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Histopathological lesions induced by stroke in the encephalon

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Abstract

Strokes are conditions with a high degree of morbidity and mortality worldwide. These conditions profoundly affect the quality of life of patients; in addition to physical disabilities, patients present various mental disorders, such as mood disorders, anxiety, depression, behavioral disorders, fatigue, etc. Microscopic lesions of the brain parenchyma explain the clinical symptoms and correlate with the severity of the stroke. Our study consisted of the histopathological (HP) and immunohistochemical analysis of brain fragments, collected from 23 patients, with a clinical and imagistic diagnosis of stroke, who died during hospital admission. The microscopic analysis showed that both neurons and glial cells are affected in the ischemic focus. Neuronal death in the ischemic focus was mostly caused by cell necrosis and only about 10% by apoptosis. Regarding vascular lesions, it was observed that the most frequent HP lesion of intracerebral arterioles was arteriosclerosis. The lumen of the arterioles was reduced, and the vascular endothelium had a discontinuous aspect, which indicates a change in the blood–brain barrier. Sometimes the arteriole lumen was completely obstructed, with fibrinoid necrosis in the internal and middle tunic, or with the proliferation of fibroblasts and the formation of young intraluminal connective tissue. Intraparenchymal blood capillaries in the ischemic area showed endothelium discontinuities, lumen collapse, and sometimes massive perivascular edema. As for neuroinflammation, the presence of numerous neutrophils, lymphocytes, plasma cells and macrophages was found in the ischemic focus, forming a complex and inhomogeneous cellular mixture. Of the inflammatory cells present in the ischemic focus and in the ischemic penumbra area, the most numerous were the macrophages. The HP analysis showed that neuroinflammation is very complex and different in intensity from one patient to another, most likely due to associated comorbidities, age, treatment administered until death, etc.

***Keywords*:** stroke, neurological disorder, brain, blood vessels, neuroinflammation.

Introduction

Cerebrovascular accident or stroke is a neurological deficit with a "rapid" onset, lasting more than 24 hours and manifesting as a focal or global brain dysfunction, caused by interruption of blood flow to the brain, of an obstructive or hemorrhagic cause, thus stopping the supply of oxygen and nutrients to the brain and causing damage to brain tissue [1].

Strokes are conditions with a high degree of morbidity and mortality worldwide. According to some studies, stroke registers a prevalence of over 100 million cases worldwide, with about 12.2 million new cases of strokes every year and a mortality of about 6.55 million per year [2, 3]. In addition to the high number of deaths, stroke causes about five million cases of permanent disability annually [4]. Statistics show that stroke is currently the second leading cause of death and the third major cause of disability globally [2, 5].

As a result of changes in the lifestyle of society and the increasingly frequent presence of cardiovascular risk factors (high blood pressure, atherosclerosis, stress, dyslipidemia, diabetes, and obesity), the incidence of stroke continued to increase in the last 50 years, affecting not only the elderly population but also young people [6]. Between 1990 and 2019, the absolute number of strokes increased by 70% and stroke deaths increased by 43% [2]. The same study showed that the group of low-income countries had a standardized stroke mortality rate 3.6 times higher than high-income countries [2].

The main risk factor for stroke is aging, and as the population ages, the overall impact of stroke continues to increase [7].

The incidence of stroke differs from one geographical

area to another and even from country to country. The incidence of stroke in Asia varies from 116 to 483 per 100 000 inhabitants, being about two times higher than in Europe, due to the higher prevalence of dyslipidemia, diabetes, and obesity [3, 8, 9].

The medical costs related to stroke are enormous. In the US alone, medical costs are expected to reach 240.67 billion USD in 2030 (represented by direct costs, necessary for medical care, but also indirect costs resulting from reduced labor productivity), with an increase of 129% compared to 2012 [10].

Stroke profoundly affects patients' quality of life. In addition to physical disabilities, patients have various mental disorders, such as mood disorders, anxiety, depression, behavioral disorders, fatigue, etc., that sometimes compromise the recovery and rehabilitation processes [11–14].

Aim

Knowledge of histopathological (HP) lesions and early and appropriate treatment to limit these lesions are essential for the survival and recovery of the stroke patient. In the present study, we aimed at assessing the microscopic lesions present in the brain in people with stroke.

Materials and Methods

The analyzed material consisted of brain fragments collected from 23 patients hospitalized in the Department of Neurology, Emergency County Hospital, Drobeta-Turnu Severin, Mehedinţi County, Romania, between 2015–2022, clinically and imagistically diagnosed (computed tomography) with stroke, who died during Hospital admission. The necropsy was performed in the Department of Pathology of the same Hospital. Necropsies were performed with the consent of relatives of deceased patients who signed an informed consent regarding necropsy and use of harvested biological material.

The harvested tissue fragments were fixed in 10% neutral buffered formalin solution at room temperature for 48 hours, after which they were processed using the classical HP technique of paraffin inclusion. The biological material included in the paraffin was then sectioned into the HMB-350 microtome, equipped with a Peltier cooling system and a water bath section transfer system (STS, microM), obtaining 4 μm thick serial sections that were stained with Hematoxylin–Eosin (HE) and trichrome with light green, using the Goldner–Szekely (GS) technique.

For the immunohistochemical (IHC) study, the encephalon sections obtained after microtome sectioning were placed on special slides containing poly-L-lysine on their surface to increase the adhesion of nervous tissue to the glass surface of which histological slides are made. Then, these sections were left for 24 hours at 32ºC in the thermostat for drying, after which they were processed according to immunohistochemistry protocols, specific to each antibody.

In our study, we used the following antibodies: anti-neuronal nuclei (NeuN) (monoclonal mouse anti-human, clone MAB377, 1/1000 dilution, Millipore); anti-neurofilament protein (NFP) (monoclonal mouse anti-human NFP, clone 2F11, 1/100 dilution, Dako); anti-caspase-3 (caspase-3 rabbit antibody, clone 9662S, 1/100 dilution, Cell Signaling Technology); anti-glial fibrillary acidic protein (GFAP) (monoclonal rabbit anti-human, clone Z0334, 1/20 000 dilution, Dako); anti-cluster of differentiation (CD)3 (monoclonal mouse anti-human CD3, clone F7.2.38, 1/25 dilution, Dako); anti-CD20 (monoclonal mouse anti-human CD20cy, clone L26, 1/50 dilution, Dako); anti-CD68 (monoclonal anti-human CD68, clone KP1, 1/100 dilution, Dako); anti-CD204 (purified – eBioscience anti-human CD204, clone J5HTR3, 1/100 dilution, Invitrogen); anti-CD34 (monoclonal mouse anti-human CD34 class II, clone QBEnd 10, 1/50 dilution, Dako); anti-ionized calcium-binding adaptor molecule 1 (IBA1) (monoclonal rabbit anti-human, clone MA5-3257, 1/1000 dilution, Thermo Fischer).

