VEGF expression in human brain tissue after acute ischemic stroke

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Abstract
Ischemic stroke is the third most common cause of death in humans, requiring further studies to elucidate its pathophysiological background. One potential mechanism to increase oxygen delivery to the affected tissue is induction of angiogenesis. The most potent proangiogenic factor is VEGF. For this reason, our study investigated immunohistochemically VEGF reactivity in different cellular brain compartments from 15 ischemic stroke patients, as well as from 2 age control cases. By enzymatic immunohistochemistry, we investigate VEGF expression in different brain cell compartments and then we quantified its signal intensity by assessing integrated optical densities (IOD). To establish the exact cellular brain topography of VEGF immunoreactivity we performed double fluorescent immunohistochemistry series (VEGF/NeuN, GFAP, CD68, CD105). In control samples, VEGF reactivity was observed especially in neurons from the Brodmann cortical layers IV to VI and in protoplasmic astrocytes from the deeper layers of gray matter and in endothelial cells from normal blood vessels because of systemic hypoxia generated after death. In acute ischemic stroke samples, this reactivity was noticed in all brain cellular compartments but with different intensities. The most reactive compartment was the neurons, the intensity of VEGF reaction decreasing with the lesion age from the core infarct toward intact adjacent brain cortex. With a lower intensity, VEGF reaction was noticed in astrocytes compartments, especially in gemistocytic astrocytes adjacent to the liquefaction zone. We also noticed a weak reaction in activated non-phagocytic microglia from the periphery of liquefaction zones, and high VEGF–CD105 colocalization values at the level of microvessels that surround the infarcted brain area. In conclusion, this reactivity could suggest that VEGF might exhibit neuronal and glial protective effects and also a neoangiogenic property in acute ischemic stroke, facts that may have significant therapeutically impact on these patients.

Keywords: acute ischemic stroke, VEGF, NeuN, GFAP, CD68, CD105.

Introduction
Being the third most common cause of death, new studies are still necessary to elucidate its pathophysiology and to develop novel therapeutical strategies for ischemic acute stroke [1]. Physiopathologically, an ischemic stroke represents the death of an area of brain tissue resulting from an inadequate supply of blood and oxygen due to the occlusion of a cerebral blood vessel. To compensate for tissue hypoxia, the organism responds by increasing the oxygen delivery to the affected tissue via induction of angiogenesis. This fact has been demonstrated to occur in brain tissues of patients surviving after an acute ischemic stroke [2].

The most potent in vivo promoter of angiogenesis is VEGF [3]. VEGF is a homodimeric glycoprotein of 36–46 kDa with four isoforms (VEGF121, VEGF165, VEGF189, and VEGF206) [4], from which VEGF165 is the most common and active form [5]. The main angiogenic functions of VEGF include promoting endothelial cell survival [6, 7], inducing their proliferation [8, 9], and enhancing their migration and invasion [10, 11].

In addition to its crucial role in angiogenesis, VEGF may be involved in several other processes in the central nervous system such as: (i) neural cell development, including migration, differentiation, synaptogenesis, and myelination [12–16]; (ii) has neurotrophic and neuroprotective effects [17–22]; (iii) stimulates adult neurogenesis [23–27]; and (iv) enhances hippocampus-dependent memory formation [23, 26].

The majority of studies assessing the VEGF expression in brain tissue following an acute ischemic stroke were performed on animal models [28–35]. In humans, such studies analyze especially the VEGF serum levels [36, 37], and to date, only one such study assessed the VEGF expression in peri-infarct region from the autopsy specimens [38].

The objective of this study was to investigate the pattern of VEGF expression in the infarct core, peri-infarct, and normal contralateral tissue from 15 patients who died following acute ischemic strokes.

