the fact that the brain is no longer supplied with blood by a drastic decrease of the blood flow, whether by a thrombosis or arterial emboli, or by a cerebral hemorrhage (Sims NR, Muyderman H, 2010; Robert AA, Zamzami MM, 2014). Within the past years, the CVA has become the main cause of serious neurological disturbances, the main cause of physical and mental handicap and one of the main causes of death. According to their nature and seriousness, CVA can be the cause of multiple cerebral lesions that leave physical, psychological, cognitive and even social dysfunctions (Kim P, Warren S, et al, 1999, Clarke P, Marshall V et al, 2002; Pollak J, Doyle KP, Mamer L, et al, 2012).

New statistical data (Adeloye D, 2014) estimated that in 2005 in the entire world there were nearly 16 million new cases of cerebrovascular accidents and that worldwide there were almost 62 million survivors of a cerebrovascular accident. Deaths caused by CVAs in 2005 represented 9,7% of the total number of deaths worldwide. Epidemiological studies estimated that in 2015 about 6,7 million people will die because of an CVA if important medical measures shall not be taken worldwide so as to fight against risk factors (Lopez AD, Mathers CD, Ezzati M, et al, 2006; Robert AA, Zamzami MM, 2014). It is expected that until 2013 the number of people affected by a CVA should increase to over 23 million new cases each year and the number of deaths should reach 7,8 million (WHO, 2004; Strong K, Mathers CD, Bonita R, 2007).

The incidence of a CVA varies from one country to another, being estimated between 100 and 200 new cases at every 100.000 inhabitant. If until a decade ago cerebrovascular accidents were, in developed countries, a major medical and social issue as they represented the third cause of deaths and the first cause of invalidity, recent data show that CVAs tend to rapidly increase in many countries with small and average income (CVAs appear in countries with small and average income, a very serious aspect, as these countries cannot afford an appropriate treatment for this disease or for its consequences).

In Romania there are registered nearly 300 new cases of CVAs for every 100.000 inhabitants compared to a European average of up to 200 CVAs/ 100.000 inhabitants. Currently, in Romania there are approximately 800.000 patients that suffer a CVA.

In this Ph.D. thesis we aimed to investigate the clinical and statistical aspects of the CVAs that have been hospitalized in the Pitesti Emergency Hospital over a 5-year period and to correlate these aspects with the etiopathogenic factors that might contribute to their initiation. We also intended to perform a histological and immunohistochemical study concerning the microscopic changes that occur with the encephala in the case of people that die because if a hemorrhagic CVA.

Chapter I. Meninges and brain circulation

The central nervous system is one of the best vascularized systems in the body. Though the encephalon only represents 2% of the body weight, it receives nearly 17% of its blood flow. The brain constantly uses about 20% of the amount of oxygen in the body (KiernanJA, 2009).

Cerebral circulation is characterized by three main elements: cerebral blood flow, cerebral perfusion pressure and local cerebrovascular resistance. The relationship between the elements mentioned can be expressed with the phrase" "cerebral blood flow (CBF) varies according to the cerebral perfusion pressure (CPP) and in reverse relation with the cerebrovascular resistance". To the three factors there can be added: trans-cerebral circulatory speed and capacity of the cerebral vascular bed (Arseni C, 1984).

Cerebral blood is supplied by a pair of large arterial flows: the main arterial flow, offered by the internal carotid arteries (ICA) (nearly 70% of the CSF) and by the vertebral arteries (VA) which provide nearly 30% of the FSC). These two arterial systems inosculate by means of the anterior and posterior communicant arteries at the base of the brain and they form together the arterial circle of WILLIS (Dănăila C, 2011). We must add that there is a considerable variability in the relative size of vertebral and communicant arteries. In normal conditions, this has no functional meaning, but it becomes important when one of the main trunks is obstructed.

The venous circulation of the CNS is exceptional because it differs from the common antiparallel orientation of the arteries and veins in many other organs. Furthermore, the cerebral venous drainage uses dural sinus as final intracranial collecting blood vessel.