Results

Changes in brain parenchyma

The microscopic aspects of the brain parenchyma were very different from one case to another, probably depending on the time passed since stroke onset and death, and on the other hand depending on the patient age, since it is known that the brain also undergoes structural changes with age.

Of the 23 cases examined, two (8.70%) were diagnosed with intracerebral hemorrhage. They appeared microscopically as either diffuse intracerebral hemorrhage or brain hematoma (Figures 1 and 2). The rest of the cases (21, representing 91.30%) were ischemic strokes.

In ischemic stroke, the morphopathological changes were very extensive, often exceeding the ischemic penumbra region, considered the boundary between the cell necrosis area and the normal area of the encephalon. The microscopic analysis showed that both neural bodies and neural extensions were affected. Most commonly, neuronal bodies arose with acidophilic cytoplasm ("red neurons", "ischemic neurons") most likely produced because of changed mitochondrial activity, changes in the cellular metabolism and neuroplasm proteins due to reduction or total lack of oxygen (Figures 3 and 4). The nucleus of neurons appeared more condensed and hyperchromic, while non-myelinated neuronal extensions in gray matter appeared fragmented, giving the neuropil a smoothly granular aspect. In white matter, myelin fibers appeared as disorganized fragments, with a varied reaction to the used stainings. Some small blood vessels (arterioles, capillaries, venules) appeared with a collapsed lumen and perivascular edema, microscopic aspects denoting damage to the blood–brain barrier (BBB) and extravasation of components from blood plasma into the perivascular space.

Some of the largest ischemic lesions consisted of vacuolization of the neuroplasm, condensation of the nucleus, fragmentation of neurons because of the presence of neural debris surrounded by astrocytes and neuropil. In some areas, empty spaces appeared, in the shape and size of oval or pyramidal neurons, lacking neurons or with few neuronal debris, with an aspect of "phantom neurons" (Figure 5). Viewed with the smallest microscopic lenses, these areas of brain parenchyma had a spongy aspect. We found that in a previous stroke, the most common "spongy" aspects of the brain parenchyma occurred in the superficial part of the brain cortex, in the external molecular and granular layers, which denotes a greater sensitivity of neurons in these areas to hypoxia.

For the study of hypoxic neurons, we performed some IHC staining. We used the anti-NeuN antibody because the NeuN protein expression is known to be associated exclusively with nerve tissue. This marker was not detected in tissues other than nerves and it is not present in glial cells, suggesting that it is a specific neural marker. Anti-NeuN

antibodies can identify most types of neurons throughout the nervous system.

As can be seen in our images (Figures 6 and 7), the reaction to NeuN is very intense and homogeneous in neurons unaffected by hypoxia, but decreases strongly, until disappearing in the neurons from ischemic areas.

Another marker we used was the NFP, a protein that enters the structure of neurofilaments or "intermediate filaments" in the neural cytoskeleton. The IHC reaction to NFP in neuronal bodies was reduced, and neuronal extensions showed discontinuities of neurofilaments in the neurofibril structure, indicating significant damage to the neuronal cytoskeleton (Figure 8).

By using the anti-caspase-3 antibody, we wanted to identify neurons in the ischemic area that enter apoptosis. As seen in our image, the IHC reaction is of low intensity and is expressed in a small number of neurons (Figure 9). The analysis of microscopic images showed that less than 10% of ischemic neurons die from apoptosis.

Other important cells that intervene in the histophysiology of neurons, under normal and pathological conditions are astrocytes. These are the most abundant types of glial cells in the central nervous system (CNS). They not only metabolically nourish neurons, but also regulate neuronal activity by buffering ions and neurotransmitters, being involved in the formation and functioning of microscopic synapses, showed that less than 10% of ischemic neurons die from apoptosis.

For the analysis of astrocyte reaction in stroke, we used the anti-GFAP antibody. GFAP is a protein that belongs to intermediate filaments in the cytoskeleton of astrocytes. The protein is the smallest of the intermediate filament proteins (8 nm) with a molecular weight of about 51 kDa. In the CNS, GFAP is expressed in astrocytes and ependymal cells, but not in other glial cells.

The microscopic study revealed various aspects of astrocytes in stroke, most likely correlated with the time between stroke onset and patient death. On some HP samples, it was noted the presence of a reactive gliosis characterized by an increase in the number of astrocytes, an increase in their volume, an increase in the size of nuclei, elongation of extensions, microscopic aspects corresponding to relatively recent acute strokes or brain areas less affected by ischemia (Figure 10). On other HP samples, numerous astrocytes with reduced IHC reaction, with pyknotic nucleus, hypochromic, finely granular cytoplasm probably resulting from cytoskeletal damage or fragmentation and fragmented astrocytic extensions, aspects of old stroke (Figure 11) were highlighted. We believe that prolonged lack of oxygen causes the death not only of neurons, but also of glial cells.

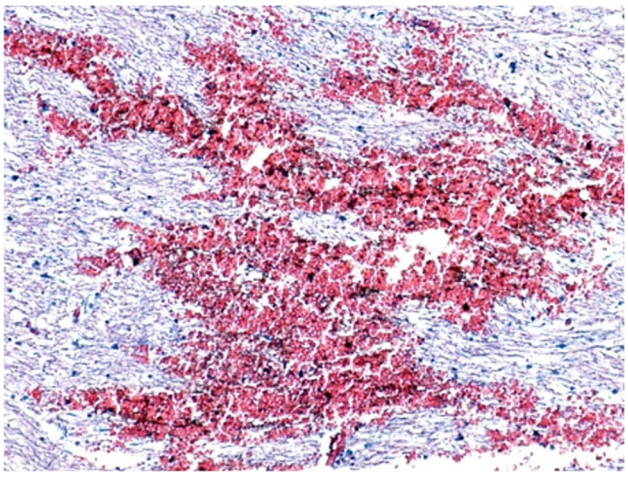

Figure 1 – ***Microscopic image of diffuse intracerebral hemorrhage. Goldner–Szekely (GS) trichrome staining, ×100.***

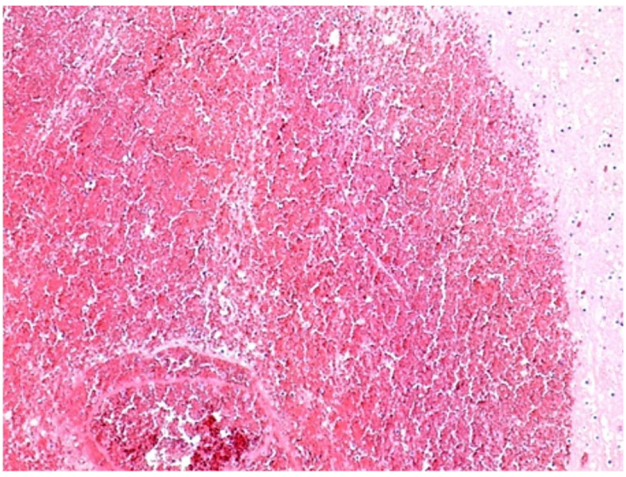

Figure 2 – ***Intraparenchymal hematoma. Hematoxylin–Eosin (HE) staining, ×100.***

