DOCTORAL THESIS

ABSTRACT

Hemorrhagic strokes - Clinical, histological and immunohistochemical study

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Importance of the topic. Worldwide, stroke is one of the leading causes of morbidity and mortality (Lopez AD, et al, 2006) as it annually "kills" five million people and causes five million other severe disabilities. In addition, stroke is the second cause of dementia and the most common cause of epilepsy in the elderly and a frequent cause of depression (O'Brien JT, et al 2003; Rothwell PM et al, 2005). According to the World Health Organization, in 2001 there were 5.5 million deaths from strokes and annually about 15 million people surviving a stroke. Lethality of stroke is 11% for women and 8.4% for men. Prospective studies show that this condition increase every year, both in incidence and prevalence, and World Health Organization experts believe that stroke will become by 2030 the leading cause of mortality.

In Europe, the incidence of stroke varies from country to country, estimated between 100 and 200 strokes per 100,000 persons, representing a huge economic burden. Romania is in the top ten in the world in incidence of stroke. Stroke mortality is three to four times higher in Romania than in EU countries and six to seven times higher than in the United States of America.

With regard to age, education statistics show that the maximum incidence of stroke in 75% of cases occur after the age of 65 (Rothwell PM et al, 2005), age associated with a more difficult recovery after stroke (Markus TM et al, 2005).

These data suggest that stroke is a real health problem, not only by high mortality rate but also motor and cognitive consequences for the performance of the survivors. Sequels of stroke can have catastrophic effects on the quality of life for patients and their families. Given these data, in this paper we propose to perform:

I. A retrospective statistical clinical study on hemorrhagic stroke in a representative hospital, in order to highlight the following aspects of the disease:
- the relationship between ischemic stroke and bleeding, overall and in each year of the study;
- the ratio of the different clinical forms of hemorrhagic stroke;
- distribution by sex;
- distribution by social environment;
- distribution by age categories
- correlation of pathology associated with hemorrhagic stroke.

II. A histological study on the human brain of people clinically and paraclinically diagnosed with brain hemorrhage, in order to highlight:
- changes in meningo-cerebral arteries;
- changes in small intraparenchimatous cerebral vessels;
- changes in the blood-brain barrier;
- changes in the affected brain parenchyma and the perilesional area

III. An immunohistochemical study, in addition to histopathological study reveal:
- reaction of the monocyte-macrophage perilezional;
- reaction of the glial system

I. The Clinico-statistical study conducted by us was a retrospective epidemiological study conducted on a period of 5 years, 2006-2010, having studied all cases of hemorrhagic stroke hospitalized in the Clinical Hospital Number 4 of Craiova. We have analyzed clinical observation sheets and statistical data provided by the Statistical Office of the Hospital. The clinico-statistical study aims to highlight the following aspects of hemorrhagic stroke: ischemic and hemorrhagic stroke ratio of both overall and in each year under study, the percentage of different clinical forms of hemorrhagic stroke, distribution by sex, according to the social distribution, distribution according to age categories, highlighting the risk factors.

Between January 1st, 2006 to December 31st, 2010 in Craiova Neurological Hospital a total of 8611 patients diagnosed with stroke were admitted. The number of patients with ischemic stroke was 7308, while strokes were hemorrhagic in 1303 cases. In percentiles, ischemic stroke accounted for 85% while hemorrhagic stroke accounted for only 15%. The most frequent location for the hemorrhagic type was the cerebral parenchyma, registering a 56.5% share of total hemorrhagic stroke, followed by 25.1% subcortical hemorrhage, bleeding in the brain stem with a 10.4%, intraventricular hemorrhages at a rate of 3.6%, intracerebellous bleeding at 3.1% and bleeding in multiple locations at a rate of 0.8%.

Regarding distribution by sex of the hemorrhagic stroke in our study, we noted that there is a minimum difference between hemorrhagic stroke occurring in males and females. Thus it was noted that some cerebral hemorrhages occurred more frequently in males (53% of cases) and less common in females (47% of cases).

Overall mortality in patients hospitalized during 2006-2010 with hemorrhagic stroke in the Neurological Hospital of Craiova, was 9%. Compared with other international statistics, mortality from Hospital no. 4 of Craiova, is very low, which does not correlate with the severity of the condition, because most authors show that mortality from hemorrhagic stroke is much higher, especially in the first month of the onset of cerebral hemorrhage. We believe that the mortality recorded in our study does not reflect reality, since most patients with severe stroke are discharged at the request of family and death is recorded at home. Distribution of 1303 cases of cerebral hemorrhage by age, revealed that this condition was met in persons aged 24 and over 85 years. By the age of 35 years, cerebral hemorrhage is rare.
(11 cases in total) representing about 0.8% and under 25 years is extremely rare (3 cases) about 0.2%.