The blood from the cortical white substance is drained through veins of different lengths which still have an anti-parallel conventional orientation with pial penetrating arteries. Generally, the number of veins is larger than the number of perforating arteries. The number and localization of cortical veins varies considerably, which makes difficult to angiographically check their permeability and functionality. Superficial veins have thin walls lacking in muscular

tunic and valves, fact which allows them to dilate and direct the venous blood flow to different directions. These characteristics, together with their numerous anastomoses guarantee an efficient collateral flow in the case of venous thrombosis.

Short interruptions of the cerebral circulation (hypoxia) can determine neurological and mental disturbances. Loss of consciousness occurs within 5 to 10 seconds if the blood flow to the brain is completely stopped; if cerebral circulation is interrupted for about 4-5 minutes, irreversible brain lesions happen (Noback R et al, 2005).

Chapter II. The physiopathology and neuropathology of cerebrovascular accidents

It is currently well acknowledged the fact that all cerebrovascular diseases have their origin in the parietal changes of cerebral vessels. Consequently, acknowledging these pathological changes which occur in the vessels and in the blood, it is crucial to understand the physiopathology of different types of cerebrovascular accidents and to plan efficient therapeutic strategies.

Changes of the vascular wall can lead to the blocking of the blood flow with the interaction between the parts of the blood and the vascular wall, thus being able to cause thrombosis and blocking of the blood flow in that vessel. Moreover, stenosis or vascular occlusion, where vascular changes occur, interrupt the blood supply, raising the possibility that consecutive heart attacks could produce by emboli resulted from vascular lesions placed close or form a source from the heart.

Changes of large arteries that supply blood to the brain, including the aorta, are mostly caused by atherosclerosis. Average and intracerebral arteries can also be affected by acute or chronic vascular diseases of inflammatory origin because of certain subacute or chronic infections (tuberculosis and lues) or as a consequence of collagen diseases (arteritis with giant cells, granulomatous angiitis of the CNS, nodular panarthritis, and, even more rarely, systemic erythematous lupus), Takayasu arthritis, Wegener's granulomatosis, rheumatoid arthritis, Sjőgren syndrome, Sneddon disease or Behcet disease. In the case of some diseases that affect cerebral vessels, the etiology and pathogenesis are still unclear, for instance, Moyamoya disease and fibromuscular dysplasia, but these disturbances are characterized by typical lesions of the vascular wall.

Atherosclerosis is the widest spread disturbance, the trouble that leads to the increase of morbidity, including in the CVA. It usually affects large meningocerebral vessels.

Atherosclerosis beings at an early age, the lesions accumulate, grow in time and become symptomatically and clinically obvious when the organs are strongly affected (Hossmann K A, Heiss W-D, 2010). Small vessels of the brain are affected by hyalinosis and fibrosis, this "small vessel disease" being able to cause gaps and if widely spread it is the sublayer of vascular cognitive disturbances and vascular dementia. Small vessel disease usually affects arterioles and it is frequently associated with arterial hypertension. It is owed to the subendothelial accumulations of a pathological protein called "hyaline" formed of mucopolysaccharides and matrix-like proteins. The sedimentation of this material on the vascular wall leads to a narrowing of the vascular lumen or even to an occlusion of these vessels.

The acute occlusion of a major cerebral artery determines a stereotypy of morphological changes which evolve over a long period of time (Auer RN, Benveniste H, 1997, Petito CK, 2005). The most sensitive cells of the brain are the neurons, followed by the oligodendrocytes, astrocytes and vascular cells. The most vulnerable regions of the brain are the CA1 hippocampal subfield, neocortical layers 3, 5 and 6, the outer segment of the striate nucleon, Purkinje's cells.

CHAPTER III. Cerebrovascular accidents – current clinical data

A cerebrovascular accident is a medical emergency because it can lead to permanent neurological lesions and even to the death of the person affected. The most commonly spread risk factors are: old age, arterial hypertension, diabetes, atrial fibrillation, smoking, etc. (Donnan GA, Fisher M, Macleod M, Davis SM, 2008). Blood pressure is the most important changeable risk factor for cerebrovascular accidents.