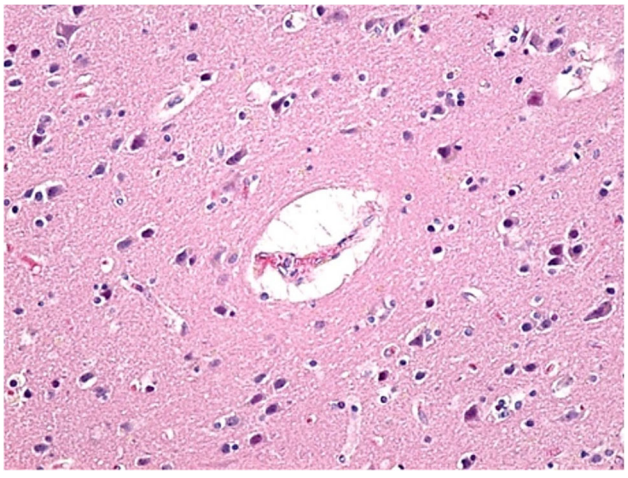

Figure 3 – ***Area of hypoxic cerebral parenchyma, outside the ischemic penumbra area with "red neurons", perivascular edema, collapse of blood vessels and fine-granular appearance of the neuropil. HE staining, ×200.***

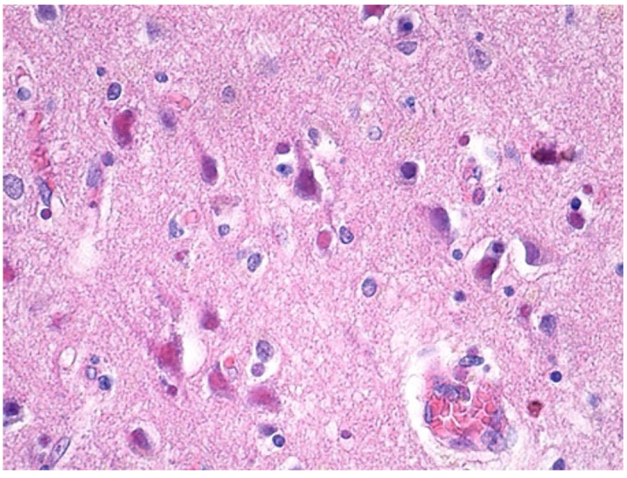

Figure 4 – ***Microscopic image of "red neurons" specific to brain ischemia. HE staining, ×400.***

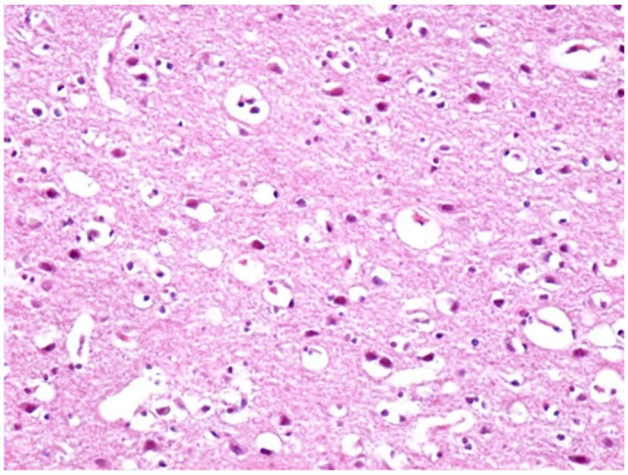

Figure 5 – ***Brain parenchyma with spongy aspect resulting from the death of neurons, collapse of blood capillaries, perineuronal and perivascular edema. HE staining, ×200.***

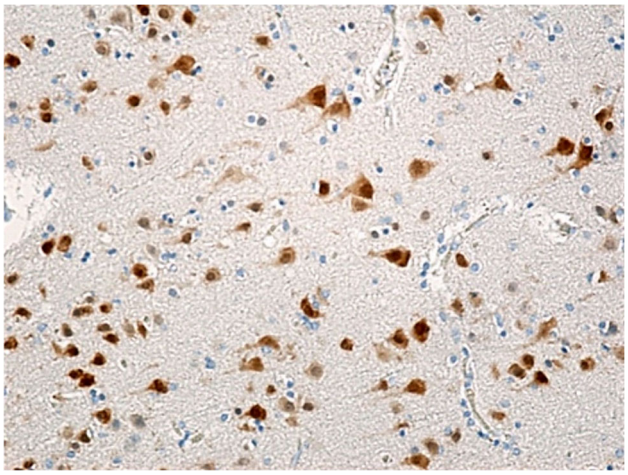

Figure 6 – ***Normal brain parenchyma (unaffected by ischemia), where an intense and homogeneous reaction of NeuN protein is observed during immunomarking with anti-NeuN antibody, ×200. NeuN: Neuronal nuclei.***

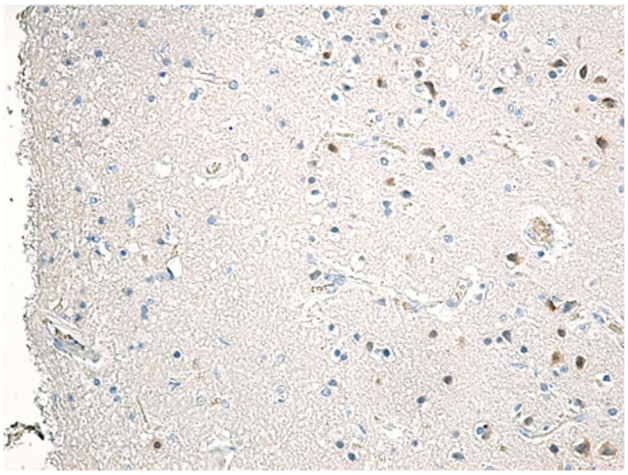

Figure 7 – ***Brain parenchyma of the ischemic area, where we observe a reduction in the number of neurons and reduction of reaction intensity to immunomarking with anti-NeuN antibody, ×200.***