After the age of 35 years the number of cases of cerebral hemorrhage has increased dramatically, registering the following situation:
- Between 35 and 44 years there have been 35 cases, representing 2.7%;
- Between 45 and 54 years there have been 157 cases, representing 12%;
- Between 55 and 64 years there have been 352 cases, representing 27%;
- Between 65 and 74 years there have been 482 cases, representing 37%;
- Between 75 and 84 years there have been 248 cases, representing 19%;
- Over 85 years there have been 18 cases, representing 1.3%.

Investigation of risk factors in patients with cerebral hemorrhage revealed remarkable correlations between risk factors and cerebrovascular disease.

*Hypertension* was one of the most common factors in people with hemorrhagic stroke. The study of hypertension by years has allowed us to note that this risk factor was found in a very high percentage, ranging between 69.91% and 80%. The average yearly hypertension rate in our investigated group was 75.28%, i.e. from 1303 patients with brain hemorrhage, 981 had hypertension.

Another risk factor we investigated was *coronary heart disease*. In our study of the 1303 investigated patients with brain hemorrhage, 341 patients were diagnosed with coronary heart disease, which is a percentage of 26.21%.

*Cardiac rhythm disorders* is another group of diseases that can be associated and may contribute to the onset of cerebral hemorrhage. In our study, from a total of 1303 patients with cerebral hemorrhage, 113 patients were diagnosed with rhythm disturbances, which represent a rate of 8.62%.

*Hyperlipidemia* is one of the most important conditions favoring the appearance of atheromatosis. In our study, hyperlipidemia was highlighted in a percentage variable from year to year, from 3.76% to 13.49% with an average of 8.67% of all patients diagnosed with brain hemorrhage. As with coronary heart disease, there is an increase in the percentage of fat from one year to another.

Another parameter that we studied was *cholesterol levels*, as cholesterol and other lipids are involved in the development of ateromatosis and atheroma plaques. Hypercholesterolaemia was found in small percentages, which ranged from 0.83% to 1.58% with an average of 0.99% in people with hemorrhagic stroke.
Obesity is one of the forms expressing clinical disorders of lipid metabolism. In our study, a relatively small percentage of patients (4.53%) were diagnosed with brain hemorrhage and was diagnosed with obesity. The yearly percentage variance was not significant, the lowest percentage value was 2.54% and 6.50% highest.

Diabetes mellitus is considered a different pathological entity which may be complicated by a cerebral hemorrhage. The number of patients with cerebral hemorrhage and diabetes mellitus was very low throughout the study period, ranging from 1 to 7 patients, which meant a very small percentage, i.e. between 0.40% and 2.54% overall average being 1.30%.

II. Histological study of cerebral vessels in patients deceased by hemorrhagic strokes.

The biological material studied consisted of encephalon fragments collected during autopsy, carried out on a number of 43 patients diagnosed clinically and by imaging techniques with hemorrhagic stroke, hospitalized in the Neurological Hospital of Craiova, between 2006 and 2010. Fixation was performed in 10% neutral formalin solution and processed in the classical technique of inclusion in paraffin.

Two staining techniques were used: hematoxylin - eosin and trichromic Masson stainings.