The most CVAs occur in the case of individuals of over 65 (Brass LM, 2006); the occurrence of CVAs in the case of people over 40 is only 0.5%; for the group with ages between 60 and 79, the percentage of spreading exceeds 6%. Concerning the morbidity and mortality rate, we must show that 8% up to 17% of the patients with ischemic attack and from 37% up to 38% of the patients with hemorrhagic attack die in the first 30 days since the accident occurred (Van der Worp HB, van Gijn J, 2007). Besides the burden of mortality, the cerebral attack is the main cause for disabilities. They drastically limit the body functions, contribute to the increase of medical expenses and reduce the patient's quality of life. Direct medical costs connected to the cerebral attack and the indirect ones were estimated in 2008 in the USA at 65.5 billion dollars.

The symptoms of a cerebrovascular accident usually begin suddenly, from a few seconds up to a few minutes. Sometimes, especially with the elder people, the beginning of the disease is

installed more slowly within several hours. Clinical symptomatology depends very much on the affected part of the brain. In most cases, the symptoms affect only a part of the body (unilateral).

Loss of consciousness and vomit usually occur in hemorrhagic CVAs and more rarely in the case of the ones of ischemic nature because of an increased intracranian pressure as a consequence of blood leaks from the intravascular segment into the parenchimatous one, with a more or less intense compression of the brain. The impact on the cerebral cortex and the maine afferent or efferent ways is translated with aphasia, dysarthria, apraxia, diminishment of the visual field, memory deficits, confusions, irregular moves, incapacity to understand words or sounds, incapacity to talk, etc. If the projection areas of the spinothalamic, cortical-spinal or lemniscus medial pathway are affected, the patients show hemiplegia, a diminishment of sensorial perceptions, hypotony, spasticity or hyperreflexia (Susan B, 2007). Patients that suffered a major cerebrovascular accident are also inclined to subsequently have several manifested or silent CVAs (Miwa K, Hoshi T, et al, 2010).

CHAPTER IV. Importance of the subject. Objectives of the study

Several epidemiologic studies helped identify the risk factors for a CVA and brought convincing evidence concerning the measures that need to be taken so as to reduce this affection. Thus, contributing factors include: arterial hypertension, diabetes mellitus, hypercholesterolemia, affections of coronary arteries, several cardiac affections (atrial fibrillation, endocarditis), smoking, excessive alcohol consumption, use of cocaine, lack of physical activity, obesity or use of some medicine such as oral birth control pills and hormonal replacement therapy with women at menopause (Kaohsiung J, 2007).

We aimed to perform the following studies and to reach several objectives:

- A retrospective clinical and statistical study concerning the CVA in a representative hospital to highlight the following aspects:
    - the relation between ischemic and hemorrhagic CVAs;
    - the distribution of the CVA according to age groups;
    - the distribution per genres;
    - the distribution of the CVA according to the social environment.
- A histological study over the human brain coming from people clinically and imagistically diagnosed with CVA who died in hospital units, so as to highlight:
    - Changes of the cerebral parenchyma with the lesion and perilesional;

- Changes of the meningo-cerebral arteries;
- Changes of small intraparenchimatous cerebral vessels;

- An immunohistochemical study in completing the histopathological study so as to highlight:
  - Neuronal changes and their vitality by using the NeuN antibodies;
  - The reaction of astrocitary cells at the environs of the hemorrhagic or ischemic focus;
  - Changes of cerebral circulation in the hemorrhagic or ischemic focus.

CHAPTER V. Clinical and statistical study of the CVA

The study was performed on 10.730 patients hospitalized in the County Emergency Hospital of Pitesti during the period January 1st 2007 – December 31st 2011, clinically and imagistically diagnosed with a CVA. The number of patients with ischemic CVAs was 9184 (85.59%), while hemorrhagic VCAs were in a number of 1546 (14.41%).