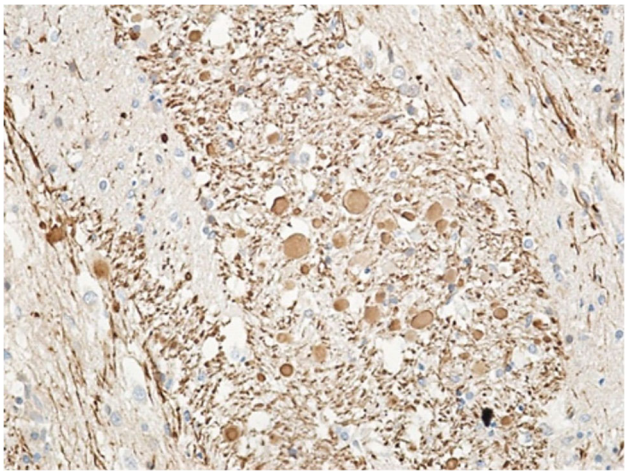

Figure 8 – ***Low uneven reaction of anti-NFP antibody in ischemic neurons and neuronal extensions. Immunomarking with anti-NFP antibody, ×200. NFP: Neurofilament protein.***

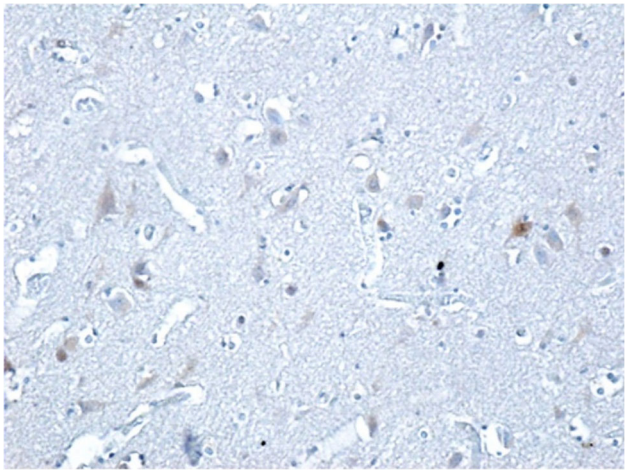

Figure 9 – ***Reduced immunohistochemical reaction of ischemic neurons to anti-caspase-3 antibody, meaning that a small number of ischemic neurons die by apoptosis. Immunomarking with anti-caspase-3 antibody, ×200.***

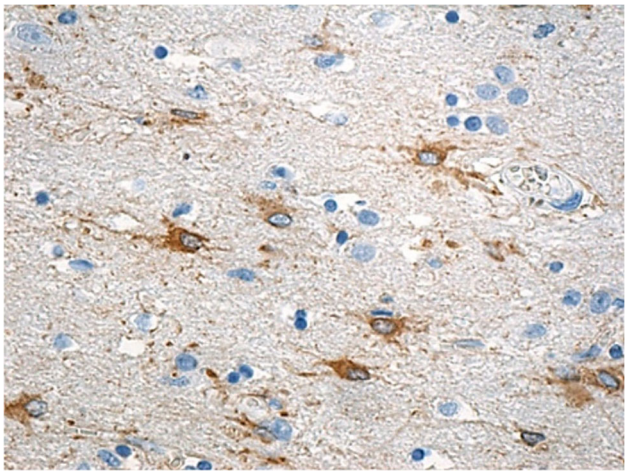

Figure 10 – ***Encephalon area with reactive gliosis. Immunomarking with anti-GFAP antibody, ×200. GFAP: Glial fibrillary acidic protein.***

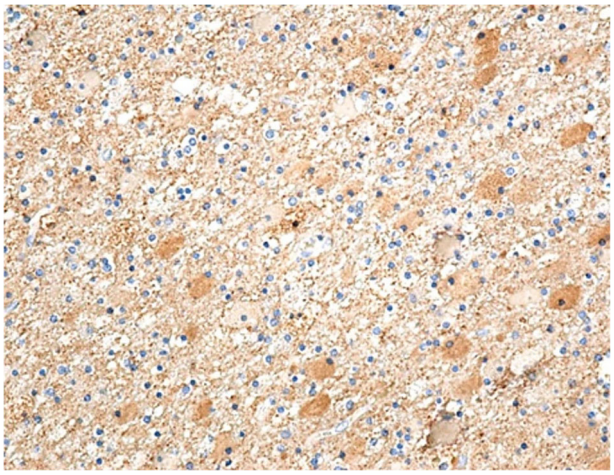

Figure 11 – ***Encephalon area with numerous astrocytes with reduced immunohistochemical reaction, pyknotic nucleus, hypochromic, fine-granular cytoplasm probably resulting from cytoskeleton damage or fragmentation and fragmented astrocytic extensions. Immunomarking with anti-GFAP antibody, ×200.***

Changes in brain vessels of stroke patients

Brain vessels play an essential role in stroke etiopathogenesis. Structural changes in blood vessels increase with age. Therefore, strokes occur mainly in the elderly. Our study aimed at assessing small intracerebral vessels, where vascular occlusion phenomena can occur causing an ischemic stroke, or rupture of the vessel wall, resulting in a hemorrhagic stroke.

The most frequent HP lesion of intracerebral arterioles was arteriosclerosis, in which the arterioles had a diminished lumen, thickened wall by deposition of Periodic Acid–Schiff (PAS)-positive hyaline material, but also by a deposition of concentric collagen fibers, "like the scales of an onion bulb". Basically, the two forms of arteriosclerosis, hyaline and hyperplastic, met, but we must mention that the hyperplastic one dominated (Figures 12 and 13). As can be seen from our images, in the middle tunic of the arterioles the muscle fibers disappeared by replacing them with collagen fibers with concentric arrangement. The same process of collagen fibrosis can be observed in the internal tunic, the subintimal area (Figure 12). The vascular lumen is reduced, and the vascular endothelium is discontinuous. Other times, the arteriolar lumen was completely obstructed, with fibrinoid necrosis in the internal and middle tunic or with the proliferation of fibroblasts and the formation of a young intraluminal connective tissue (Figure 13).

Intracerebral venules showed arteriole-like changes: the lumen was narrowed unevenly, the wall appeared thicker by fibrillar collagen deposition, smooth muscle cells in the middle tunic disappeared, and the trajectory was irregular. Frequently, around the venules in the ischemic area, the existence of an important perivascular edema was observed (Figure 14).

Intraparenchymal blood capillaries in the ischemic area showed endothelium discontinuities, lumen collapse, and sometimes massive perivascular edema (Figure 15). The IHC study using anti-CD34 antibody showed a significant reduction in vascular density in areas with ischemia (Figures 16–18).

Stroke-induced inflammatory changes in the encephalon

It is known that brain damage after ischemic stroke leads to necrosis and apoptosis of neurons and glial cells, which in turn lead to neuroinflammation mediated by reactive oxygen species (ROS), chemokines, and cytokines.

