Injury of the brain and liver capillaries in lethal car accidents

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Abstract: The study is reporting about the range of changes in the brain and liver capillaries in lethal automobile collisions. We present five case studies, where there was no compression of the head and abdominal organs, but the cause of death was hypovolemic shock associated with excessive blood loss. During the autopsy we removed tissue samples which were subjected to standard processed in order to permit histological and immunohistochemical examinations. Slices of liver and brain tissue were immunostained for Bcl2L1, GFAP, IL-12, S100 and Vimentin. We found a wide range of changes in this material, with microbleeding, bloodstaining, relaxation of capillaries with release of erythrocytes into the periphery, as well as neuron, axon and supporting glial cells damage. We observed a relationship between the microruptures of brain capillaries and the death of adjacent astrocytes.

Key Words: arteries, vessels, bleeding, shock wave, veins, capillaries.

Due to a strategical support of industry expansion, involving car industry, has been routinely using passenger cars in the whole of Europe. Their concentration on the roads has been risen several-fold over the last ten years [1]. These trends directly correlate with an increased incidence of traffic accidents. Currently statistics show that incidence of serious and mortal car accidents is in the Czech and Slovak republics remain high. According to official statistics released by the Czech government, in the year 1996 there was 201 697 car accidents registered, including 1386 fatalities. In the year 2006 there was 187,965 car accidents registered, of which 956 were fatal. If we look at official statistics from Slovakia, in the year 1996 there were 12,823 car accidents registered, from which 543 were fatal, and in the year 2006 there were 62 040 car accidents, from which 579 were fatal. Primarily, the cause of death was most often damage of the brain or internal organ injuries, whose activities are necessary for sustaining [2]. Through constantly improving automobile safety standards, there has been visible a stagnation in the accident rate, together with a decrease in the mortality rate comparing last decades. In 2014, there were 84 398 car accidents in the Czech Republic, resulting in 540 fatalities and 25 359 injured people and in the 2015 there was 93,067 car accidents registered, including 660 fatalities. Isolated, but also serious poly traumatic injuries are therefore a constant challenge for clinical medicine and clinical research. The relationship between mechanical force and degree of internal organ damage, including alterations in organ integrity and compactness, has been extensively studied. Herein we assess not the relationship between mechanical attack and direct damage of mentioned organs, but rather the influence between physical wave

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propagation emanating from the contact point and its effect on organ morphology. After a sudden crash and subsequent stopping of an automobile, pressure waves develop as a result of the rapid deceleration associated with impact. This rapid change of speed causes a pressure wave, where its depth of penetration depends upon its amplitude, can penetrate to, possibly even passing through organs completely. Although shock waves can be used for therapeutic effect, such as in the treatment of tendonitis or to break up kidney and gallstones [3, 4], higher intensity shock waves can have a negative effect with predilection for mechanical trauma [5, 6]. In the study of Micunek [7] it was documented, that after collisions, the internal organs impact each other through inertial forces. During a collision the developing shock waves can damage the internal structure of organs at the cellular level. We hypothesize that such shock wave-induced cellular changes could be manifest in vitally important parenchymal organs: thoracic and abdominal organs, as well as the brain. In the dissertation work of Krajsa [8], who examined the relationships between abdominal and thoracic gunshot wounds and pericapillary haemorrhages within the brain, we have learned about the internal spread of shock waves and the occurrence of microbleeding in brain tissue. These results have a wider significance also for medical practice, as shock-wave-induced distal trauma could produce significant pathologies. Although damage to individual organs by the way of crash waves was shown at the microscopic level, the underlying causal mechanism was not satisfactorily explained. Starting from the hypothesis that shock-waves may occurred during car accidents with effects to the capillaries we examined their impact on the brain and liver in lethal cases of automobile accidents from our practice. The aim is to describe the morphological changes in the capillary system of the brain and liver following a car crash.

MATERIAL AND METHODS

Anamnestic data

Case no. 1: Driver of passenger car, who according to a witness, was driving without headlights and, without any braking, suddenly turned into the path of an oncoming car, resulting in a side-on collision. The driver was after rapid medical help arrival without signs of life and the injuries were incompatible with life. The cause of death was ascribed to hypovolemic shock. The accident occurred at a speed of 70 km/h (calculated to 65–70 km/h).