If the annual distribution of CVAs did not show significant variations, the distribution of the cases *depending on the genre* showed major differences between the genres. Thus, the number of ischemic CVAs registered with women was 4634 (50,46%), while the number of patients that were men was 4550 (49,54%); the number of women with a hemorrhagic CVA was 458 (29,65%), while the number of hemorrhagic CVAs registered with men was 1088 (70,35%).

The study of the CVA distribution according to the origin showed that ischemic CVAs were somewhat more increased in the urban areas, where there were registered 5281 cases, representing 57,2%, while in the rural areas there were only 3930 cases representing 42.8%. instead, hemorrhagic CVAs prevailed in the rural areas where there were recorded 967 patients, representing 62,5%, while in the rural areas there were 579, representing 37,5%.

HTA (arterial hypertension) was identified with more than 50% of the patients with CVA each year and the numbers remained relatively the same. These data confirm the fact that vascular changes underlie CVAs and HTA is the main risk factor that determines these changes.

In our study, of the 6132 cases of CVA with HTA, pressure values were:

- 2423 (39.51%) patients had TA (blood pressure) with values over 180/mmHg, but under 200/120 mmHg;
- 1058 (17.25%) patients had TA with values > 200/120 mmHg, aged 6-8

- 2651 (43,24%) cases had TA with values >200/120 mmHg, aged approximately 8-10.

Heart rhythm problems, especially atrial fibrillation, represent another group of affections which can be associated or can contribute to the occurrence of a CVA, especially an ischemic one. In our study, heart rhythm problems were present in a quite big percentage in the case of people with a CVA. They varied from 13,46% to 17.19%, with an average of 15,31%. In other words, one in 6 patients with a CVA had heart rhythm problems, which makes us conclude that these affections are strongly correlated one with the other and that they are a part of the CVA ethiopathology.

Dyslipidaemias are one of the most important conditions for the occurrence of vascular atheromatosis. In our study, the incidence of dyslipidaemias increased from 17,66% in 2007 to 46,30% in 2011. One can say that in 5 years' time the number of dyslipidaemias increased more than 2,5 times and their trend has remained in progress during the entire studied period. This aspect can be explained by unhealthy food, an increase of the consumption of food rich in animal fat (non-saturated fat), high daily stress and low physical effort. Of the 10.730 cases of ischemic and haemorrhagic CVAs within the 5 years a number of 1.160 patients, respectively 10.81% also showed diabetes mellitus.

CHAPTER VI. Microscopic aspects of cerebral lesions in patients with CVA

The histologic study was performed on a number of 49 patients clinically and imagistically diagnosed with CVA who died in the Emergency Hospital of Pitesti in 2011. Of these, 11 were diagnosed with haemorrhagic CVA and 38 with ischemic CVA.

For haemorrhagic CVAs, the microscopic aspect was very variable, according to the localization of the lesion, the quality of blood flowed out of the vessels, possibly according to the blood pressure values, the general state of the vascular system, the associated organic illness, etc. The intraparenchimatous hematoma showed an irregular external line, with diffuse extensions in the surrounding nervous system of various shapes and sizes, most often the diffusion of the haemorrhagic infiltrate being produced along the blood vessels through the perivascular covering or the Virchow-Robin spaces, as these spaces contain small quantities of lax conjunctive tissue and represent an area of minimum resistance. In the cerebral parenchyma there were affected all the cell structures equally: the neurons, the astrocytes, the oligodendroglia, the microglia. Also, the entire neuropile in the grey substance was altered in the haemorrhagic or ischemic focus.

Though there is strong evidence that in the CVA cell death is produced by necrosis and apoptosis, in the centre of the ischemic or haemorrhagic focus all cells die by necrosis. The apoptosis process was highlighted at the fringe of the haemorrhagic or ischemic focus, where the lack of oxygen and nutritive substances is maintained at a decent level for neuronal survival.