Most cells present in the blood, neutrophils, B- and T-lymphocytes, monocytes, penetrate the brain parenchyma by breaking the BBB and can produce cytokines to regulate neuroinflammation, in particular, proinflammatory cytokines, participating in numerous processes associated with the occurrence of ischemic stroke. As such, although neuroinflammation can promote brain damage repair, excessive infiltration of inflammatory cells can cause additional ischemic brain damage, leading to higher heart attack outbreaks and more severe clinical symptoms.

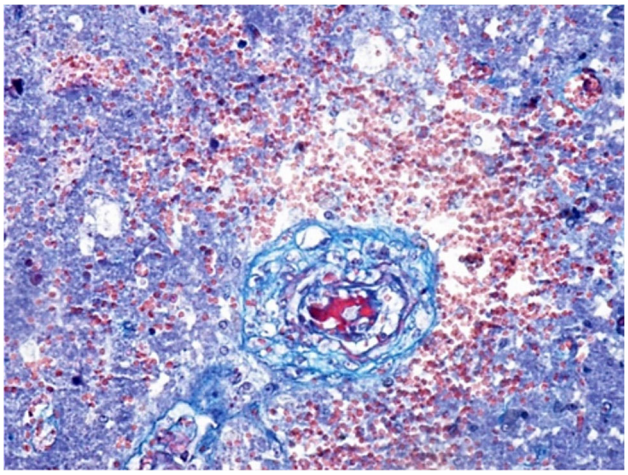

Figure 12 – ***Intracerebral arteriole with thickened wall, with fibrinoid necrosis in the internal and middle tunics, and reduction of vascular lumen. A perivascular, abundant hematic infiltrate associated with necrosis of the cerebral parenchyma is observed. GS trichrome staining, ×200.***

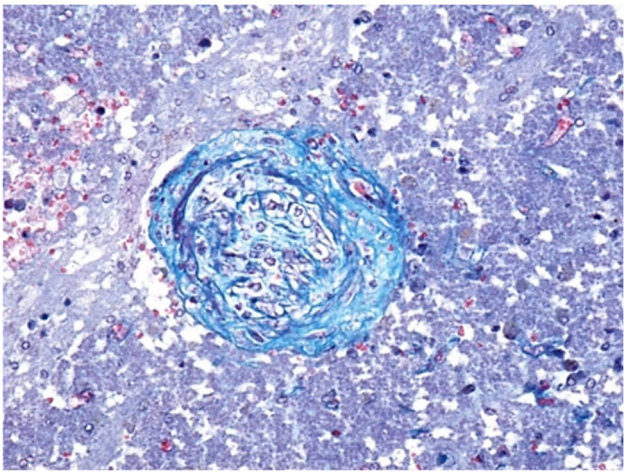

Figure 13 – ***Intraparenchymal arteriole with obstructed lumen by proliferation of fibroblasts and formation of young connective tissue occupying all the lumen of the arteriole. In the vessel wall, the replacement of smooth muscle fibers with collagen fibers of concentric arrangement is observed. GS trichrome staining, ×200.***

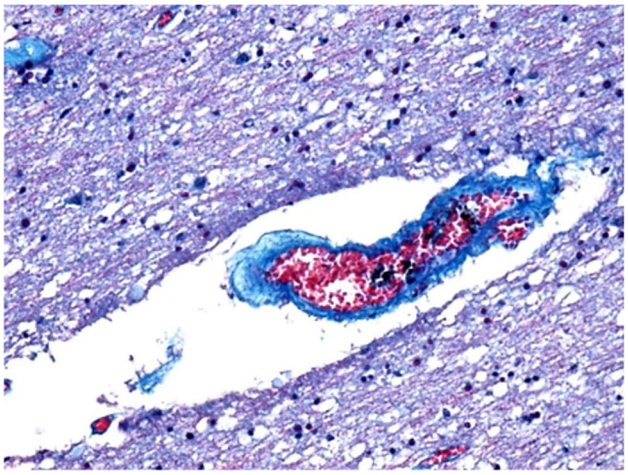

Figure 14 – ***Image of intraparenchymal venule, with irregular trajectory and perivascular edema. The wall is heterogeneously thickened by fibrillar collagen deposition in the internal and middle tunic. GS trichrome staining, ×200.***

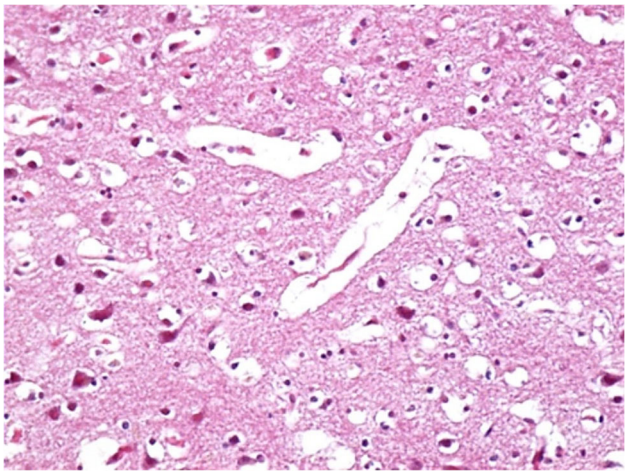

Figure 15 – ***Intraparenchymal capillaries, with discontinuous wall, collapse, and important perivascular edema. HE staining, ×200.***

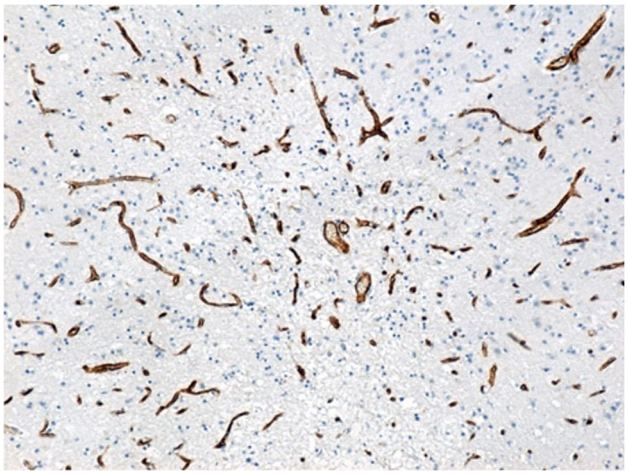

Figure 16 – ***Image of brain microvascularization in an area unaffected by cerebral ischemia. Immunomarking with anti-CD34 antibody, ×100. CD34: Cluster of differentiation 34.***