Case no. 2: Driver of lorry, who mishandled a curve and overturned the vehicle. The cause of death was hypovolemic shock. The driver was after rapid medical help arrival without signs of life and the injuries were incompatible with life. The accident occurred at a speed of 35 km/h (calculated to 65–70 km/h).

Case no. 3: Driver of passenger car, not wearing a seatbelt. He fled a police patrol at high speed, subsequently losing control of the vehicle. The driver was after rapid medical help arrival without signs of life and the injuries were incompatible with life. The cause of death was hypovolemic shock. The accident occurred at a speed of 97 km/h (calculated to 91–103 km/h).

Case no. 4: Passenger on the rear seat of a passenger car, which went into a skid and crashed sideways into a tree. The passenger was after rapid medical help arrival without signs of life and the injuries were incompatible with life. The cause of death was hypovolemic shock. The collision occurred at a speed of 48 km/h (calculated to 45–53 km/h).

Case no. 5: Driver of passenger car who failed to negotiate a mild right-hand curve and, without any signs of braking, crashed directly into a mature tree. Prior to the collision, the driver suffered a myocardial infarction with cardiac rupture and intra-thoracic haemorrhage. The driver died just before the collision itself and it was not possible to revive his. The collision occurred at a speed of 55 km/h (calculated to 52–57 km/h).

Sampling and methodology of sample processing

During the autopsy, samples were taken from individual organs, specifically the brain and liver, for histological examination. One samples was taken from frontal lobe from grey and white matter and one samples from central part of the right part of the liver. Specimens were fixed in 10% formalin solution, then standardly processed and embedded into paraffin blocks. Serial sections were cut 3 – 5 µm thick using a microtome. The first series were stained with haematoxylin-eosin (DiaPath, Italy) on standard slides (Bamed s.r.o., Ceske Budejovice, Czech Republic). Serial sections of the brain were also stained immunohistochemically, using Anti-Bcl2L1 (Anti-Bcl2L1 antibody, SPMS37, ab54396, Abcam), which marks proteins inhibiting apoptosis, rabbit anti-GFAP (Gial Fibrillar Acidic Protein, RBK037, Clone EP672Y, Zytomed Systems, Germany), which marks astrocytes and astrocyte-like cells, Anti-IL 12 antibody (ab9992, Abcam), mouse anti-S100 (MSK050, Clone 4C4.9, Zytomed Systems, Germany), which marks cells with S100 protein, and mouse anti-Vimentin (MSK023, Clone V9, Zytomed Systems, Germany), which marks proteins inhibiting apoptosis, rabbit anti-GFAP (Gial Fibrillar Acidic Protein, RBK037, Clone EP672Y, Zytomed Systems, Germany), which marks mesenchymal cells. For immunohistochemical procedures we used special glass (DAKO, Denmark). The samples were evaluated and photographed using a light microscope Carl Zeiss Axio Scope A1 (Zeiss, Germany).

RESULTS

Macroscopic report

Case no. 1: Brain: 1695 g; soft cranial pia mater is gentle, focally slightly soaked; the gyri are flattened; sulci...
are narrowed in whole scope; it is marked out occipital and temporal cone; the area of medulla oblongata with conspicuous cone is basically bruised. The neocortex: 5 mm, sticky white matter, with tendency to adhere to the blade; chamber system slightly narrowed, the fluid clear, red-violet plexus; nuclei basales, cerebellum and pons Varolii are intact; medulla oblongata with a noticeable pulpy structure.

Liver: 32x24x10 cm, 2830 g; the coat is soft, surface is smooth, tissue is adequately stiff, homogenous, with rare, more yellow lesions, intrahepatic bile ducts undamaged, from the cut surface flow the larger amount of purplish liquid. Gall bladder is adequately large, approximately with a spoon of honey coloured bile, mucous fine, unimpaired.

**Case no. 2:** Brain: 1340 g; soft cranial pia mater is gentle, slightly soaked, gyri are arched, with the flattened base, sulci are narrowed. The cut surface is soft, focally with adherence to the blade, in the place of corpus callosum are red-brown bloody effusions, organ was prominently pale, chamber system normal, fluid clear, red-violet plexus, cerebellum and pons Varolii are intact, medulla oblongata markedly soft, the cut surface without noticeable injury changes.

