Diffuse Axonal Injury—A Distinct Clinicopathological Entity in Closed Head Injuries

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Abstract: The knowledge about the diffuse axonal injury (DAI) as a clinicopathological entity has matured in the last 30 years. It has been defined clinically (immediate and prolonged unconsciousness leading to death or severe disability) and pathologically (the triad of DAI specific changes). In terms of its biomechanics, DAI is occurring as a result of acceleration forces of longer duration and has been fully reproduced experimentally.

In the process of diagnosing DAI, the performance of a complete forensic neuropathological examination is essential and the immunohistochemistry method using antibodies against β-amyloid precursor protein (β-APP) has been proved to be highly sensitive and specific, selectively targeting the damaged axons.

In this review, we are pointing to the significant characteristics of DAI as a distinct clinicopathological entity that can cause severe impairment of the brain function, and in the forensic medicine setting, it can be found as the concrete cause of death. We are discussing not only its pathological feature, its mechanism of occurrence, and the events on a cellular level but also the dilemmas about DAI that still exist in science: (1) regarding the strict criteria for its diagnosis and (2) regarding its biomechanical significance, which can be of a big medicolegal importance.

Key Words: diffuse axonal injury, traumatic axonal injury, diffuse brain injuries, closed head injuries

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Although the knowledge about the diffuse axonal injury (DAI) as a separate clinicopathological entity has matured in the last 30 years, as early as 5 decades ago, Strich[1] had first pointed to the "diffuse degeneration of the white brain matter" in a series of patients with severe posttraumatic dementia. Since then, this type of brain damage has been described under different names, such as "shearing injury," "diffuse damage of immediate impact type," and "diffuse white matter shearing injury."[2] By those descriptions, the authors mainly wanted to emphasize the basic mechanism of the occurrence of the axonal damage, that is, "the shearing and strain" of the axonal fibers after the impact. This was the emergence of the idea that axons in the white brain matter are susceptible to mechanical trauma, an idea that was further developed later.

The term *diffuse axonal injury* was introduced in the 1980s, and it was defined as a clinicopathological entity by Adams et al.[3] The grading of the pathological findings of DAI was soon carried out.[4] In this phase, when the only methods available for the visualization of the damaged axons were the conventional staining techniques and also the methods of impregnation with silver, the axonal damage in the white brain matter was accepted as an indicator of brain trauma. With the introduction of the immunohistochemistry in the process of diagnosing of DAI, it became clear that the axonal damage does not occur only as a result of trauma, but can be also caused by other conditions, such as hypoxia and ischemia, multiple sclerosis, human immunodeficiency virus encephalitis, infarcts, hypoglycemia, and some authors have demonstrated the existence of a high degree of axonal damage in cases of intoxication with the opiates.[5–11] This has been a new momentum that casted a shadow on the diagnostic relevance of the axonal damage. However, soon thereafter, prominent authors reported that there is a certain difference in the pathological finding (appearance, pattern, and distribution of the damaged axons) that is indicative of the origin of the axonal damage.[12–14] They preferred the term traumatic axonal injury instead of DAI for 2 reasons: first, they suggested that the etiology of any axonal damage should always be indicated, and second, because of the fact that the axonal pathology is disseminated and/or multifocal throughout the brain, rather than truly diffuse, which means it is everywhere.[10,12–14] Hence, some authors consider that the term DAI is a misnomer.[15]

Thanks to the extensive clinicopathological and experimental studies, several major discoveries were made about DAI.

(1) DAI has been experimentally reproduced by acceleration, and with no direct impact to the head, which has clearly shown the role of acceleration as a mechanism of its occurrence.[16]
(2) The introduction of the concept of focal and diffuse brain damage has shown that the final outcome of one particular closed head injury does not depend on the extent of a focal brain injury as much as it depends on the occurrence of diffuse brain injury.[17,18] Some authors have pointed to DAI as an almost universal consequence in the fatal closed head injuries.[7]
(3) Investigations of events on a cellular level have shown that the concussion of the brain, as the most frequent injury of the brain considered to be a pure functional and reversible impairment of the brain, where no pathological features can be found, can obviously be explained as the mildest grade of the DAI.[19–21]
(4) One of the most important discoveries is that in almost 50% of patients with closed head injury leading to death and a severe impairment of the brain function, no clinical evidence of a massive intracranial lesion can be found.[22,23] What is more, using the routine postmortem medical examination, it can be very difficult to define the injury unless the brain is appropriately fixed before the dissection and appropriate histological examinations are undertaken.[24]