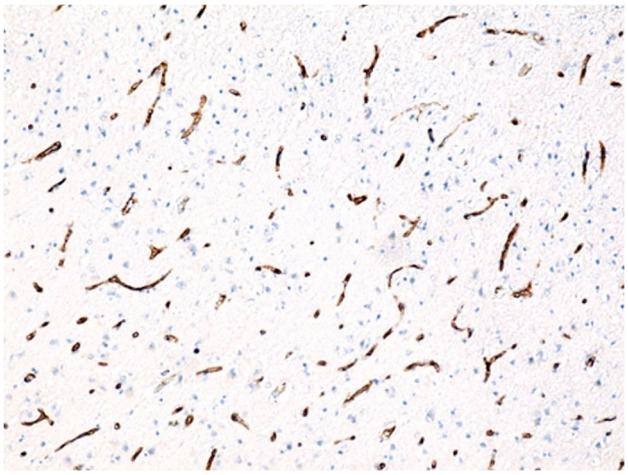

Figure 17 – ***Brain cortex from the ischemia region, where a reduction in microvascular density is observed. Immunomarking with anti-CD34 antibody, ×100.***

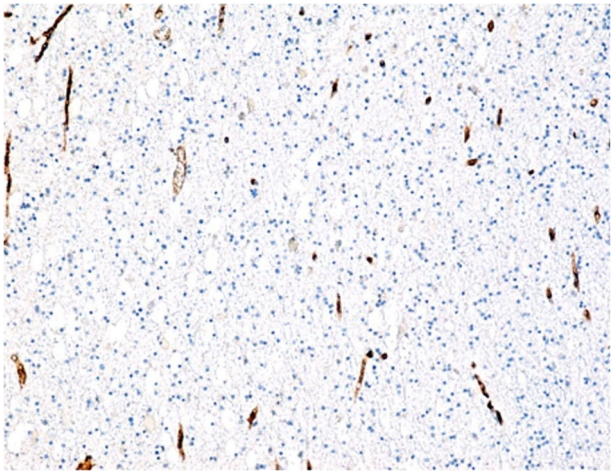

Figure 18 – ***White matter from the area affected by brain ischemia; a significant reduction in vascular density is observed. Immunomarking with anti-CD34 antibody, ×100.***

In our study, most inflammatory cells were identified around ischemia and ischemic penumbra (Figures 19 and 20). The death of neurons and glial cells in the ischemic focus led to the release of denatured proteins that constitute antigens for the immune system, which attracted numerous neutrophils, lymphocytes, plasma cells and macrophages into the ischemic foci, forming a complex cellular mixture. Moreover, local ischemia caused extravasation of some plasma components and activated perivascular fibrocytes that turned into activated fibroblasts capable of synthesizing and excreting into the extracellular space multiple components of the connective matrix. Among inflammatory cells, there were also identified activated fibroblasts, necrotic cells, extruded, intact or hemolyzed red blood cells and large cells with acidophilic, granular, siderophage-like cytoplasm.

The IHC study showed that the most numerous inflammatory cells in the ischemic focus and ischemic penumbra were macrophages (Figure 21). They showed

increased size, hypochromic nucleus, enlarged volume, abundant, vacuolar cytoplasm, spongy aspect due to capture and phagocytizing of numerous neural debris (Figure 22). The macrophages identified using anti-CD68 antibody are type 1 (M1) macrophages. They mainly produce pro-inflammatory cytokines and have intensely bactericidal properties.

To highlight type 2 (M2) macrophages, we used the anti-CD204 antibody, which plays a role in tissue remodeling and angiogenesis. In our study, in ischemic foci and ischemic penumbral areas we identified increased amounts of M2 macrophages (Figure 23). The origin of M1 and M2 macrophages is the blood monocyte that arises in the red bone marrow.

Among the cells of the nervous system involved in the local inflammatory reaction, we mention microglia that have the same origin as macrophages in other organs, but which are specific to the nervous system. In our study, microglia highlighted using anti-IBA1 antibody in ischemic brain tissue had a moderate IHC reaction (Figure 24), which leads us to believe that in the brain inflammatory reaction, most of the cells of the macrophage system come from the blood, more precisely, from blood monocytes.

Regarding the presence of B- and T-lymphocytes, they were found in reduced numbers in ischemic foci (Figures 25 and 26) compared to macrophages.

The HP analysis of brain fragments from stroke patients showed that the inflammatory reaction is very complex and different in intensity from one patient to another, most likely due to associated comorbidities, age, treatment administered until death, etc.

Discussions

Stroke is one of the leading causes of death worldwide and the most common cause of disability among adults, having a serious impact on the patient and family, but also upon the social and economic life [15]. The average cost of ischemic stroke per person, which includes inpatient care, rehabilitation, and aftercare, is estimated at 140 048 dollars in the United States [16, 17].

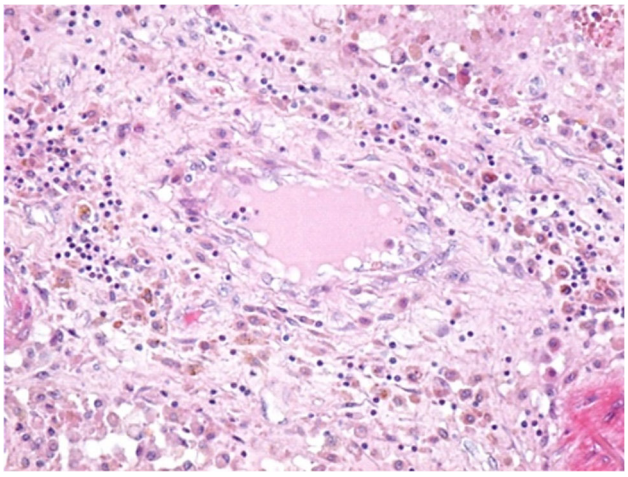

Figure 19 – ***Inflammatory cells (lymphocytes, plasma cells, macrophages) accumulated perivascularly, but also intra-parenchymal, in a case of ischemic stroke. HE staining, ×200.***

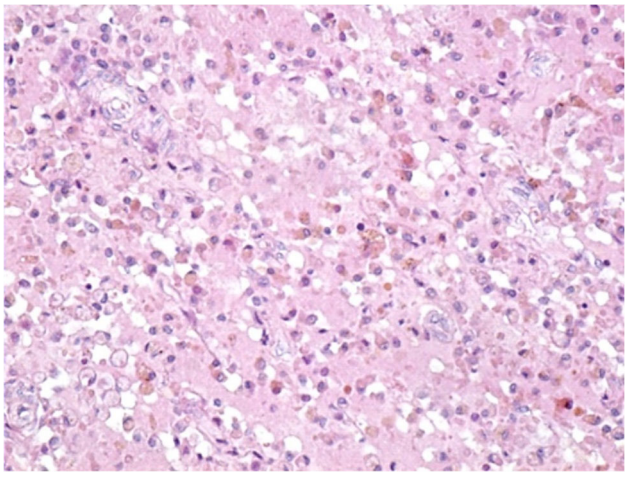

Figure 20 – ***Ischemic penumbra area with a complex cellular component (neurons, neural debris, glial cells, inflammatory cells). HE staining, ×200.***