Liver: 28x17x6 cm, 1650 g; the coat is soft, surface is smooth, in the area of left lobe, at its transition to the diaphragm area (area nuda) are found irregular wounds on a base with small bridges, surrounded by blue-violet effusions, with totally length 5 cm, the next even 7 cm, according to cut surface not overly interfere in the tissue. Intrahepatic bile ducts are not widened.

**Case no. 3:** Brain: 1420 g; soft cranial pia mater is gentle, the gyri are slightly flattened, sulci are narrowed, especially at the base, according to cut surface it is soft tissue, design is normal, nuclei basales, cerebellum and pons are intact; medulla oblongata with pulpy material at transition to a medulla spinalis. Surrounding tissue with red-brown bloody effusions.

Liver: 27x22x11 cm, 1630g; the coat is soft, surface is smooth. Tissue is adequately stiff, the cut surface brown, homogenous; intrahepatic bile ducts are not widened. Gall bladder is adequately large with a film of honey coloured bile; mucosa is greenish, with a large number of yellow spots.

**Histology results**

**Case no. 1:** Brain: The part of brain with grey and white matter with partly relaxed structures and initial autolysis of neurons, which are situated in the environment of shrinking and clumping eosinophil material. In the whole material are capillaries, which are comprising from thin-walled blood vessels and numerous erythrocytes in lumen. Individual vessels are in the appearance and morphology without significant changes with sporadic signs of markedly wavy cell and with signs of micro ruptures with releasing of erythrocytes and clear eosinophil matter in periphery. There were erythrocytes in contact with outer cell of vessel in some places, free erythrocytes and sporadic present free groups of erythrocytes. According to immunochemistry, Bcl2L1 nuclear positivity of neurons occurs in some places, including glial elements in this scope. GFAP satisfactory visualizes astrocytes, including their projections, involving perivascular visualization of well branched adjacent astrocytes. In the areas with vascular pathology is indicated loss of GFAP positivity. IL-12 shows sporadic positivity in cytoplasm of supporting cells. In reaction of S100 is occurred widespread positivity in preserved areas with predominant link to cytoplasm of supporting cells. In reaction with vimentin is well visible positivity in the cell of vessels.

Liver: Liver steatosis (G1) and in the same place is occurred proliferated mature fibrous tissue is visible.

**Case no. 2:** Brain: The cross-section of brain with grey and white matter with partly relaxed structures and numerous neurons, which shows signs of initial autolysis, stroma is composed from eosinophil and clumping material. Individual neurons are gently inflated with soaked diffusion reaction to a stain with incoherent axon projections. In part of material is visible conspicuous, twisting each other, glial fibre. Distribution system is well formed with presence of numerous capillaries, which are full of erythrocytes, including presence of clear, pinkish mass and erythrocytes, falling to pieces. Part of
erythrocytes is in the close contact with capillaries and part is localized out of capillaries, smaller part of material is bleeding. According to immunohistochemistry Bcl2L1 shows sporadic nuclear positivity of glial cells. GFAP shows good visible response in cortex part with declining positivity, tending towards negativity, in white matter. S100 indicates predominantly diffusion, well visible positivity, which is visible in cytoplasm of glial elements. In reaction with vimentin is well visible the positivity in cell vessels.

Liver: Material, which consists of liver parenchyma with preserved lobe structure and morphological changes which imitate a spectrum of autolytic activities. Individual lobes blend each other, but central and portal areas are well visible. Hepatocytes are radial arranged and are rather irregular in shape with markedly eosinophil, clumping cytoplasm and with one or two spherical nuclei, alternatively residual glycogen granules in cytoplasm. Part of cells occurs with noticeable loss of nuclei color ability, falling apart of cytoplasm, alternatively occur only remaining pinkish clumping structures. In portal areas are present deposits of fibrous material with focal inflammatory spherical-cell reaction. Central veins, portal arteries and veins are without noticeable cluster of erythrocytes. In sinusoids are visible numerous breaking up erythrocytes and free erythrocytes between areas of hepatocytes.

