Study of cerebral vascular structures in hypertensive intracerebral haemorrhage

I. E. PLEŞEA[1], A. CAMENIŢĂ[2], C. C. GEORGESCU[3],
S. D. ENACHE[4], B. ZAHARIA[1],
CLAUDIA VALENTINA GEORGESCU[4], MIHAELA ŢENOVICI[5]

[1]*Department of Pathology, University of Medicine and Pharmacy of Craiova*
[2]*Department of Neurosurgery, University of Medicine and Pharmacy of Craiova*
[3]*Department of Pharmacology, University of Medicine and Pharmacy of Craiova*
[4]*Department of Pathology, Emergency County Hospital, Craiova*
[5]*Department of Pathology, Railroad University Hospital, Craiova*

Abstract

Aims. The study was performed in order to assess the alterations of extraparenchymal and intraparenchymal vascular structures in 82 hypertensive patients suspected of primary intraparenchymal hematoma, which died and were autopsied in order to confirm the diagnosis. *Material and methods*. The studied material consisted of nervous tissue situated near and distant from the haemorrhagic lesion. The specimens of nervous tissue were processed by the classical histological technique and stained with the usual stainings and with immunohistochemical stains for basement membranes and endothelial cells. *Results*. Extraparenchymal arteries showed classic lesions of atherosclerosis. Atheromatous lesions were of all types, even the extension towards the media being encountered a complication with thrombosis. At the level of the intraparenchymal blood vessels, the spectrum of the lesions due to arterial hypertension included all steps of vascular wall degeneration, from hypertrophy of smooth muscle layer to complete hyalinization of arterial wall, but with a focal irregular distribution, not related with the proximity of haemorrhagic focus. High arterial blood pressure also influenced the capillary walls which showed focal or circumferential thickening due to the densification of the type IV collagen material from the basement membrane structure. The CD34 immunostaining showed that endothelial cells kept their structural integrity. *Conclusions*. The sequence of degenerative lesions of the cerebral vascular wall culminates with the hyalinization of excessive fibrillar material form arteriolar wall or from basement membranes. Hyalin material is weakening the wall resistance to the stress determined by the high values of blood pressure in hypertension, and, correlated with a minimal resistance of the surrounding cerebral parenchyma, can explain why the cerebral parenchyma is the only tissue in which blood pressure variations can determinate vascular rupture and cerebral haemorrhage. The more adequate term for describing the vascular wall changes seems to be sclerosis (arteriolar and even capillary) with hyalinosis.

Keywords: *hypertensive intracerebral haemorrhage, arteriolosclerosis, hyalinosis, immunohistochemistry.*

⊟ Introduction

Intracerebral haemorrhage (ICH) is thought to be one of the most frequent diseases of nervous system, representing 3.5–5% of all organic diseases of this system and 5–15% of the total number of cerebral vascular strokes. Its incidence in patients younger than 40 is 0.3/100.000 [1, 2]. IHC is the result of a haemorrhage of arterial origin directly within cerebral tissue, which involves mainly the cerebral parenchyma but with possible extention to ventricular spaces and occasionally to subarachnoid space [3, 4].

IHC etiology is diverse, complex and dynamic [5–7]. Thus, IHC can be the result of many pathological conditions and usually accompanies other nervous system lesions [8–13].

There are two types of cerebral haemorrhages: traumatic and non-traumatic. The later are also known as "spontaneous" haemorrhages even almost all of them are caused by a lesion of arterial wall [14].

Nontraumatic ICH is divided into two main groups, depending on etiopathogenesis [4]:

▪ Primary IHC representing 78–88% of all IHC. It is the result of spontaneous rupture of small vessels altered either by chronic hypertension, in 80%, or by amyloid deposits [15–18].

▪ Secondary IHC representing 12–22% of IHC patients. It is associated with vascular abnormalities (i.e, arteriovenous malformations and aneurisms), tumors and coagulopathies [4].

The study of the main cause of primary intracerebral haemorrhage is difficult because the vascular rupture hides or at least modifies the subsistent vascular pathology [19].

Taking into account the above mentioned facts, we propose to evaluate, in hypertensive patients who died from intracerebral haemorrhage, the morphological changes of the vascular wall at the level of the arterial structures of all sizes and of the capillary situated both in the proximity of the haemorrhagic lesion and distant from it.

⊟ Material and methods

This study was performed on 82 hospitalized hypertensive patients suspected of primary intraparenchymal hematoma, which died and were autopsied in order to confirm the diagnosis.

The studied material consisted of nervous tissue situated near and distant from the haemorrhagic lesion and it was obtained during the autopsy carried out at the same hospital.

The fragments were obtained using the following algorithm: a fragment which included the periphery of the haemorrhagic lesion, a fragment situated at a distance of 2–3 cm from the periphery of the haemorrhagic lesion and another fragment situated in the controlateral hemisphere.

The specimens of nervous tissue were processed by the classical histological technique (fixed in 10% neutral buffered formalin and embedded in paraffin) and stained with the usual staining Haematoxylin & Eosin and with specific stains for different components of the mesenchymal structures (van Gieson and Goldner stains) in the Pathology Laboratories of the Emergency County Hospital and of the Railroad University Hospital in Craiova, as well as with immunohistochemical stains using LSAB/HRP technique and antibodies against vascular basement membranes and endothelial cells (i.e., Colagen IV and CD 34) in the Histopathological and Immunohistochemical Laboratory of the Craiova University of Medicine and Pharmacy Pathology Department.