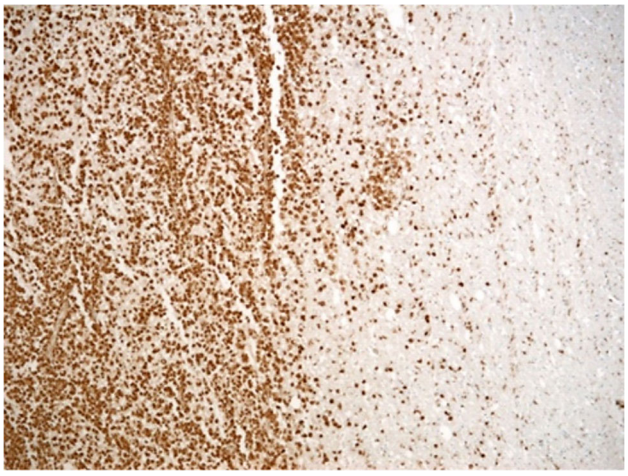

Figure 21 – ***Ischemic penumbra area with large amount of macrophages. Immunomarking with anti-CD68 antibody, ×100. CD68: Cluster of differentiation 68.***

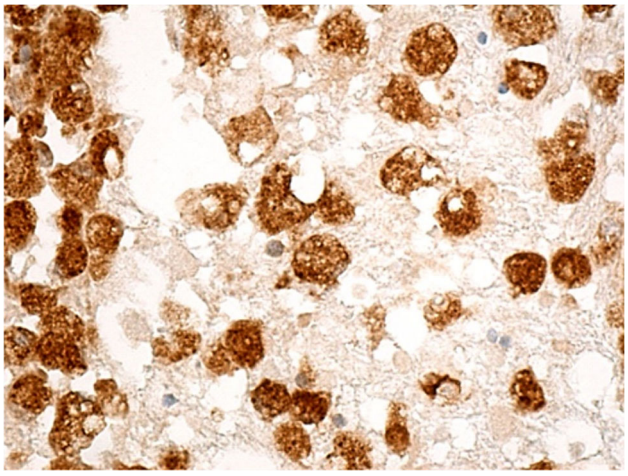

Figure 22 – ***Enlarged image from the previous figure in which cellular and tissue remains of brain nervous tissue are observed, as well as the spongy aspect of macrophages due to endocytization of fragments of necrotic brain tissue. Immunomarking with anti-CD68 antibody, ×400.***

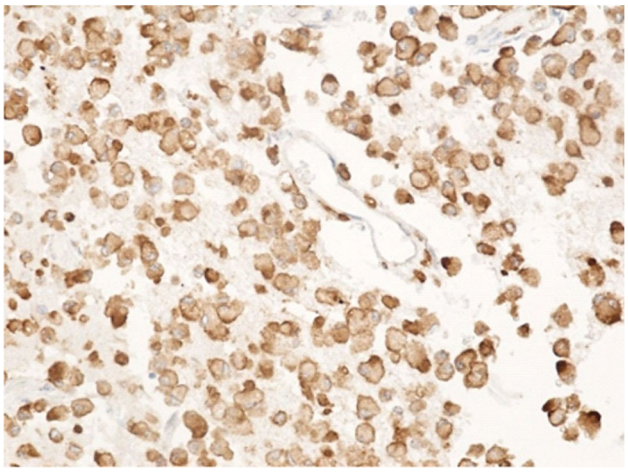

Figure 23 – ***Type M2 macrophages disseminated in the ischemic penumbra area. Immunomarking with anti-CD204 antibody, ×200. CD204: Cluster of differentiation 204.***

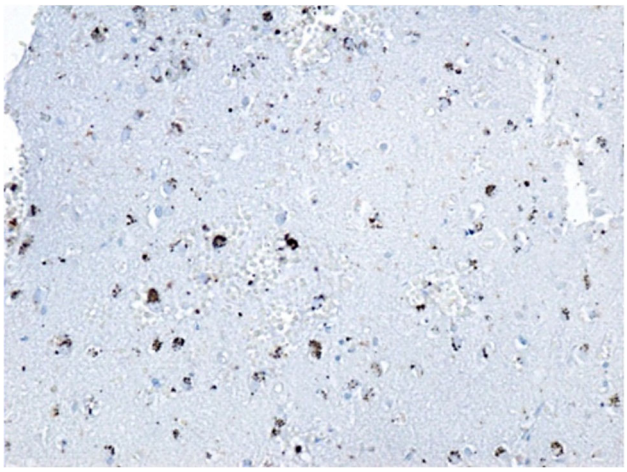

Figure 24 – ***Moderate immunohistochemical reaction of microglia in the ischemia area. Immunomarking with anti-IBA1 antibody, ×200. IBA1: Ionized calcium-binding adaptor molecule 1.***

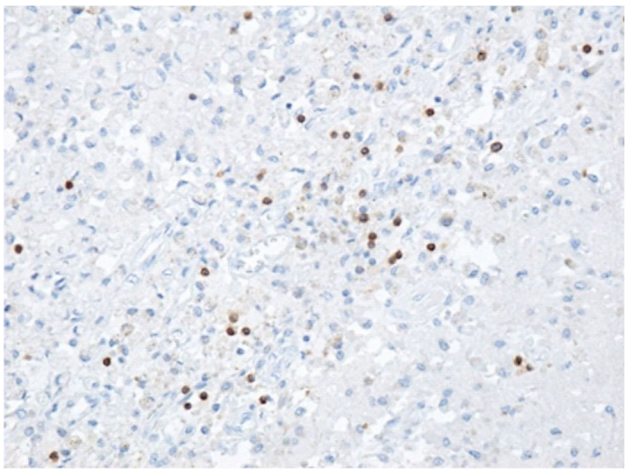

Figure 25 – ***Moderate reaction of T-lymphocytes in stroke. Immunomarking with anti-CD3 antibody, ×200. CD3: Cluster of differentiation 3.***

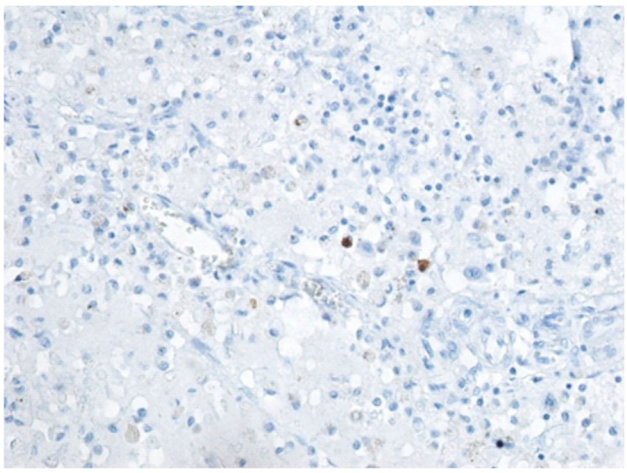

Figure 26 – ***Brain parenchyma in the ischemic area with rare B-lymphocytes present in the ischemic stroke area. Immunomarking with anti-CD20 antibody, ×200. CD20: Cluster of differentiation 20.***

Most strokes (87%) are ischemic, followed by intracerebral hemorrhage (10%) and subarachnoid hemorrhage (3%) [18]. Comorbidities, such as ischemic heart disease and atrial fibrillation, are commonly associated with stroke. According to some studies [19], ischemic heart disease and stroke together caused 15.2 million deaths worldwide in 2015. If until a few decades ago the disease mainly affected the elderly, now there is a tendency to stroke in younger people, especially in low and middle-income countries [20–22]. Due to these reasons, stroke is a priority for health systems, but also for researchers who seek to bring new data for early diagnosis and a much better treatment of stroke.