Materials and Methods
Formalin-fixed, paraffin-embedded archived brain tissue blocks were selected from both lesional and perilesional areas of 15 ischemic stroke patients, as well as tissue from 2 age control cases (Table 1).
A written informed consent was obtained for each patient from the relatives or the caretakers, before any material could be harvested. This is a retrospective study based on the material archived in the brain bank and could be harvested. This is a retrospective study based on the material archived in the brain bank

Enzymatic immunohistochemistry

Five-µm-thick sections were deparaffinized in xylene, rehydrated through graded alcohol series, and then processed for heat-induced epitope retrieval (HIER) using 0.1 M citrate pH 6 for 20 minutes, followed by cooling at room temperature for 20 minutes. After endogenous peroxidase block and skim milk incubation, Five-µm-thick sections were deparaffinized in xylene, rehydrated through graded alcohol series, and then processed for heat-induced epitope retrieval (HIER) using 0.1 M citrate pH 6 for 20 minutes, followed by cooling at room temperature for 20 minutes. After endogenous peroxidase block and skim milk incubation, for one hour with a goat anti-mouse biotinylated secondary (1:300, Dako), and visualized with an HRP Streptavidin (LSAB®2 System-HRP, Dako, Redox, Romania). The signal was detected with 3,3’-diaminobenzidine chromogen substrate solution (Vector Laboratories, Cheminkpress, Craiova, Romania) and counterstaining was done using Hematoxylin. Negative controls were obtained by omitting the primary antibody.

Fluorescent immunohistochemistry

Double immunohistochemistry was performed for VEGF in combination with anti-NeuN, GFAP, CD68 and CD105 antibodies (Table 2).

For VEGF-NeuN after endogenous peroxidase block and skim milk incubation, the anti-VEGF (mouse) antibody was added on the slides in a 1:200 concentration for 18 hours at 4°C. Next day, the signal was amplified using a one hour incubation with an anti-mouse peroxidase labeled secondary (1:100, Dako), and detected using an Alexa 488 labeled tyramide (Perkin Elmer). For the VEGF–CD68/CNP-ase double labeling, after detecting VEGF and blocking the biotin situses as described above, the CD68/CNP-ase mouse primary antibody was detected with an anti-mouse biotinylated

<table>
<thead>
<tr>
<th>Name</th>
<th>Clone</th>
<th>Epitope</th>
<th>Dilution</th>
<th>Retrieval</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD68</td>
<td>Mouse, IgG1k</td>
<td>Macrophages, monocytes</td>
<td>1:100</td>
<td>0.1 M citrate, pH 6</td>
<td>Dako</td>
</tr>
<tr>
<td>CD105</td>
<td>Rabbit, polyclonal</td>
<td>Proliferative endothelial cells</td>
<td>1:50</td>
<td>0.1 M citrate, pH 6</td>
<td>Thermo Scientific</td>
</tr>
<tr>
<td>GFAP</td>
<td>Rabbit, polyclonal</td>
<td>Astrocyte cytoskeleton</td>
<td>1:20,000</td>
<td>0.1 M citrate, pH 6</td>
<td>Dako</td>
</tr>
<tr>
<td>NeuN</td>
<td>Mouse, IgG1</td>
<td>Vertebrate neuron-specific nuclear protein</td>
<td>1:1000</td>
<td>0.1 M citrate, pH 6</td>
<td>Millipore</td>
</tr>
<tr>
<td>VEGF</td>
<td>Mouse, IgG2</td>
<td>Vascular endothelial growth factor</td>
<td>1:200 (chromogenic)</td>
<td>1:30 (fluorescence)</td>
<td>Santa Cruz</td>
</tr>
<tr>
<td>VEGF</td>
<td>Rabbit, polyclonal</td>
<td>Vascular endothelial growth factor</td>
<td>1:50</td>
<td>0.1 M citrate, pH 6</td>
<td>Santa Cruz</td>
</tr>
<tr>
<td>CNP-ase</td>
<td>Mouse, IgG1</td>
<td>Cytoplasm and extensions of oligodendrocytes and Schwann cells</td>
<td>1:20</td>
<td>0.1 M citrate, pH 6</td>
<td>Thermo Scientific</td>
</tr>
</tbody>
</table>