Results. Microscopic study of meningo-cerebral vessels revealed multiple changes in the wall and their size in all studied subjects. The medium layer of cerebral arteries showed a marked fibrosis, smooth muscle cells were partially or completely replaced by collagen fibers. Tunica media seem to continue without any distinction from the internal tunic of the vessel by the disappearance of loose connective tissue layer in the subendothelial structure and its replacement by fibrous tissue. We also noted in some patients the disruption of the elastic tissue of the internal elastic limitant, which has made it more difficult to precisely delimit the boundary between the tunica and intimate medium. Adventicace, in most studied cases, appeared thickened, but thickness varied from case to case, with a relatively homogeneous structure, with a greater number of fibroblasts and lymphocytes in the wall. Meningo-cerebral arteries less than 0.5 mm caliber, unlike the large-caliber arteries, showed no atheromatosis plates. Their wall presented a relatively uniform thickness throughout the circumference. In some areas, however, we noticed a trend to thickening of the subendothelial layer through the development of a slide of loose connective tissue, poor in cells and fibers but rich in basic substance. In these vessels we observed the presence of larger amount of collagen fibers in the structure of the three tunics.
A novel feature of the studied cases was the presence of figurative elements around the meningo-cerebral vessels. Blood count items were found both in fused intraparenchimatous or subarachnoid hemorrhages and in subarachnoidian bleedings. Meningo-cerebral hemorrhagic infiltrate showed a varied cytology from case to case, depending on the age of stroke occurrence. In most cases, polymorph infiltration was noted, with fragmented, partially hemolyzed and intact red blood cells with morphological features still preserved. We also noticed the presence of many large macrophages containing debris of red blood cells or intact haematties phagocytized in their cytoplasm, ferric pigment and cytoplasmic vacuoles of various sizes and shapes, resulting from specific and nonspecific phagocytosis processes in which these cells were involved. Very seldom have we noted the presence of granulocyte or lymphocyte cell type elements in the hemorrhage infiltrate.

Small brain vessels, arteries, arterioles, capillaries, veins and venue, had bleeding in the joints forming true perivascular sheaths. At this level some blood vessels have shown signs of vascular thrombosis.

A histological picture that has emerged in almost every case we studied was that of perivascular edema. Around arteries, arterioles, venules, veins and capillaries even some intraparenchimatous capillaries, we observed a clear space, of variable thickness, or occupied by acellular hematic debris, located between the advent and nervous tissue itself. The microscopic images allowed us to conclude that perivascular edema remains long after the great vascular drama.

We also observed mineral deposits, calcium salts in the wall that cerebral vessels of small arms in the form of rosettes, in Advent.

III. Immunohistochemical study on the response of macrophage system cells and astrocytes in hemorrhagic stroke.

The studied biological material was fragments of encephalon collected at the autopsy carried out a total of 24 patients with hemorrhagic stroke. As the histological technique, biological material was fixed in 10% neutral formalin solution, 3 to 7 days at laboratory temperature and processed in conventional histological techniques for paraffin inclusion. Methods used. Two techniques were used for staining:

- Technique for highlighting macrophages using CD68 antibody;
- Technique for evidence astrocytes using GFAP antibody.

Results. Immunohistochemical study allowed us to highlight the intense reaction of the monocyte-macrophage cells in the immediate vicinity of the outbreak of bleeding. The presence of these cells indicates an activity of phagocytosis of debris resulting from the
degradation of blood cells and plasma components extravasated into brain parenchyma. Away from the hemorrhagic focal point, we have identified numerous reactive perivascular macrophages, which suggest that the blood-brain barrier alterations are early to the episode of acute cerebral hemorrhage.

In the white matter in perilesional areas we could also highlight the numerous large macrophages with foamy cytoplasm, which have phagocyted nerve fibers surrounded by myelin, axons of neurons destroyed by hematic overflow.

Astrocyte reaction was intense near the lesion core. Here, numerous astrocytes were identified by increased in size, with extra-long, thick, elongations, with enlarged, round, centrally disposed, hypochromic nucleus, highly reactive to GFAP. The reaction of glial cells is the main limitation of neuronal damage induced by hemorrhagic stroke.

**General conclusions**

In our study, hemorrhagic stroke accounted for 15% of all strokes recorded over a period of five years at the Neurological Hospital of Craiova.

The most frequent location of cerebral hemorrhage was intaparenchimatous, followed by subcortical hemorrhage, hemorrhage of the brain stem, cerebellar hemorrhage and intraventricular hemorrhage.

Regarding gender, no significant changes were found, the percentage of men diagnosed with hemorrhagic stroke was 53% while females were affected at a rate of 47%. Decade of age most affected by the hemorrhagic stroke between were 65-74 years (37%), followed by decades 55-64 years (27%) and the decade 75-84 years of age (19%).

Hemorrhagic stroke were correlated in 75% cases with hypertension, in 26.21% to ischemic cardiopathy, in 8.62% with cardiac arrhythmias.

Histopathological changes seen in patients with hemorrhagic stroke were atherosclerosis in major blood vessels and small vessel atherosclerosis. We frequently met deposits of calcium salts in the wall of cerebral vessels. Immunohistochemistry studies have shown a strong reaction by both macrophage system cells and astrocitary cells.

AVC hemoragice s-au corelat în 75% din cazuri cu HTA, în 26.21% cu cardiopatia ischemică i în 8,62% cu tulburările de ritm cardiac.