Case no. 3: Brain: It is partly preserved and partly markedly relaxed tissue, which is comprising of white and grey matter with quite well visible neurons, which are located in a base of gently clumping, predominantly homogenous, eosinophil stroma. Individual neurons are preserved, similar to polygonal elements with several peripheral projections, imitating dendritic cells. These cells have a dark cytoplasm and one spherical nucleus with one prominent nucleolus. Part of cells is accompanied by initial autolytic changes and a part is total autolytic with disappearance of individual cell contour and fusion with surroundings. Axon projectiles are bordered by markedly inflated, tearing and pale layer of material. In part of this material is visible glial fibres twisting each other and highlighted distances of projectiles from individual neurons. Distributional system is well visible and consists predominantly of capillaries with numerous erythrocytes in lumen. Part of erythrocytes are placed free, out of vessels, and part are in contact with outside of vessels, with signs of relaxation and damage of cell wall. Bcl2L1 positivity shows a clear nuclear positivity, the mature nuclear positivity is visible in supporting cells and in smaller scope in nerve cells too. GFAP mature positivity is visible too, with the image of well branched astrocytes, in places with micro trauma in distribution system occurs a declining of positivity with fragments of grainy remaining astrocytes. IL-12 shows visible positivity in cytoplasm of supporting cells and S100 shows a similar response, the positivity of cytoplasm in supporting and nerve cells. In reaction with vimentin is well visible positivity of wall vessels.

Liver: There is a partly preserved morphology of liver with a semi-vital and several necrotic parts. Individual lobes are blended each other with visible central and portal areas. Hepatocytes are pressed closely each other. They are light, pinkish cells with clustered, markedly clumping cytoplasm. The part of hepatocytes is noticeable distanced with remaining deposits of glycogen material, but the bigger part is composed of rather semi-vital cells with diffusion response to staining and weakly stained nuclei. Between cells are present free erythrocytes and it is present dispersal inflammatory material in the smaller scale. Central veins, portal artery and veins are without marked extravasation of erythrocytes, but there are visible numerous releasing, falling apart erythrocytes in sinusoids.

Case no. 4: Brain: Normal structure of tissue with totally increased eosinophilia, section with cracks, with extravasations of red blood cells, noticeable are tiny and bigger cavities around veins and cells. Individual neurons are broadened, there are marked sign of standard composition of neurons and in larger part of section are noticeable autolytic processes. Part of erythrocytes is localized out of vessels. Bcl2L1 nuclear positivity of supporting cells is only sporadic. GFAP visible reaction is noticeable in cortex area with declining positivity, tending towards negativity, in white matter. IL-12 shows only sporadic positivity in cytoplasm of supporting cells. Antibody S100 has predominantly a well visible positivity in cytoplasm of glial elements. In reaction with vimentin is well visible positivity in wall of vessels.

Liver: In transparent magnification is apparent standard arrangement of liver tissue, at a higher magnification are borders between cells and their structure imperceptible. Surroundings of central veins become congested with blood, there is in one place the small number of eosinophil matter with absence of structure.

Case no. 5: Brain: It is a well preserved structure of brain tissue with cross-section of cortex part, including detected white matter. In cortex parts are reggressively changed, but well visible numerous neuron bodies, which are shapeless, with polygonal form, light cytoplasm and one dark eccentric nucleus. In places are highlighted groups of neurons with addition of melanin granules and well visible projection. Stroma consists of gently, fibrous and clumping structure with presence of numerous predominantly thin-walled vessels, which are fulfil of erythrocytes, including presence of clear eosinophil material from erythrocytes, which breaking apart. There are in some places gently wavy contours of vessels and several released erythrocytes. Bcl2L1 shows almost total negativity. GFAP shows very good visible mature positivity of astrocytes, including their projections without unequivocal signs of positivity decline, located
close to microtrauma. IL2 shows a total negativity. S100 occurs with significant cytoplasm positivity in supporting and nerve cells. In reaction with vimentin is well visible positivity in wall of vessels.

Liver: There is a well differentiated liver steatosis (G2), which impacts the whole liver parenchyma with a badly visible liver lobe and fibrous reconstruction of portal areas. The smaller part of hepatocytes has preserved morphology with shapeless, polygonal, markedly eosinophil cytoplasm and with one or two dark, oval nuclei. Other hepatocytes are with markedly intracytoplasmatic vacuoles, which are bigger than the cell. Part of cells is semivital, towards to a necrotic with remaining dark, granular structures and disintegration of karyoplasm. Extracelular areas are noticeable widened with presence of gently, mature, fibrous component and some free erythrocytes with predominant localization in central parts of lobes with presence of a few pinkish matter.