In our study, we were concerned with highlighting HP lesions that occur in the brain nervous tissue following a stroke. The relatively small number of cases studied by us is because, according to Romanian legislation, an autopsy is not performed on all deceased cases, but only where necessary, to establish the cause of death or if there is suspicion of death by physical aggression.

The cases analyzed by us presented a multitude of lesions, which completely changed the architecture of the nervous tissue at the site of cerebral ischemia. The largest changes were found, as normal, at the center of ischemic lesion and often resulted in cell necrosis, the presence of neurons with acidic cytoplasm (ischemic neurons), cellular debris or "neural ghosts". All these neural changes are due to lack of oxygen resulting from blockage of arterial blood flow through a thrombus or embolus. Local hypoxia triggers a cascade of biochemical events that ultimately result in the death of neurons and glial cells in the affected area. Decreased blood flow to a region of the brain causes severe stress and premature cell death (necrosis). Neuronal death is the underlying manifestation of stroke. Cell necrosis is followed by destruction of the plasma membrane, swelling of intracellular organelles and leakage of cellular contents into the extracellular space [23], lesions that trigger other pathophysiological mechanisms. In acute ischemic stroke, neuronal damage occurs in a very short time after the ischemic episode [24]. Neurological dysfunction occurs within seconds to minutes of vessel occlusion; the progression of ischemic damage and cell death continues in stages for minutes, hours and even days, depending on the vulnerability of the specific brain region, its cellular constituents, and the extent of residual perfusion [25].

According to some studies, there are three major mechanisms underlying neuronal damage caused by ischemic stroke and brain infarction: loss of neurons through cell necrosis and apoptosis induced by ischemia and infarcts is

one of the most direct causes of neuronal damage; vascular obstruction caused by ischemia excessively produces ROS that exacerbate oxidative stress and exacerbate neuronal damage, leading to severe functional deficits; ischemia-induced inflammation is an additional factor leading to further neuronal damage after strokes [26–28].

In our study, we captured various microscopic aspects of neuronal death, without being able to correlate pathological aspects with local or blood biochemical changes. Some studies showed that ischemic neuronal damage causes a significant release of glutamate and a massive flow of Ca^{2+} ions into cells, causing neuronal death through excitotoxicity mechanisms [29]. However, we should mention that the neurons and astrocytes themselves in the ischemic area, immediately after the onset of stroke, produce ROS, which deplete glutathione, one of the essential antioxidant agents that prevent deoxyribonucleic acid (DNA) damage mediated by ROS [30]. Moreover, damaged neurons and glial cells cause local inflammation through biochemical products resulting from their degradation. Under these conditions, oxidative stress and the inflammatory process contribute to the rupture of the BBB, allowing activated immune cells in the blood, such as neutrophils and T-cells, to more easily reach the brain parenchyma and accumulate in tissue affected by ischemia [31].

BBB is a very important structure in maintaining the homeostasis of brain parenchyma, its damage seriously affecting the life and functions of neurons. As we showed, in the first stage, a change of the BBB permeability occurs in the ischemic area, leading to cytotoxic edema. In the acute phase of stroke, neuroinflammatory responses exacerbate BBB lesions, resulting in greater permeability, which causes in the ischemic focus lesions of neurons and glial cells to accentuate and accumulate a large amount of inflammatory cells [32].

Following BBB damage and accumulation of activated immune cells in the blood, the microglia in the brain also become activated after the onset of the ischemic process, due to the increase in extracellular adenosine triphosphate (ATP) resulting from the depolarization of neurons and glial cells and the subsequent release of biochemical compounds, resulting from the disintegration of neurons and glial cells [31, 33]. Activation of microglia has beneficial effects because proinflammatory agents, such as cytokines, promote the production of growth factors, such as neurotrophic factors derived from the brain and through phagocytosis, contribute to the removal of dead tissue and cellular debris released after ischemia [34].

In our study, within the inflammatory reaction, we highlighted several types of cells belonging to the immune system (neutrophils, B- and T-lymphocytes, plasma cells, macrophages) in the ischemic focus and in the ischemic penumbra area. Of these, the most numerous were macrophages and phagocytizing cellular and tissue debris. We believe that most of these cells come from blood monocytes, which are activated and attracted into the ischemic focus by a series of cytokines, secreted by the very neurons and glial cells affected by hypoxia.

If at the moment of ischemia, the synthesis and cytokine release is a beneficial process, subsequently the release of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), ROS and nitric oxide (NO) after activation of microglia is harmful [34]. This process leads to an increase in neuronal cell death, causing a larger area of cerebral infarction. In other words, excessive neuroinflammation can promote additional damage that causes neuronal cell death [35, 36].

Conclusions

Of the 23 cases examined, two (8.70%) cases were diagnosed as intracerebral hemorrhages, and 21 (91.30%) cases were ischemic strokes. The microscopic aspects of cerebral parenchyma in stroke deceased persons were very different from one case to another, probably depending on the time elapsed since stroke onset and death, and on the other hand depending on the patient age and pre-existing comorbidities. The nerve cells showed varying damage, from ischemic neurons to neuronal necrosis, depending on the distance from the ischemic focus. In old foci, the long lack of oxygen caused the death not only of neurons, but also of glial cells. The most common HP lesion of intracerebral arterioles was arteriosclerosis, in which the arterioles had a diminished lumen, thickened wall by deposition of PAS-positive hyaline material, but also by deposition of concentric collagen fibers. The intraparenchymal blood capillaries in the ischemic area showed discontinuities of the endothelium, lumen collision and sometimes massive perivascular edema. Inflammatory cells identified around ischemia and ischemic penumbra were represented by neutrophils, lymphocytes, plasma cells and macrophages, making up a complex and heterogeneous cellular mixture.

Conflict of interests

The authors declare that they have no conflict of interests.

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