Results and discussions

Changes of extraparenchymal large arteries wall

Extraparenchymal large arteries showed, before entering the cerebral parenchyma, only classical lesions of atherosclerosis. The atheromatous lesions were found in most of the patients. The severity and extention of atherosclerotic lesions presented wide individual and inter-individual variations and were not correlated with the patients' age.

The "key" morphologic changes in atherosclerosis are: focal thickening of intima and lipid accumulation, producing the characteristic atheromatous plaques.

On gross specimens, the atherosclerotic lesions usually involved the arterial wall only partially along its circumference (eccentric lesions) (Figures 1a and 2a), had a non-uniform distribution and different sizes.

They had the aspect of whitish-yellow endoluminal outgrowths which sometimes were confluent, generating larger lesions. On cross section, the area situated towards the vascular lumen (fibrous cap), tended to be more firm and white. The deeper soft yellow area, found especially in the center of the larger plaques, contained yellowish viscous debris, called atheroma (Figure 1, a and b).

Microscopically, the atheromatous plaques contained all three main components: cells, connective tissue extracellular matrix and intracellular and extracellular lipid accumulations their amounts varying in different plaques, and thus, resulting in a wide spectrum of lesions.

We found usually enough, old lesions, with a superficial fibrous cap consisting of smooth muscle cells, relatively dense connective tissue and a few leukocytes. The plaques had a large amount of collagen fibres in the basal and central areas with only a small amount of atheromatous material below the superficial fibrous cap (Figures 2a and 3a).

There was a cellular area below and around the fibrous cap edges, consisting of a mixture of macrophages, smooth muscle cells and lymphocytes in extracellular matrix elements such as collagen, elastic fibres and proteoglycans.

We found also plaques where cellular and vascular components of the granulation tissue were present around the "anchoring zone" and the basal regions of the lesions. They were invading the plaque and organizing it (Figures 2b and 3b). In some of the more advanced lesions, the progressive fibrosis transformed the fatty atheroma into a fibrous scar.

Typical atheromatous plaques were common, with a necrotic core, placed deeply in the vascular wall, consisting of an amorphous mass and a relatively abundant quantity of lipid material (mainly represented by cholesterol crystals), cellular debris and fibrin. Characteristic components of this necrotic core were the swollen lipid-laden macrophages, with small nuclei frequently in an eccentric position and a reticular cytoplasm, known as foamy cells (Figures 2c and 3c).

In many cases, the inner elastica was intact (Figures 3b and c), the atheromatous lesion being still situated at the intimal level, but with an important extension towards the vascular lumen, which became significantly narrowed.

However, although atherosclerosis is initially restricted to the intima, in some cases, especially in large extraparenchimal vessels, the atheromatous lesion became severe and involved the media, producing the destruction of the inner elastica and the decrease of the middle layer thickness, these alterations being visible especially on Goldner stained specimens (Figures 2d and 3d).

The atrophy of the middle layer resulted in significant loosening and subsequent aneurismal spindle-shaped dilatations in certain extracerebral arterial segments.

Thrombosis, the most feared complication, usually occurs on fissured or ulcerated lesions. In some cases, we found in the lumen of large arteries thrombi which partially occluded it (Figure 4a and b).

Some of these thrombi were incorporated within the intimal plaque by organization.

Changes of penetrating arteries and intraparenchymal arterioles

The lesions spectrum modified in the next vascular level: the intraparenchymal arterial segments. From this level, no atheromatous lesion was observed found in any of the cases. We observed instead, in all the microscopically examined cases different stages of evolution of the vascular wall degenerative process determined by the hypertensive disease.

The first stage consisted in the hypertrophy, in different degrees, of the penetrating arteries wall (Figure 5, a and c).