The aforementioned facts provoked the development of new methods for the postmortal diagnosis of DAI and also the implementation of a complete forensic-neuropathological examination in the daily practice. The procedure for the complete forensic-neuropathological examination has been scientifically ascertained and affirmed in the forensic medicine and already implemented in the recommendation no. 99 of the Council of Europe about the medicolegal autopsy rules.[25–27]

Yet, there are still some open questions in the scientific community concerning the DAI entity.[28]

The first group of questions arise from the different criteria for diagnosing DAI: are there certain differences which can be

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distinguished between axonal damage caused by ischemia and axonal damage caused by trauma? Does the secondarily occurring ischemia mask, to a greater or lesser extent, the traumatic axonal damage? What can be concluded with certainty from the proper interpretation of axonal damage?

The second group of questions is especially important from the aspect of the medicolegal relevance of DAI in the process of reconstruction of the mechanism of injury. Is DAI more characteristic of certain traumatic events? Does DAI have any specific biomechanical relevance which can be of some advantage in the forensic medicine practice? Can the presence of DAI indicate the type of traumatic event that caused the head trauma?

In a death caused by a closed head injury, the task of the forensic doctor is not only to recognize the brain injury as a cause of death, but also the detailed analyses and reconstruction of all processes and mechanisms leading to the death are of utmost importance. In this mission, it is his right and obligation to use all available scientific methods and achievements to explain and clarify the circumstances of death. That is also of great interest to the judicial system. Thus, the motif for this review arose from the huge medicolegal importance of these vital issues associated with the DAI phenomenon.

What is DAI? DAI as Clinicopathological Entity

It seems that the most constant clinical indicator of the occurrence of DAI is the impairment of the consciousness, that is, the occurrence of immediate and prolonged coma, with no evidence of the presence of any intracranial lesion.[3,29] As already described, this coma occurs in almost half of the patients with a severe head injury, and its etiology is considered to be DAI.[30]

Adams et al[3] have first pointed out that these patients fall into immediate and prolonged unconsciousness and stay severely disabled or in a vegetative state until death. This description is not only a part of the definition of DAI as a clinicopathological entity in their early papers[3,4,16] but also in recent studies.[10,18,31] It has been emphasized that the structural base for the vegetative state after a head injury is the diffuse damage of the white brain matter.[31–33]

"This cannot be. The patient is vegetating after severe head injury, but his CT is normal. How should I explain this to his family?"[34] The patient actually has DAI, and this citation is a frequently heard conversation between colleagues who are faced with DAI in clinical conditions.

Hence, some authors are considering DAI by the means of clinical diagnosis to be a diagnosis of exclusion, based on the impossibility of detecting a brain lesion where there is a clear image of a severe brain failure.[10]

Pathologically, DAI is defined by the feature of the "diffuse axonal damage" in the white brain matter, as originally described.[3,4,14,35] In fact, the axonal pathology is not truly diffuse but widespread and disseminated through many brain regions.[10,15]

Hence, the diagnosis of DAI is clinicopathological:

The DAI is the clinicopathological entity clinically characterized by the immediate and prolonged unconsciousness after the mechanical impact to the head, typically without any lucid interval, leading to severe brain failure, vegetative state and death, and pathologically defined by the feature of the widespread and disseminated damage of the axonal fibers inside the white brain matter, including the fiber tracts and the brain stem.

The Mechanism of Occurrence and the Pathogenesis of DAI

The mechanism of the occurrence of DAI has been fully explained so far. The DAI has been reproduced experimentally, and this led to a profound understanding of the mechanisms causing the diffuse damage of axons in the white brain matter.

Experimental studies have shown that 2 major mechanisms play a key role in head injuries: (1) the contact phenomenon, and (2) acceleration and deceleration.[30,36] Acceleration, which is a result of the sudden movement of the head, causes the pressure gradients in the intracranial cavity, thereby initiating the forces of shearing and strain. Those inertial phenomena typically produce: (1) acute subdural haematoma (ASDH) caused by the tearing of the subdural bridging blood vessels, and (2) DAI in the white brain matter produced by the strain and tearing of the axonal fibers.[16]

Furthermore, it has been observed that ASDH and DAI, as 2 typical acceleration injuries, differ in the duration of the acceleration forces to the head. Experimentally, it has been shown that ASDH is caused by a relatively short duration (5–10 milliseconds) of the angular acceleration loading at high rates of acceleration, whereas DAI occurs most readily where the head moves in the coronal plane and when the acceleration duration is longer (20–25 milliseconds), and the rate of acceleration is lower than the conditions that produce ASDH.[16,37] The role of the angular or rotational acceleration in the occurrence of DAI, especially the one in the coronal plane, has been emphasized and recently confirmed by other authors.[16,38,39] Nowadays, there are some novel studies which point to the combination of the translator and angular acceleration as most responsible for the occurrence of DAI.[40]