**DISCUSSION**

In the introduction it was mentioned that under the influence of a crash, there can occur precapillary ruptures, or alternatively haemorrhages in individual organs. In relation to our cases it is necessary consider also other aspects. It is obvious that the histopathological markers detected in the brains or in the livers were not resulted from focal injury, perhaps due to diffused shock wave from rapid deceleration. However, the cause of death in all the five cases was ascribed to hypovolemic shock, indicating loss of blood or loss of body fluid. It should be taken into consideration, that this hypovolemic shock can be resulted from the primary wound injury during the accidents that can lead to shear stress. Thus, rupturing and bleeding of the capillaries could be initiated by shear stress or by pure diffused shock wave. It is not controlled experimental study, but real cases from our practice. Nevertheless, we believe that it is relevant to look to the shock wave propagation dynamics and the results are basis for other controlled experimental studies. All the cases were collected for a number of years and here are selected only cases without head or abdominal injuries (Fig. 1, A-D and F). Anyway today, modern medicine can save a greater proportion of lives following variable trauma, but hardly anybody asks, what is the downstream sequelae arising from vascular changes, and if they predispose the patient to the development of other diseases [9, 10]. Medical treatment primarily focuses upon loss of hearing, compression, and alternatively, other mechanical damages. If we consider war veterans, many have overcome trauma, including mechanical trauma, originating from explosion-induced shock waves [11, 12]. Some researchers are of the opinion, that it would be useful to monitor the consequences of these changes in regard to the development of other diseases, which primarily could be the result of damage to the brain by mechanical crash [13, 14]. It has been proven that mechanical damage of brain is accompanied by apoptosis of neurons and glial cells [15] and it was

![Figure 1](image_url). Selected macroscopic images of individual cases. A: The view of the corpse of man of case number 2 before the autopsy. Please note that on the head and body no visible traumatic injuries; B: The view of the soft cover of skull from case number 3. Notice, please, that in these parts is apparent a head with absence of trauma; C: The view on the brain from case number 3. It is obvious, that it is not occurs intracranial damage; D: The view on the basal part of the cranium without any visible changes from case number 4; E: The view of the liver from case number 4. The location of subcapsular hematoma (already after bursting) indicates a significant violent in abdomen area; F: In case number 5, some wounds were visible on the limbs.
estimated, that this increased rate of apoptosis begins one hour after trauma, and persists for several days [16]. In our work, we similarly found the initiation of apoptosis within the limited substrate material, while in two cases it was already well-developed affecting neurons and supporting cells (Fig. 2, C). Results of the work of Li et al. [17] documented, that positivity of S100 in astrocytes is dependent on the cause of death, while the supporting cells are more damaged, compared to neurons, in fatal brain injuries. We found that where there was damage to the membrane of vessels, there were a decrease in GFAP expression, including in the remaining fragments of astrocytes (Fig. 2, D), but the expression of S100 was unchanged (Fig. 2, F). It should be noted that it is possible to consider the relationship between membrane microruptures of capillaries, and the death of astrocytes, followed by the damage of neurons and their projections (Fig. 2, E). Besides, it brings into consideration also some other hypotheses. Here are especially pathologies as a result of a contact of antibodies and neurons, as well as products of endothelium and neurons, and eventually also the product of cellular blood cells and neurons included. According to Sonden et al. [18] endothelial damage can alternatively be caused by attack of reactive oxygen species. In the study of Haghighi et al. [19] it was shown that shock waves increased DNA methylation and expression of the Aanat gene decreases. This gene is involved in the degradation of serotonin and melatonin, which are well known to be associated with the sleep cycle and the occurrence of sleep disorders. According to Elder et al. [20] transmitted to the brain are even waves of weaker intensity, and these can have possible negative effect on the nervous system. In one experimental model, mice were subjected to explosion-induced shock waves [21]. A wide spectrum of changes was observed, associated with axon abnormalities of the cerebellum, corticospinal system and visual cortex. Hlavac et al. [22] in an in vitro culture model, studied the activation of astrocytes upon exposure to overpressure caused by a shock wave generator. They found that 72 hours after shock exposure, glial cells were activated, supporting a hypothesis of mechanical brain damage. This can be result of glial accumulation, alternatively the result of reparation processes in damaged neurons. The glial cells have a whole spectrum of changes, as a damage and reorganization of cytoskeleton, changes of permeability of membranes, including an initiation of apoptosis [23]. In literature are occurred speculations that these changes can lead even to neurobehavioral symptomatology [24, 25]. We found that crash, alternatively a shock wave, has an influence on damage to the capillary system. This effect is explained by an expansionism of blood and