In the ischemic half-shade area there were identified several neuronal changes such as an increase of the size of the neurons, a vacuolisation or granulation of the neuroplasm, the disappearance of the nucleon or of the nucleoli, changes of nuclear or cytoplasmic colourability. These microscopic aspects indicate significant changes both of the neuronal hyaloplasm and of the intracellular organelles and especially of the Nissl particles and of the mitochondria. The increase in volume of the nerve cells can be owed to the increase of water and catabolites at an intracellular level as consequence of the disturbance of the neuronal metabolism with the alteration of the selective barrier function of the neuronal membrane. Another cause of the change in the form, size and tinctoriality of the nerve cells represents the modification brought to the intracellular proteins, with the aggregation of some and the dissipation of others.

Neuronal death sometimes occurs by autolysis, aspect which, from a histological point of view, leads to the occurrence of some “neuronal ghosts”. These ghosts are nothing but neuronal rests or even only the place held by the degenerated neuron. We consider that the formation process of “neuronal ghosts” is quite complex and a consequence of a state of chronic hypoxia which progressively disturbs cellular metabolism, membrane-type transportation, alters intracellular organites and sets free the lysosomal proteases which destroy the neurone completely.

Where the ischemia did not cross the critical line and even in other hypoxic areas there were identified signs of neuronal suffering with the occurrence of some neurons characteristic for cerebral ischemia, “red neurons” or ischemic neurons which are the expression of a severe hypoxia. The acidophilic characteristic of the cytoplasm is given by the disintegration of the ribosomes, of the endoplasmic reticulum and of the Nissl particles, with the appearance in the cytoplasm of numerous protean structures which are eosinophils.

The most sensitive ones to hypoxia seem to be the neurons from the superficial part of the grey substance, where there occur associative processes, fact which explains in a great measure the psychological behaviour of the patients that survived an ischemic attack. Furthermore, by destroying the nerve cells and the neuroglia, the superficial part of the neuronal cortex, it is

determined a porous aspect. The neuronal depletion was associated to a porous aspect of the white substance as consequence of the axons' process of necrosis and of the processes of demyelination of the nervous fibres, process known as leukoaraiosis.

The reaction of the cells of the immunity system in a CVA was extremely variable from one patient to another and from one area of the cerebral parenchyma to another. Among the first immune-type cells which go into the lesional focus are the neutrophil polymorphonuclear leukocytes. They were diffusely spread at the fringe of the haemorrhagic or ischemic focus. These cells set free a multitude of proinflammatory factors which amplify the cerebral inflammatory response and keep exacerbating cerebral lesions. The inflammatory reaction can have negative consequences on the clinical evolution of a CVA. It is known the fact that patients with cerebrovascular accidents and systemic inflammation show lower results from the clinical point of view.

CHAPTER VII. Immunohistochemical aspects of cerebral lesions on patients with CVA

The study of neuronal viability by using the NeuN antibody showed that in the old ischemic focus the reaction to NeuN was totally negative, which proves the fact that all the neurons in this area lost their vitality. In the ischemic half-shade area, even though on usual colourations the neurons do not seem to show great morphological changes, the reaction to NeuN was reduced, which proves a significant impact over the neuronal population in this area. The reaction to NeuN was more and more reduced as the hitopathological examination included areas closer to the ischemic focus. In the case of haemorrhagic CVAs, as well, there was noticed a diminishment of the neuronal reaction to NeuN until the total disappearance around the haematoma. A diminished reaction also occurred in the neurons from the counter-lateral hemisphere, which proves that the cerebral haematoma produces ischemia in the counter-lateral hemisphere, thus being able to disturb the metabolism and the normal functioning of the neurons.