One characteristic feature of this step of the vascular reactive process induced by high pressure values was the thickening and folding of the inner elastica, a phenomenon best seen on Goldner stained samples (Figure 5, b–d).
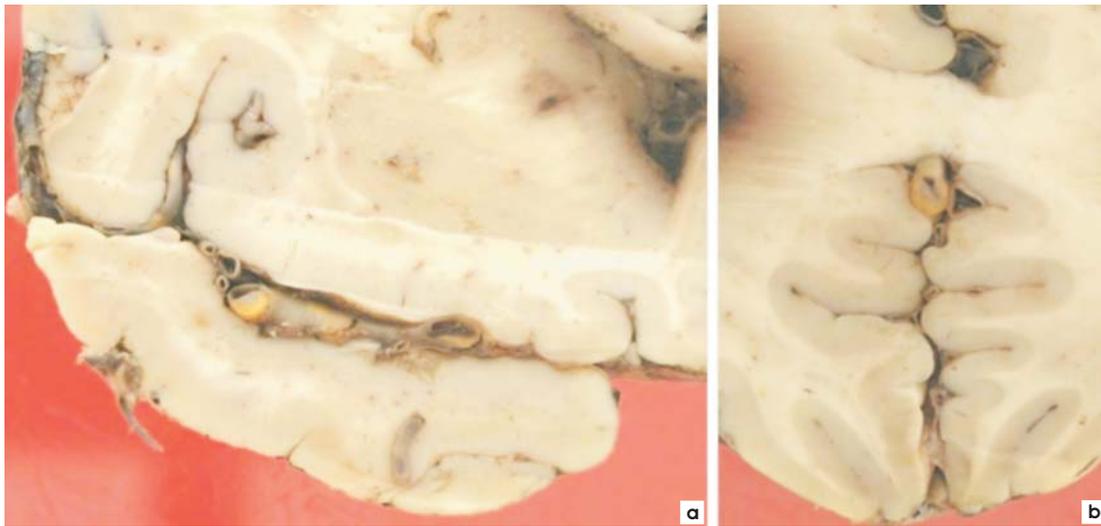
Figure 1 – *Atheromatous plaque in posterior cerebral artery (Pitres section on necroptic sample fixed in formalin)*
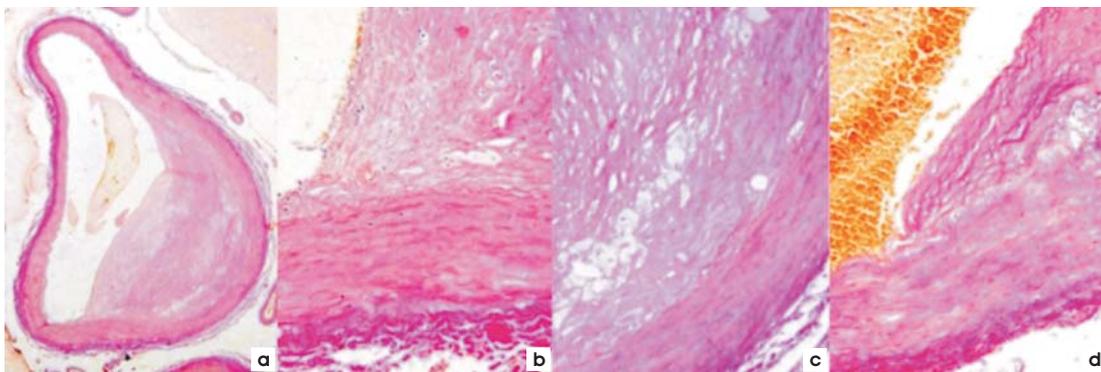

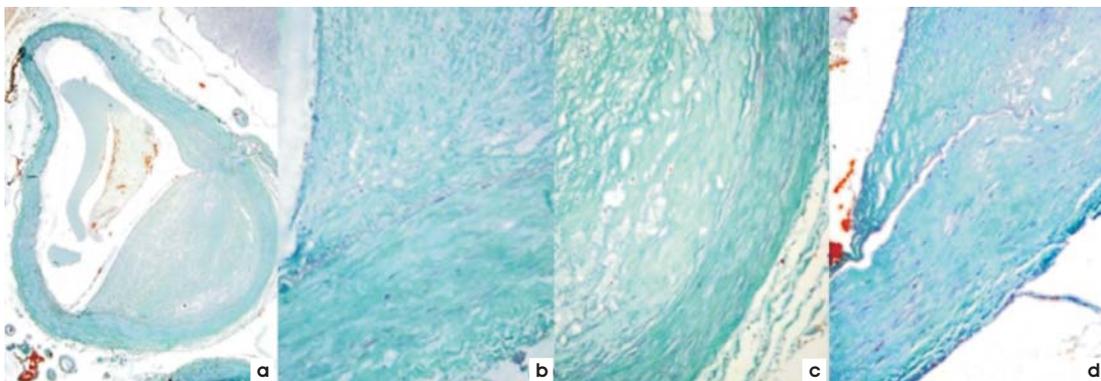**Figure 2** – *Atheromatous plaque (van Gieson stain, oc. ×10, ob. ×4; ob. ×20)*

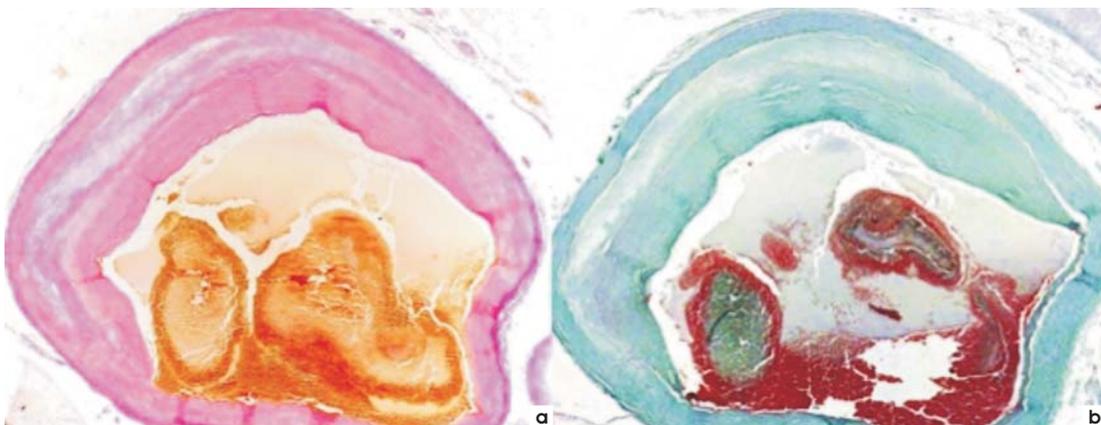**Figure 3** – *Atheromatous plaque (Goldner stain, oc. ×10, ob. ×4; ob. ×20)*