**Figure 2.** Selected microscopic images of individual cases. A: This is a view of the relaxed vessel with erythrocytes in lumen and the mass of erythrocytes out of vessels. Legend: HE, 200x; B: Several miniature vessels, which have impaired a membrane with release of erythrocytes into periphery. Legend: HE, 200x; C: The view of several astrocytes and neurons with nuclear positivity in reaction with Bcl2L1. Legend: Bcl2L1, 200x; D: The part of brain tissue with several impaired vessels and periphery localized astrocytes with conspicuous projections. Notice, please, that in parts where is impaired a vessel, is visible only remaining positivity GFAP. Legend: GFAP, 200x; E: The view into a centre of brain and of a wavy contours of vessels with periphery blood staining. Legend: VIM, 200x; F: Well visible positivity of S100 in the whole brain tissue. Legend: S100, 200x.
subsequent entry of erythrocytes and leukocytes into adjacent tissue. The effect can be fatal and is and a search for internal injuries caused by shock wave is important in all types of blast injuries [26]. According to Hirt et al. [27] this is a mechanism whereby a shock wave spreading through the blood and into thin-walled capillaries, can, under the increased pressure, burst. If we take a look at the statistics of car accidents mentioned in the introduction, we can see, that the numbers of seriously injured people are very high. From these numbers it is possible to infer that in many survivors, there has occurred trauma, damage to the walls of vessels, including the influx of vessel material into the vascular periphery, and other findings, which we described above. In clinical practice, shock waves have a wider therapeutic significance, and are specifically oriented to the bounded localization of individual organs for therapeutic benefit [28]. A common example is the destruction of kidney stones. There was described damage to the liver parenchyma with the formation of a hematoma after shock wave therapy [29]. In our work comparable to trauma to liver in case 4 (Fig. 1, E). Other example still in using conventional ultrasonography (USG) examination causing progression of foetal abnormalities and foetal mortality [30, 31]. In the study of Forer et al. [32] it is written, that the application of shock waves can have negative effect on the liver, because after their application for removal of kidney stones, there is an elevation of transaminases with their stabilization of the levels in two weeks. The experimental study of Delius and Gambihler [33], where 3000 shock waves were applied to the wall of the gallbladder in six dogs, found bleeding into the lumen in three dogs and increasing echogenicity in the liver parenchyma as well as post-mortem finding of hematoma in the liver. Yokota et al. [34], similarly described subcapsular hematoma and bleeding in the liver parenchyma of hamsters, after experimental application of shock waves. This is similar to our case no. 4, where we observed subcapsular hematoma rupture due to trauma. A detailed histological analysis of the liver parenchyma and gall bladder, following experimental application of shock waves on the canine model is described by Fusijaki et al. [35], who found haemorrhagic necrosis with vacuolar degeneration of cells, oedema and bloody bile ducts. In another study by Shim et al. [36], it is stated that after the experimental application of shock waves on a rabbit model, there occurs haemorrhagic necrosis of the liver with the view of sinusoidal extension of portal circulation and an increased amount of thromboses, involving damage of thin-walled veins. Nardi et al. [37] states that after the application of shock waves on experimental rats, there occurs a whole spectrum of changes which are visible macroscopically. These lung, spleen and liver, where results demonstrate changes in the level of the capillaries. Please also look to the Figure 2 (Fig. 2, A and Fig. 2, B).

CONCLUSION

Our study, focusing on the brain and liver, revealed changes in the level of the capillaries were detectable and increasing impact velocities, the observed changes became more apparent. In the material we find a wide spectrum of changes, including micro bleeding, blood staining, relaxation of blood vessels with releasing of erythrocytes into the periphery, neuronal and axonal damage and the damage to the supporting glial cells. We also found a relationship between micro rupture of vessels and astrocyte death.

Conflict of interest. The authors do not have any disclosures to report.

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