Table 1 – Neuropathologic data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age [years]</th>
<th>Sex</th>
<th>Survival [hours / days]</th>
<th>Lesional topography</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>78</td>
<td>F</td>
<td>6 days</td>
<td>Carrier of an 8-year-old cortical and subcortical ischemic event (left frontal lobe), together with a recent lesion (hippocampus and the occipital lobe).</td>
</tr>
<tr>
<td>2.</td>
<td>51</td>
<td>F</td>
<td>36 hours</td>
<td>Recent cortical and subcortical event of the left frontal and temporal lobes (involving corpus callosum and striatum).</td>
</tr>
<tr>
<td>3.</td>
<td>68</td>
<td>M</td>
<td>12 hours</td>
<td>Old (cystic) ischemic event in the subcortical white matter of the superior temporal lobe, left hemisphere. Recent ischemic event in the left parietal lobe.</td>
</tr>
<tr>
<td>4.</td>
<td>67</td>
<td>M</td>
<td>40 hours</td>
<td>Ischemic event involving both occipital lobes, and the left hippocampus and temporal isocortex.</td>
</tr>
<tr>
<td>5.</td>
<td>51</td>
<td>F</td>
<td>36 hours</td>
<td>Early ischemic event in the left frontal lobe, callosal body and basal nuclei of the left hemisphere.</td>
</tr>
<tr>
<td>6.</td>
<td>NA</td>
<td>M</td>
<td>20 hours</td>
<td>Old ischemic lesion in the right temporal lobe and basal nuclei.</td>
</tr>
<tr>
<td>7.</td>
<td>66</td>
<td>M</td>
<td>30 hours</td>
<td>Large right tempo-parietal ischemic stroke.</td>
</tr>
<tr>
<td>8.</td>
<td>77</td>
<td>F</td>
<td>7 days</td>
<td>A 4-year-old cystic cavitation in the left superior parietal lobe, with a recent softening involving adjacent parietal tissue.</td>
</tr>
<tr>
<td>9.</td>
<td>68</td>
<td>F</td>
<td>2 days</td>
<td>Cortical ischemic event of the frontal-temporal lobes, left hemisphere.</td>
</tr>
<tr>
<td>10.</td>
<td>82</td>
<td>M</td>
<td>12 hours</td>
<td>Small parietal cortical and subcortical lesion of the right hemisphere.</td>
</tr>
<tr>
<td>11.</td>
<td>77</td>
<td>F</td>
<td>5 days</td>
<td>Cortical ischemic event with hemorrhagic transformation (all lobes of the right hemisphere). The right dentate cerebellar nucleus is also involved.</td>
</tr>
<tr>
<td>12.</td>
<td>64</td>
<td>F</td>
<td>28 hours</td>
<td>Left striatal and thalamic infarction.</td>
</tr>
<tr>
<td>13.</td>
<td>74</td>
<td>M</td>
<td>12 hours</td>
<td>Left striatal infarction.</td>
</tr>
<tr>
<td>14.</td>
<td>65</td>
<td>M</td>
<td>2 days</td>
<td>Right parietal softening.</td>
</tr>
<tr>
<td>15.</td>
<td>83</td>
<td>F</td>
<td>–</td>
<td>Right temporal ischemic event in the superior temporal lobe.</td>
</tr>
<tr>
<td>16.</td>
<td>68</td>
<td>M</td>
<td>–</td>
<td>Lung tumoral pathology.</td>
</tr>
<tr>
<td>17.</td>
<td>85</td>
<td>M</td>
<td>–</td>
<td>Bilateral tuberculosis bronchopneumonia.</td>
</tr>
</tbody>
</table>

Lesional topography

- Bilateral tuberculosis bronchopneumonia.