Figure 4 – *Atheromatous plaque. Recent red thrombus (van Gieson and Goldner stains, oc. ×10, ob. ×4)*
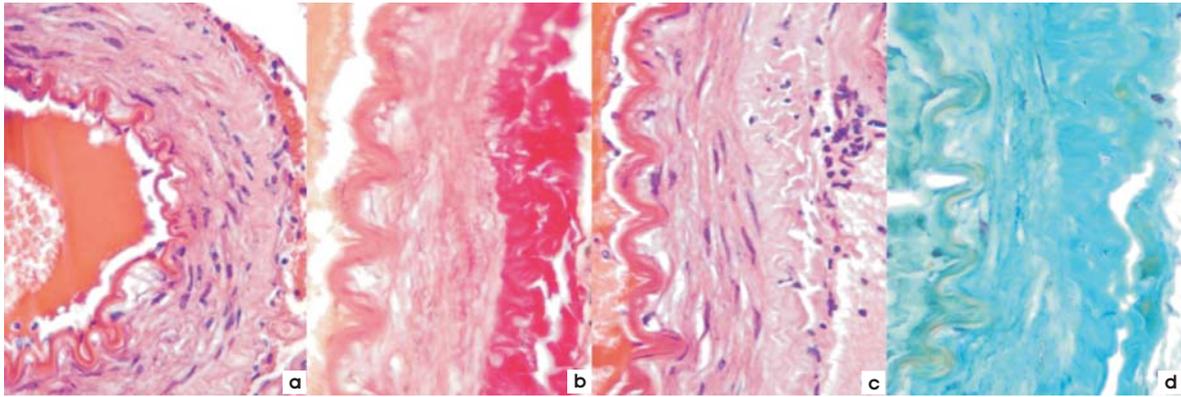
Figure 5 – *Intraparenchymal artery. Smooth muscle hypertrophy; thickening and folding of inner elastica:*
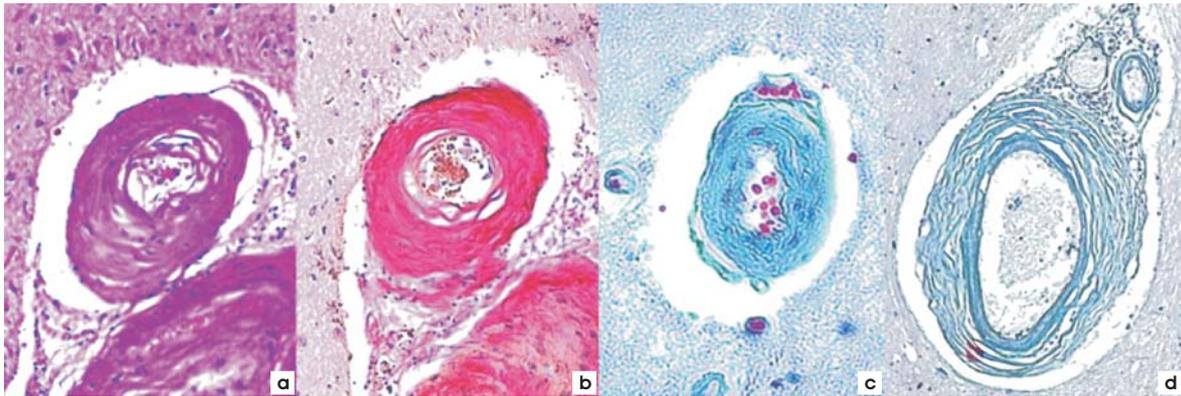*(a), (c) HE stain; (b) van Gieson stain; (d) Goldner stain (oc. ×10, ob. ×65)*

Figure 6 – *Intraparenchymal artery. Complete sclerosis of the vascular wall:*
(a) HE stain; (b) van Gieson stain; (c), (d) Goldner stain (oc. ×10, ob. ×65)
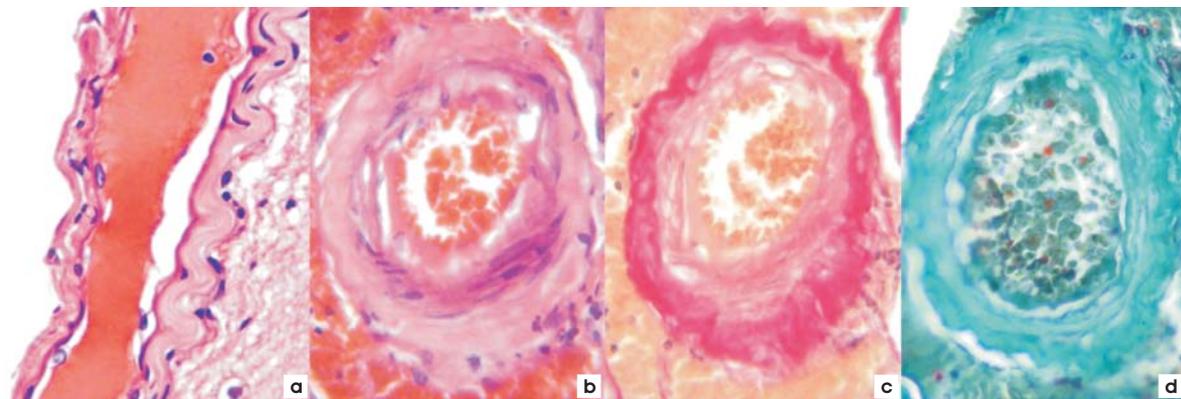