In real life, the circumstances for the occurrence of ASDH appear in falls where the head rapidly decelerates against a firm surface, whereas the conditions for producing DAI appear in vehicle traffic accidents where the impact to deformable and padded surfaces lengthens the deceleration and decreases its rate. Our own results have shown that ASDH was most frequently found in cases of simple fall, assault cases, where the blow was often associated with falling on a firm surface, and in those kinds of traffic accidents where a great part of the injury mechanism has been attributed to a fall on a firm surface, represented by cyclists.[41] On the other hand, ASDH was least frequently found in drivers and not found at all in passengers, which makes it an injury not so typical of vehicle traffic accidents.[41] Analyzing the occurrence of DAI in the same study, the results have shown the overall presence of DAI in 40% of the investigated cases with a survival time of at least 2 hours, its exclusive occurrence in drivers, and its high frequency in cases of a fall from a considerable height. It was not detected in the cases of a simple fall or in assault cases.[41]

Although it has been clearly shown experimentally that the forces of acceleration and deceleration are sufficient for producing the injury, with no direct impact to the head, we are aware that it rarely happens in the real world. Almost always, the direct impact to the head (either a blow or a fall) comes first. The point of great medicolegal significance is that the forces of the initial contact do not need to be strong (they do not need to be intensive enough to cause fractures of the skull), but can still produce a serious, even lethal diffuse brain injury.[38,42]

The Events on a Cellular Level and the Role of the β-Amyloid Precursor Protein

The first scientific considerations about axonal injuries indicated that the underlying pathological mechanism of traumatic axonal damage, the tearing of the axons with the subsequent retraction of the torn fiber into the so-called retraction bulb, occurs at the moment of the injury, that is, the primary axotomy.[1,2] Yet, subsequent studies showed that axonal damage is not an immediate, but rather a delayed consequence of the impairment of

axoplasmic transport (secondary axotomy).[43,44] According to this theory, the initial impact on the brain causes focal perturbation in the axon, resulting in focal disruption of the axoplasmic transport and subsequent accumulation of some substances that are normal contents of an axolemma, that is the β-amyloid precursor protein (β-APP). As some axons are not permanently injured in this process, only their function is disrupted, some authors see a "potential time-window for therapeutic intervention".[45]

Even primary axotomy seems to be a rare phenomenon, but can still happen at the moment of the impact and proof for that are typically for DAI tissue-tearing in the white brain matter "gliding" hemorrhages. The rest of the events occur in the hours or days after the injury and are attributed to the progressive disorganization of the axonal cytoskeleton. Hence, the more recent studies do not reject completely the concept of primary axotomy, but consider it as a rare phenomenon probably restricted to the highest levels of injury to the axon.[35,46]

Furthermore, there has been an argument that DAI has a major microvascular component and that the true traumatic lesion is a penumbra around a small vessel.[47] Typical areas of injury in this pattern of "inner cerebral trauma" include: the corpus callosum, septum pellucidum, subcortical region, the periventricular and parasagittal white matter, and the brain stem with the pontocerebellar complex.[48,49]

The β-APP is a transmembrane glycoprotein, widely represented in the central neuron system, being a constituent part of all membranous structures. It is genetically determined by the gene located in the chromosome[21,50] The β-APP is part of many normal cellular functions. In the neuron, the β-APP is synthesized in the perikaryon and then, it moves through the neuron with fast anterograde transport (100–400 mm/day).[50] Hence, in normal circumstances, β-APP is not accumulated in a degree that can be detected in the tissue. However, in the case of structural axonal injury, the accumulation of β-APP occurs in the proximal and the distal axonal segment to a degree where it can be detected by means of immunohistochemistry.[50]

Thus, it has been demonstrated that all the changes that result in the degeneration of the axon and the formation of retraction bulbs occur in a time frame of about 12 to 24 hours. That is the reason why the detection of DAI in cases with short survival time can be difficult or impossible when using the conventional staining techniques. Using the method of routine haematoxylin-eosin staining, the damaged axons can be seen at least 24 hours after the injury, and using the methods of impregnation with silver, they can be detected 12 to 18 hours after the injury.[4,35]