Table 2 – The antibodies utilized in this study

- Lung tumoral pathology.
antibody (1:200, Dako), and visualized with an Alexa 488 Streptavidin (1:200, Invitrogen). In case of VEGF–CD105 double labeling we proceeded in the same manner as previous reactions but we kept in mind that first primary antibody was produced in mouse serum and the second in rabbit serum. For the VEGF–GFAP double labeling, after detecting VEGF (mouse antibody) with the same type of species-specific protocol, the slides were incubated with GFAP (rabbit), and later on with a goat anti-rabbit Alexa 488 antibody (1:400, Invitrogen). In all cases, the slides were counterstained with DAPI (Invitrogen) and coverslipped with a fluorescence anti-fading mounting medium (Dako).

Microscopy and image analysis

Chromogenic immunohistochemistry slides were imaged with an Eclipse E200 microscope (Nikon; Apidrag, Romania) equipped with a 5 Mp CCD camera and the Nikon Nis Elements software.

First, we were interested to establish VEGF immunoreactivity and topography in normal and infarcted brain samples, and then we compared its presence in different brain compartments (neurons, astrocytes, microglia/macrophages and endothelial cells–brain microvessels). VEGF expression intensity was quantified as the sum of the integrated optical densities (IOD) of the threshold pixels for all signal measured in each image. Average values were calculated for each pathological type considered. All image analysis was done using Image ProPlus 7 AMS software (Media Cybernetics Inc., Buckinghamshire, UK).

Fluorescently labeled sections were next imaged with a Nikon Eclipse 90i microscope equipped with a QImaging Rolera cooled CCD camera and with narrow-band fluorescent filters centered for Alexa 594, Alexa 488 and DAPI excitation and emission wavelengths. Fluorescent images were captured and archived using the Image ProPlus 7 AMS software.

VEGF colocalization degree with different cellular markers was reported as the Manders’ M1 and M2 coefficients, which show the fraction of the intensity in each channel that coincide with some intensity in the other channel. Thus, M1 coefficients represent the percentage of pixels in the red channel that intersect with some signal in the green channel, and M2 other way around.

All data were collected and plotted in Excel (Microsoft Corp.) sheets and statistics was performed in SPSS 11 (SPSS Inc.).

Results

Histopathological data

All 15 investigated cases were cerebral ischemic strokes involving especially the vascular territory of left middle cerebral artery (n=7), right middle cerebral artery (n=5), left internal carotid artery (n=1), right internal carotid artery (n=1), and left anterior cerebral artery (n=1). In two cases, the acute ischemic stroke developed adjacent to old infarcts (four years and respective eight years ago). The evolution time of these strokes varied from 12 hours to seven days (determined as the time passed between the initial clinical presentation and the date of death).

According to the classification of Mena H et al. [39] for the human histopathological cerebral infarctions, the majority of our cases (11 cases) where classified as: (1) stroke under the organization phase (or acute inflammation, at the age of 3–7 days), characterized by coagulative necrosis, acute inflammation, neuronal injury, red neurons and macrophages; and (2) as acute neuronal injury (four cases), at the age of 12 hours–2 days, characterized by the presence of neuronal changes, spongiosis of the neuropil and absence of neuronal ferrugination, chronic inflammation, macrophages, neo-vascularization and cavitation. Two of the recent acute strokes developed near to old cerebral infarcts (or the phase of resorption), with ages of four and respective eight years, characterized by the absence of an inflammatory reaction, with the presence of cystic cavitations, astrogliosis and mononuclear cells infiltrates.

Immunohistochemical data

VEGF expression in neuronal compartment

Both in normal brain samples and in normal contra-lateral specimens, VEGF expression was seen in the cytoplasm of most neurons. The intensity of the reaction was higher in the cortical layers IV to VI, especially in stellate and pyramidal neurons (Betz cells showing the highest reactivity) (Figure 1A, A and B). Also, in neurons, VEGF reactivity was present both in the body but also neurites, with a higher intensity in the neuronal body (Figure 1C). In the gray matter, VEGF was also present with high intensity in neurites of both fusiform and pyramidal neurons. A lower VEGF reactivity was observed in the white matter (Figure 1D).