Figure 7 – *Smooth muscle atrophy. Medio-adventitial fibrosis. Incipient hyalinosis:*
(a), (b) HE stain; (c) van Gieson stain; (d) Goldner stain (oc. ×10, ob. ×65)

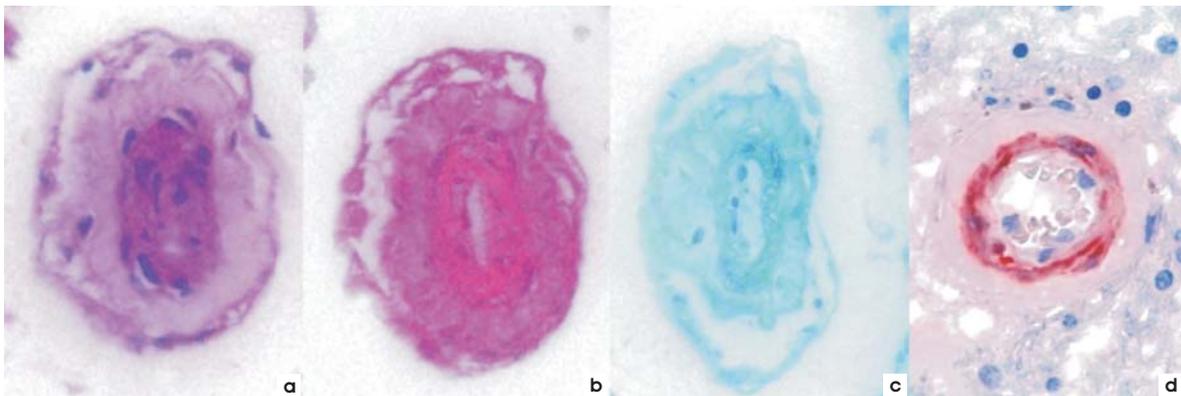**Figure 8 – *Complete hyalinisation of vascular wall: (a) HE stain; (b) van Gieson stain;***
(c) Goldner stain; (d) CD34 stain (oc. ×10, ob. ×100)

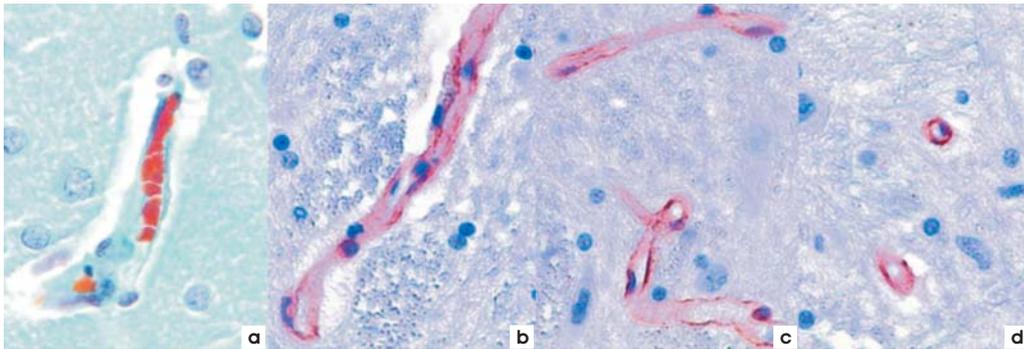**Figure 9 – *Capillaires with focal thickening of the basement membrane:
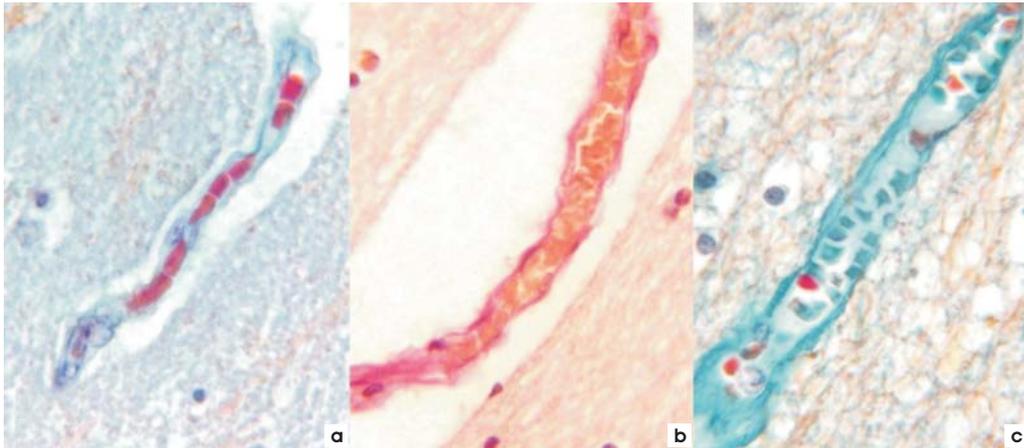(a) Goldner stain; (b), (c), (d) Collagen IV stain (oc. ×10, ob. ×65)***

**Figure 10 – *Capillaires with increasing and extention of basement membrane thickening:
(a), (c) Goldner stain; (b) Van Gieson stain (oc. ×10, ob. ×65)***