The introduction of the immunohistochemical technique that uses antibodies against β-APP represented a watershed in the elucidation of the DAI phenomenon.[5,6] This method enables the visualization of damaged axons as early as 2 to 3 hours after injury. This proved to be a highly specific and an extremely sensitive method targeting selectively damaged axons.[6,37] The application of antibodies against β-APP made the visualization of axons even in cases of short survival time (2–3 hours) possible, unlike the conventional methods. Since 1994, when Sheriff introduced the method for the first time, shorter and shorter survival time has been reported where the positive β-APP immunoreactivity has been evidenced: 3 hours, 2 hours, 1.75 hours, and 35 minutes.[8,19,37,51]

Hence, the β-APP has proved to be a very useful early marker of the axonal damage, and it has become one of the most useful markers in the forensic neuropathology science.

Neuropathological Findings of DAI

Regarding the neuropathological features of DAI, this triad of specific pathoanatomical changes has been defined, which provided the base for the grading system that was soon conducted.[3,4,29]

(1) Focal lesion in the corpus callosum, as grade II of DAI (Fig. 1);
(2) Focal lesion in the dorsolateral quadrant of the rostral pons, as grade III of DAI (Fig. 2);
(3) Microscopically detected diffuse axonal damage in the absence of any macroscopic lesion, as grade I of DAI.

Having explored the material of 60 cases with fatal closed head injuries, 30 of which were diagnosed with DAI, we have previously reported that a focal lesion in the corpus callosum has been detected in 9 cases (Fig. 1), and a focal lesion in the dorsolateral quadrant of the rostral brain stem has been detected in 10 cases (Fig. 2).[28]

The diagnosis of DAI must always be confirmed by the microscopic finding of the damaged axons, the swollen "varicosity-like" axons, and the torn axons in the form of retraction bulbs. The criteria for the microscopic determination of the traumatic axonal damage are discussed in detail bellow.

Dilemmas in Science Still Surrounding DAI

At the time when DAI was originally introduced, it was considered a pure indicator of the traumatic damage of axons.[1,3] After the introduction of β-APP immunohistochemistry, many studies have documented β-APP immunoreactive axons in the nontraumatic cases.[7–11] In this new era of knowledge about the axonal injuries, it has been shown that axonal damage occurs with the same frequency in cases of brain trauma and brain ischemia as well. However, it has been emphasized in these reports that axonal damage as a local isolated phenomenon should not be equalized

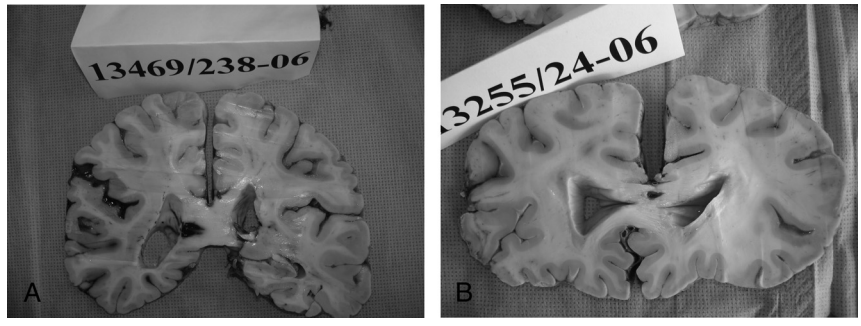

FIGURE 1. Focal lesion in the corpus callosum, grade II of DAI. A, Case with a survival time of 6 days, where a hemorrhagic focal lesion is discovered in the splenium of the corpus callosum. B, Case with a survival time of 1 month, where the cystic formation in the genu of the corpus callosum can be detected.

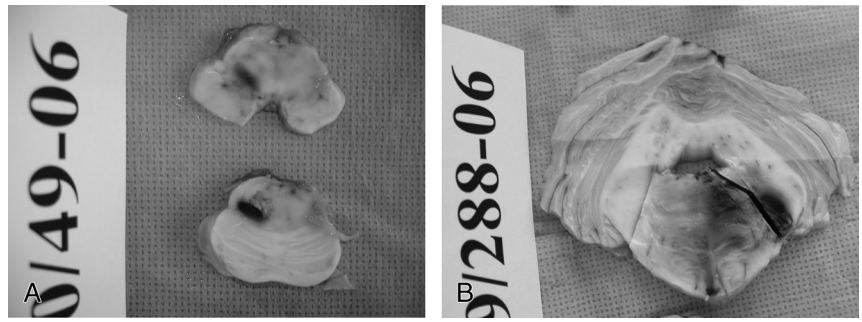

FIGURE 2. Focal hemorrhagic lesion in the dorsolateral segment of the rostral brainstem and in the cerebellar peduncles. A, Case with a several hours of survival time, where the lesion is in the transition area between the midbrain and the pons. B, Case with 24 hours of survival time, where the lesion is seen in left upper cerebellar peduncles.