In the acute neuronal injury phase of stroke, the neurons from the ischemic zones showed the highest VEGF reactivity. This reactivity decreased with advancing age of the lesion, when the neurons from the peri-lesional area become more reactive for this marker (Figure 1, E and F). We observed a progressive increase of VEGF IOD from normal tissue to contralateral samples and finally to ischemic lesions (8.2×10³ ± 7.5×10⁵, 463.6×10⁴ ± 275.4×10⁴, 127.4×10⁵ ± 98.5×10³, F(2, 85)=10.482, p<0.001) (Figure 2). Contrary, the NeuN–VEGF colocalization decreased slowly from control samples to ischemic lesion from 0.039 ± 0.017 and 0.043 ± 0.021 to 0.013 ± 0.012 and 0.026 ± 0.025 for M1 and respective M2 indexes) (Figure 3), and increased abruptly in the distant perilesional areas (0.079±0.028 and 0.101±0.045), per pair Student t-tests, p<0.05 (Figure 1, G–I and J–L). Also, a high VEGF reactivity was noticed to adjacent neurites (Figure 4A), and this was proved by VEGF–CNP-ase signal colocalization (Figure 4B).

VEGF expression in astrocyte compartment

In non-lesional samples, VEGF reactivity was observed in astrocytes but with less intensity than in neurons. The reaction was more obvious in the protoplasmic astrocytes from the deeper layers of gray matter (Figure 4C).

In lesional samples, VEGF astrocyte reactivity was
most obvious first in gemistocytic astrocytes adjacent to the liquefaction zone (0.146 ± 0.045; 0.173 ± 0.035) (Figures 3 and 4, D–F) and latter in multiplying fibrous astrocyte with long slender processes from the edge of a so called glial “scar” (0.056 ± 0.027; 0.087 ± 0.022), per pair Student t-tests, p<0.001 (Figure 4, G–I). In old lesions, VEGF reaction was also observed at the level of the dense tangles of delicate astrocytic processes that forms the glial “scar” (Figure 4, J–L).

Figure 1 – VEGF reactivity in neuronal compartment: (A and B) Control brain tissue sample/contralateral lesional tissue, cytoplasmic VEGF expression in stellate and pyramidal neurons with NeuN nuclear positivity; (C) Control tissue, fusiform and pyramidal neurons with VEGF reactivity both in the bodies but also in the neurites; (D) Control sample, with a lower VEGF reactivity in the white matter; (E) Lesional sample, high VEGF expression in Betz cell; (F) Lesional sample, VEGF expression in neurons adjacent to infarct core; (G–I) Lesional sample, VEGF expression in neurons adjacent to infarct core, notice the low/absence of VEGF–NeuN signal colocalization (arrows); (J–L) Lesional sample, VEGF expression in neurons away from the glial “scar”, notice the high VEGF–NeuN signal colocalization. Scale bars represent 50 µm.

Figure 2 – VEGF expression assessment as IOD scores. There is a clear-cut decrease of VEGF IODs from the lesion areas, to contralateral hemispheres and control patients’ tissue. * represents significance on ANOVA testing (p<0.001).
**Figure 3** – VEGF colocalization with cell markers. Stars represent significance on Student t-test (*, p<0.05; **, p<0.01; ***, p<0.001).

**Figure 4** – (A and B) VEGF reactivity in neuronal compartment: (A) Lesional sample, VEGF expression in neurites within the gray matter adjacent to the glial “scar”; (B) VEGF–CNP-ase signal colocalization in neurites from the gray matter adjacent to glial “scar”; VEGF reactivity in astrocyte compartment: (C) Normal sample, VEGF reactivity in an astrocyte from gray matters; (D–F) Lesional sample, VEGF reactivity in gemistocytic astrocytes adjacent to the liquefaction zone; (G–I) Lesional sample, VEGF expression in and around fibrous astrocytes at the edge of glial “scar”; (J–L) Lesional sample, VEGF expression in the dense tangle of delicate astrocytic processes that form the glial “scar”. Scale bars represent 50 µm.
VEGF expression in microglial cell/macrophage compartment