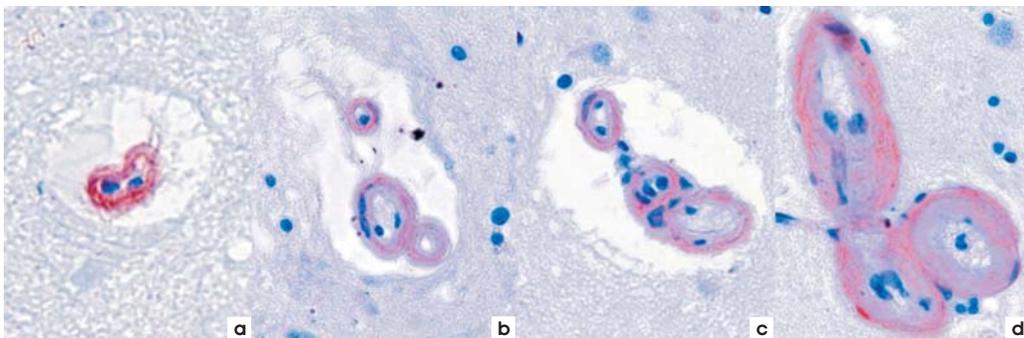**Figure 11 – *Capillaires with increasing and extention of basement membrane thickening
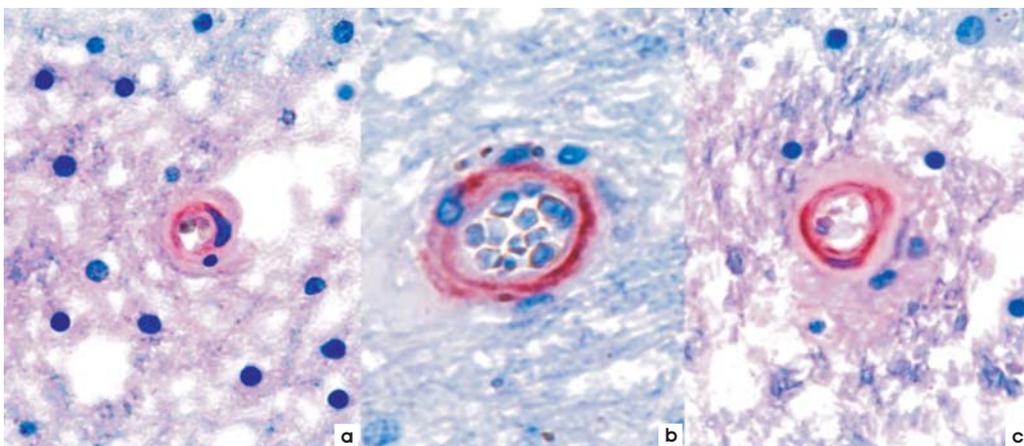(Collagen IV stain, oc. ×10, ob. ×65)***

**Figure 12 – *Hyalinisation of the outer layers of the thickened basement membrane
(Collagen IV stain, oc. ×10, ob. ×65)***
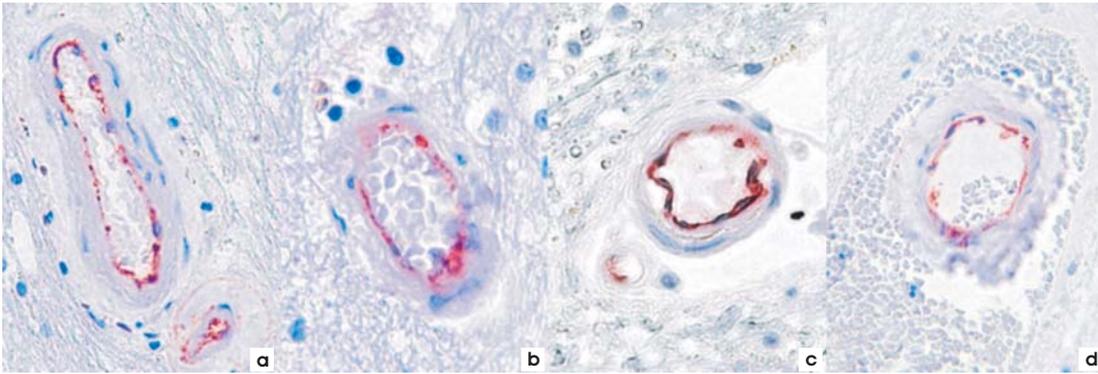
**Figure 13 – *Intraparenchymal arteriolae with continuous endothelial layer
(CD34 stain, oc. ×10, ob. ×65)***