An assessment of the reaction of the astrocytes by using the GFAP marker showed that the astrocytes, as well as the neurons, are sensitive to the acute lack of oxygen. In the centre of the ischemic focus, in the cerebral attack area and in the cerebral haemorrhagic area all the glial cells suffered an intense process of cellular necrosis. In a relatively old CVA, at the fringe of the lesional focus there was noticed an increase in the number of astrocytes, but also an intensification of the immunohistochemical reaction. Most often, astrocytes showed long, thick

extensions, sometimes irregular as calibre and spatial distribution and the cellular body increased in its size. The cellular cytoplasm was abundant, intensely reactive to GFAP and the nucleus appeared big, hypochromic, with the chromatin laid non-evenly, microscopic aspects which betray an intensification of the astrocitary activity. Also known as "reactive gliosis", the reaction of the glial cells was extremely intense in the "ischemic half-shade" area. At the limit between "the ischemic half-shade area" and the cerebral tissue, the number of astrocitary cells, their volume, the number of extensions and the immunohistochemical reaction were much reduced comparing to the immunohistochemical aspect from the ischemic half-shade area.

The assessment of the cerebral microcirculation by using the CD31 antibody showed that in a CVA it is produced a rapid deterioration of the microcirculation from the affected area, which determines the discharge of water, plasmatic proteins and even blood cells. In an old CVA, in the ischemic half-shade area there were noticed angiogenesis vessels. The recovering of the vascular device is a physiological process, essential for the diminishment of the state of cerebral hypoxia and for the diminishment of the vascular parenchyma. The angiogenesis vessels showed an irregular tract, a variable calibre and they were laid mostly around the ischemia focus.

Selective bibliography

1. Sims NR, Muyderman H. Mitochondria, oxidative metabolism and cell death in stroke. Biochimica et Biophysica Acta, 2009, 1802 (1): 80–91.

2. Robert AA, Zamzami MM. Stroke in Saudi Arabia: a review of the recent literature. Pan Afr Med J. 2014 Jan 15;17:14. eCollection 2014.

3. Kim P, Warren S, Madill H, Hadley M. Quality of life of stroke survivors. Qual Life Res. 1999;8 (4):293-301.

4. Clarke P, Marshall V, Black SE, Colantonio A. Well-being after stroke in Canadian seniors: findings from the Canadian Study of Health and Aging. Stroke. 2002; 33 (4):1016-21.

5. Pollak J, Doyle KP, Mamer L, Shamloo M, Buckwalter MS. Stratification substantially reduces behavioral variability in the hypoxic-ischemic stroke model. Brain Behav. 2012;2 (5):698-706.

6. Adeloye D. An estimate of the incidence and prevalence of stroke in Africa: a systematic review and meta-analysis. PLoS One. 2014 Jun 26;9(6):e100724.

7. Adeloye D. An estimate of the incidence and prevalence of stroke in Africa: a systematic review and meta-analysis. PLoS One. 2014 Jun 26;9(6):e100724.

8. Strong K, Mathers CD, Bonita R. Preventing stroke: saving lives around the world. Lancet Neurol (2007) 6: 182–187.

9. Arseni C, Popoviciu L. Bolile vasculare ale creierului si maduvei spinari. Bolile vasculare ischemice. Partea I, Editura Academiei, 1984, pag.11-52; 465-483;

10. Arseni C. Tratat de Neurologie, partea I, Editura Medicala, Bucure□ti 1982, pag.21-36.

11. Kiernan JA. Barr's-The human Nervous system-An anatomical viewpoint. 9th edition. Baltimore: Lippincott, Williams & Wilkins. 2009, 367-382

12. Dănăilă L, Golu M. 'Vascularizația arterială şi venoasă a creierului' - In Dănăilă L, Golu M, Tratat de Neuropsihologie, vol 1, Ed medicala, Bucuresti, 2001.

13. Noback R, Strominger NL, Ruggiero DA. The human Nervous System-Structure and functions-sixth edition. Humana Press, 2005, 77-89.

14. Hossmann K A, Heiss W-D - Neuropathology and pathophysiology of stroke. In Textbook

of Stroke Medicine. Edited by Michael Brainin, Wolf-Dieter Heiss - Cambridge University Press, 2010, pg.1-27,