In all controls we did not observed any VEGF reactivity in the resting microglial cell compartment (Figure 5, A and B). The most intense microglial VEGF reactivity was seen at the periphery of the liquefaction zones (Figure 5, C). In these areas, starting from 24 hours after onset of the infarct takes place the microglial proliferation and activation. In these activated non-phagocytic microglia, VEGF expression was present both in the body and its extensions unveiling their hyper-ramified morphology (Figure 3) (0.049 ± 0.033; 0.036 ± 0.028) (Figure 5, D–F). A low expression of VEGF was next observed in the cytoplasm of some activated phagocytic microglia with foamy cell appearance (Figure 3) (0.023 ± 0.016; 0.036 ± 0.024) (Figure 5, G–I). Some of these cells could represent vacuolated macrophage coming from circulating monocytes, fact proved by an increased blood influx of monocytes in the infarcted area (Figure 5, J and K). In resting microglia adjacent to the infarct lesions, we did not observed any VEGF reactivity (Figure 5, L).

Figure 5 – VEGF reactivity in microglial cell/macrophage compartment: (A and B) Control sample, VEGF expression in microglial cell from the gray/white matter; (C) Lesional sample, VEGF reactivity within microglia from the periphery of the liquefaction zones; (D–F) Lesional sample, VEGF reactivity in activated non-phagocytic microglia from the glial “scar”; (G–I) Lesional sample, VEGF expression in activated phagocytic microglia with foamy cell appearance from the edge of the glial “scar”; (J and K) Lesional sample, VEGF expression in foamy macrophage from the infarcted area; (L) Lesional sample, absence of VEGF expression in activated but not phagocytic microglia adjacent to the infarct lesions. Scale bars represent 50 µm.

VEGF expression in endothelial cells – microvessels compartment

A weak VEGF reaction was observed even in endothelial cells from non-lesional samples (Figure 3) (0.026 ± 0.029; 0.030 ± 0.007) (Figure 6, A–C). The most intense reaction was seen in the endothelial cells of microvessels that surrounded the infarcted brain areas (Figure 3) (0.065 ± 0.027; 0.058 ± 0.019) (per pair Student t-tests, p<0.05) (Figure 6, D–F). VEGF endothelial reactivity was noticed even in the resting
brain capillary caught inside the necrotic areas (Figure 6, G–I). Also, endothelial cells from vessels located away from the necrotic areas showed VEGF reactivity (Figure 6, J–L), including those of the meninges. We did not observe any VEGF reactivity in the glial limits of the Virchow–Robin spaces that surrounds perforating arteries and veins in the parenchyma of the brain.

Figure 6 – VEGF reactivity in endothelial cells – microvascular compartment: (A–C) Control sample, VEGF expression in endothelial cell from the white matter; (D–F) Lesional sample, VEGF reactivity in endothelial cells of microvessels that surround the infarcted brain area; (G–I) Lesional sample, VEGF reactivity in resting brain capillary from inside the necrotic area; (J–L) Lesional sample, VEGF expression in endothelial cells of the vessels located away from the necrotic area. Scale bars represent 50 µm.

Discussion

Immunohistochemical studies regarding VEGF expression in the human brain tissue are very few in the literature, our study being the first to characterize by immunofluorescence the spatial distribution of VEGF immunoreactivity in the penumbra compared with infarcted brain and contralateral hemisphere.