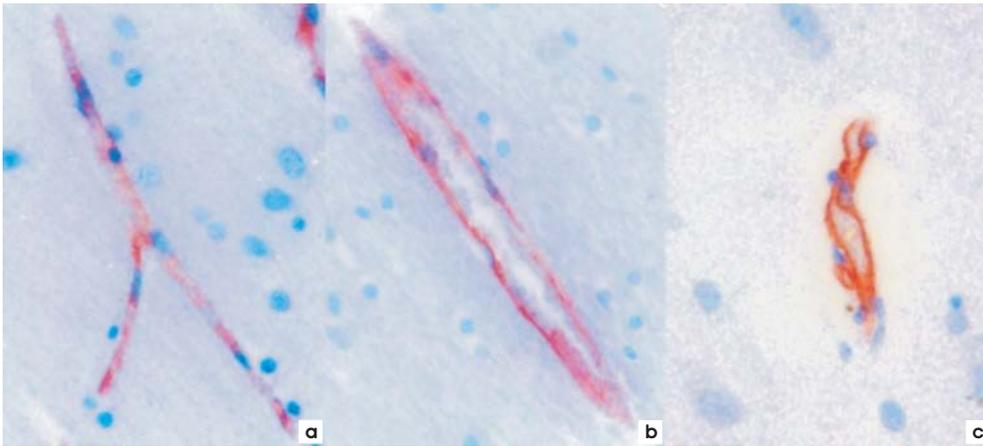**Figure 14 – *Intraparenchymal capillaries with continuous endothelial layer
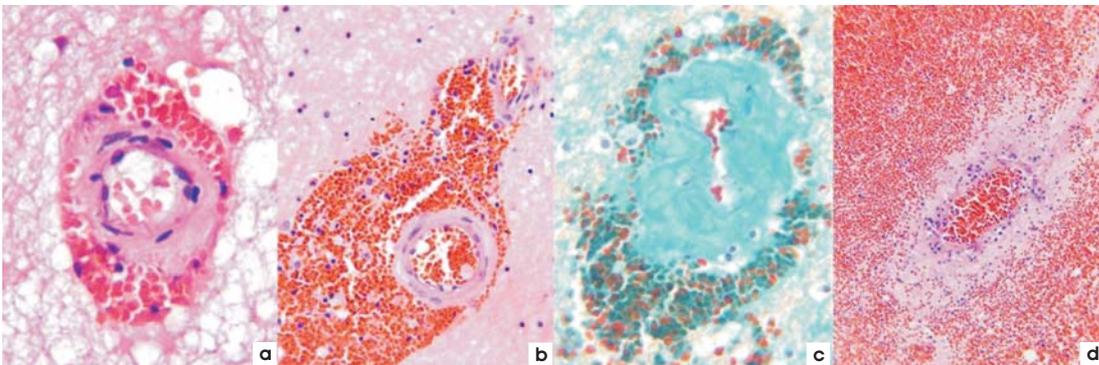(CD34 stain, oc. ×10, ob. ×100)***

**Figure 15 – *Arteriolae with hyalinised walls centering microhaemorrhagic foci
(a), (b), (d) HE stain; (c) Goldner stain (oc. ×10, ob. ×65)***

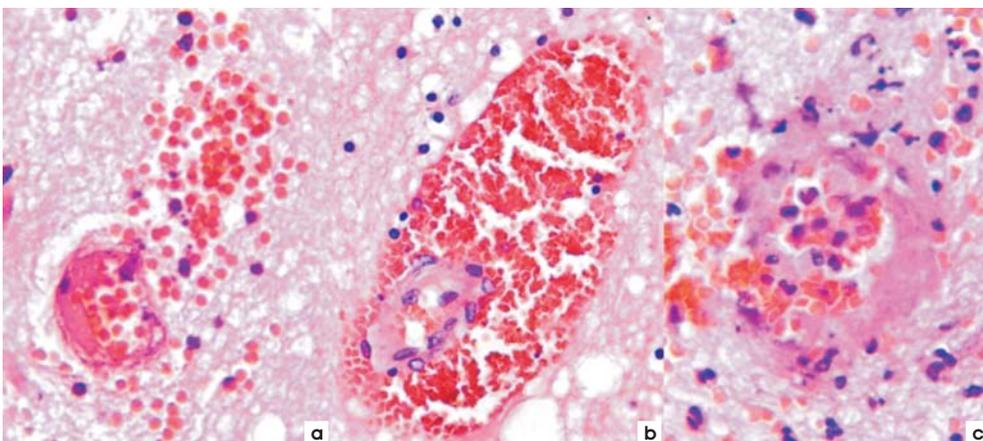**Figure 16 – *The onset of haemorrhage by efraction of hyalinised vascular wall
(HE stain, oc. ×10, ob. ×65)***

A second significant aspect was the hypertrophy of the middle layer smooth muscle cells (Figure 5, a and c).

The third important phenomenon was the beginning of fibrosis, initially inside the vascular adventitia, leading to its thickening and thinning of the outer elastica (Figure 5, b and d).

The second stage of the degenerative process consisted in the progressive atrophy of smooth muscle cell and their replacement by a multilayered collagen network with concentric arrangement produced by fibroblasts. Thus, the vascular wall was transformed into a fibrous sleeve extending from the subendothelial space to the external adventicial layer (Figure 6, a–d).

The IIIrd stage is characterized by the onset and the extension of the hyaline degeneration of the collagen fibers which replaced the middle vascular layer and are in excess in the adventitial layer. Thus, the smooth muscle layer is progressively dissociated and reduced concomitantly with a medio-adventitial fibrosis (Figure 7b).

Figure 7 – a, c and d – shows the disappearance of the outer elastica, extensive fibrosis and hyaline degeneration of collagen fibers, especially of those outlying the vascular wall. The final stage of the degenerative process is represented by the complete hyaline transformation of the vascular wall (Figure 8, a–d).

Unlike collagen fibrillary structures, the amorphous hyaline has a low resistance to mechanical stress induced by high pressure variations that are no longer corrected by the muscular sleeve. Thus, hyaline material represents the sight of a possible vascular rupture.

Capillary changes

By extending the morphological analysis to capillary-type vascular structures we have observed some interesting aspects we did not find to be described in the literature.