Sentilhes L et al. [14], studying the spatial and temporal sequences of expression of VEGF in the human forebrain and cerebellum from the beginning of the second trimester of gestation to the 13th postnatal month, proved that immunoreactivity was present in progenitor cells (radial glial cells and external granular layer), in neurons that have migrated to their final positions, and in astrocytes and mature oligodendrocytes. These results indicate that cortical neurons are exposed to VEGF during the most important developmental stages (migration, maturation, axonal and dendritic sprouting) proving its neurotrophic properties. VEGF expression in astrocytic cells was observed from 23 weeks of gestation until birth, proving that neurotrophic effects of VEGF/VEGFR-2 are not restricted to neurons but are also involving the generation and maturation of telencephalic and cerebellar astrocytes. The authors also observed VEGF immunoreactivity in mature oligodendrocytes from 24 weeks of gestation, in the cerebellar white matter and from the moment of birth in the
telencephalon. Also, a high expression of VEGF and VEGFR-2 was observed in most fibers of the internal capsule, in the telencephalic commissures, and in cerebellar white matter fibers; this transient expression being thought as preceding and perhaps facilitating initiation of fiber connections and synaptogenesis. VEGF was weakly and focally expressed after 34 weeks of gestation most likely by decreased angiogenesis stimulation, when the blood-brain barrier becomes functional.

One of the two previous studies on the VEGF reactivity of cerebral infarctions showed an upregulation of VEGF levels in the penumbra, with neurons, endothelial cells and astrocytes expressing the marker [38]. Both VEGF165 and VEGF189 mRNA transcripts were upregulated in all brain cellular compartments in the penumbra compared with the infarcted tissue and the normal looking contralateral hemisphere. In the other article, VEGF did not appear to be overexpressed in the ischemic cases, neuronal expression being more intense in the control series, while its microvascular expression was equal between stroke and control cases [40]. In addition, the authors observed a parallel increase in VEGF expression with chymase in neurons; and the microvascular VEGF expression with Fas and also with the post-admission patient survival times.

Our study showed VEGF reactivity in both lesional and normal brain samples. The VEGF reactivity on normal brain samples was noticed especially in neurons and normal brain samples. The VEGF reactivity on macrophage from the liquefaction zones, VEGF reactivity with foamy cell appearance, as well in vacuolated liquefaction zones. In activated phagocytic microglia also in the glial “scar”. In the microglial cell/macrophage compartment, VEGF reactivity was obvious in activated astrocytes adjacent to the liquefaction zone and the microvascular VEGF expression with Fas and also with the post-admission patient survival times.

In the study of Slevin M et al. [41], VEGF protein expression assessments by western-blotting were not influenced by patient age, sex, time of survival after stroke, stroke sub-types, associated clinical pathologies or any measured blood parameters. Also, the authors suggested that chronic activation of MAP kinase and tyrosine phosphorylation pathways, together with up-regulation of VEGF protein, may help explain the mechanism of neuronal protection in the penumbra tissue undergoing angiogenesis, and could be of relevance when considering clinical management of patients to maximize recovery after an acute ischemic stroke.

Considering its antagonistic roles, such as stimulating of angiogenesis and neuroprotective effect on one hand, and the potent vascular permeability effect with a role in brain edema formation on the other hand, the treatment of stroke with VEGF is controversial. Thus, some authors showed that early post-ischemic administration of VEGF (one hour after the insult) led to significantly increased blood brain barrier leakage as well as enlarged ischemic lesions, while late therapy (48 hours after stroke) resulted in augmented angiogenesis in the ischemic penumbra and significantly improved neurological recovery [42, 43]. In addition, treatment with a VEGF neutralizing antibody proves to be able to reduce brain edema formation in animal models of experimental cerebral ischemia [44, 45].

Conclusions

Investigating VEGF reactivity on both normal brain tissue and acute ischemic cerebral infarcts we conclude that the high VEGF reactivity in the peri-lesional brain tissue at the level of all cellular compartments could have both neuronal and glial protective effects and neoangiogenic properties, limiting thus the effects of
stroke hypoxia. These facts could be of relevance when considering clinical management of these patients, treatment with a VEGF neutralizing antibody being able to significantly improve their neurological recovery.

Acknowledgments
This paper is partially supported by the Sectoral Operational Programme Human Resources Development, financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/89/1.5/S/64109 to Dr. D. Pirici.

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Received: August 30th, 2011
Accepted: December 11th, 2011