The first observation, even on usual stains, is the focal thickening of the basement membrane (BM) of cerebral capillaries which appears to be more obvious at the branching sites and not to extend to the entire circumference of the capillary (Figure 9, a).

Anti-collagen IV immunostaining showed that the focal and/or circumferential thickening was the result of the densification of the collagen material present in the BM of the capillary walls (Figure 9, b–d).

The second stage of this process seems to be the increase of the BM densification, associated with different degrees of circumferential and longitudinal extension (Figure 10, a–c). Collagen IV immunostaining offered a better view of circumferential basal membrane densification (Figure 11, a–d).

It is interesting to notice that even this BM specific collagen material underwent an initial process of densification, followed by a hyalinisation phenomenon (Figure 12, a–c).

Endothelial changes

Two mechanisms were described in the genesis of the intraparenchimal sanguine extravasation: vascular wall rupture and erythrodiapedesis.

The second mechanism requires the presence of an alteration at the level of the vascular endothelium.

One of our objects was to analyze the status of the vascular endothelium in all types of intraparenchymal vessels using anti CD34 antibody.

We observed that arteries and arterioles did not show any alterations with discontinuities in the vascular endothelium (Figure 13, a–d).

The same integrity of endothelial structures was also seen in intraparenchymal capillaries (Figure 14, a–c).

Genesis of the haemorrhagic focus

We present, in the end, some suggestive images showing vascular structures of arterial type within the hemorrhagic focus or in its proximity that presented some of the above mentioned changes and especially hyaline degeneration, and also their involvement in intraparenchymal overflow.

Figure 15, a–d, shows arteriolar-type vessels with different degrees of hyalinisation surrounded by erythrocytic overflows in the proximity of massive hemorrhagic foci.

Figure 16, a–c, presents clear images with the efraction of the completely hyalinised arteriolar wall, massive erythrocyte outflow in the perivascular area and subsequent flooding of the surrounding cerebral parenchyma. The same phenomenon could also be observed in capillaries except that the wall rupture implied both normal capillaries and capillaries with the above mentioned changes.

The microscopic study involved only the patients deceased before the completion of investigating procedures, usually in the first 24 hours of hospitalization. On the other hand, hypertensive disease was, in most cases, either a major risk factor or a determining factor. Thus, at least in the cases where we performed a necroptic examination we found no evidence of a vascular malformation to explain the dramatic intraparenchymal hemorrhage.

Arterial and arteriolar degenerative changes did not suggest the presence of abnormal deposits of focal amorphous material that would lead to a possible diagnosis of amyloid angiopathy and thus requesting the completion of the morphological investigation algorithm by performing a special stain for amyloid (Congo red).

Thus, vascular lesions that were encountered and analyzed were interpreted as being the result of the harmful effect of chronic hypertension on intraparenchymal vascular structures.

We also did not find any signs of fibrinoid necrosis in healthy vascular wall.

Finally, we have to mention that the lesions we described, in different stages, were encountered both in vascular structures in the proximity of the hemorrhage, in the same hemisphere, as well as in its counterpart.

⯐ Conclusions

In conclusion, the vascular wall changes are different depending on vessel histological structure and function.

We propose the following evolution sequences for the degenerative lesions involving arterial and capillary-type intraparenchymal vascular structures under the influence of hypertensive disease:

▪ *Penetrating arteries and arterioles*

– Smooth muscle degeneration / fibrinoid necrosis.

– Substitutive progressive sclerosis in of the media and later on of the entire wall.

– Hyalinisation of the substituting collagen fiber network.

– Spindle-shaped dilatations.

– Rupture of impaired vascular wall in hyalinization areas.

▪ *Capillaries*

– Thickening of the basal membrane

– Hyalinisation of collagen fibers inside the thickened basal membrane.

– Rupture of normal capillary wall / impaired capillary wall in hyalinisation areas.

– Spindle-shaped dilatations.

– Rupture of impaired vascular wall in areas with hyalinization.

Hyalin material areas in the vascular wall are points of low resistance and therefore, possible site of wall rupture in case of abrupt increasing of blood pressure.

The sequence of evolutive lesions culminating with vascular wall hyalinization, rendering it vulnerable to high pressure waves associated with minimum resistance of the surrounding parenchyma which doesn't allow the vessel to withstand large pressure variations or constantly increased values can explain why the cerebral parenchyma is the only parenchyma in which variations in blood pressure can lead to intraparenchymal hemorrhage subsequent to vascular wall rupture.

The term lipohyalinosis was first introduced by Fisher C. M. (1969) and considered to be a healed vascular wall lesion [20]. It is characterized by complex structural disorganization with or without aneurismal dilatations and is more and more often subject to controversy [19, 21].

Thus, we propose to be used no longer in morphological description of cerebral vascular structures lesions generating ICH. In turn, we recommend the already acknowledged term of arteriolosclerosis with hyalinosis which describes more accurately one of the stages of the vascular wall degeneration which can lead to vascular wall rupture with subsequent intraparenchymal bleeding.

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Mailing address

Iancu Emil Pleşea, Assistant Professor, M. D., Ph. D., Department of Pathology, University of Medicine and Pharmacy, Street Petru Rareş no. 2–4, 200 349 Craiova, Romania; Phone: +40251–524 441(2), E-mail: emp@umfcv.ro, pathology@umfcv.ro