with the entity of DAI and that "simultaneous demonstration of axonal injury in the corpus callosum and pons must be regarded as evidence of DAI".[37]

A new dilemma has occurred: is the feature of the axonal damage an indicator of DAI as a traumatic entity, or are there also situations where axonal damage is not the evidence of trauma? How to make the distinction? Is DAI a distinct clinicopathological entity of traumatic origin which must be diagnosed by strict criteria?

To resolve this dilemma, many prominent authors have reported and explained the differences in the pathological feature of axonal damage that is indicative of its origin.[10,12–14] These explanations have been altogether pointing out that in the pathological determination of DAI, the diffuse axonal damage must be found in the white brain matter of many brain regions, of which at least one is located above, and one is below the tentorium, especially including axonal tracts, such as the corpus callosum and the internal capsule. Furthermore, the axonal damage must be with a typical traumatic appearance, pattern, and distribution. These authors also emphasize that the interpretation of the pathological findings has to be in accordance with the clinical history of the head trauma. If there is a lack of appropriate clinical antemortem information, only the proper and correct sampling of the brain, from the exact brain regions, can help in the determination of the trauma as a cause of the axonal damage.[10]

All the current discussions are directed toward specifying the term *axonal injury* and also the diagnostic criteria where DAI can be confirmed. Axonal injury is a nonspecific term referring to the axonal damage of any etiology and it does not have any specific biomechanical relevance.[14,37] A DAI is a distinct clinicopathological entity which is clinically defined by the occurrence of immediate and prolonged coma leading to death or severe disability, and it is pathologically defined by the widespread and disseminated axonal damage throughout the brain, including the brainstem.[14,15]

Based on the abovementioned information, there is a difference in the appearance, the distribution, and the pattern of the axonal damage that is indicative of a traumatic or ischemic etiology.

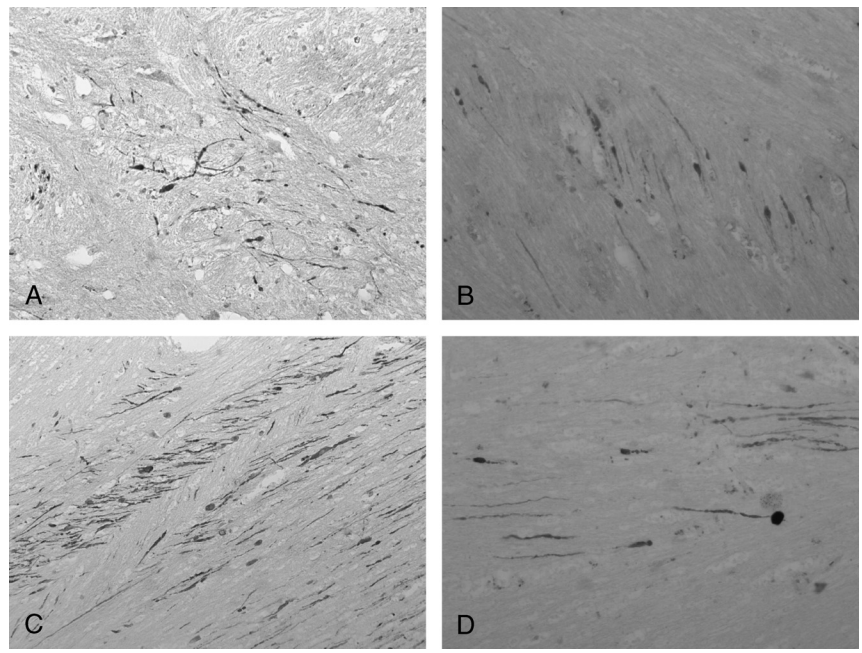

FIGURE 3. Typically, traumatic pattern and distribution of β-APP immunoreactive damaged axons. A, A case with a 10 days of survival time. B, A case with 2 days of survival time. C, D, A strong β-APP immunoreactivity is present in the corpus callosum, in a case with an 8-day survival time. The scattered β-APP–positive axons and the clearly present retraction bulbs can be noticed as well (immunohistochemical staining using the monoclonal antibody against β-amyloid precursor protein, clone 4C11, ×200).

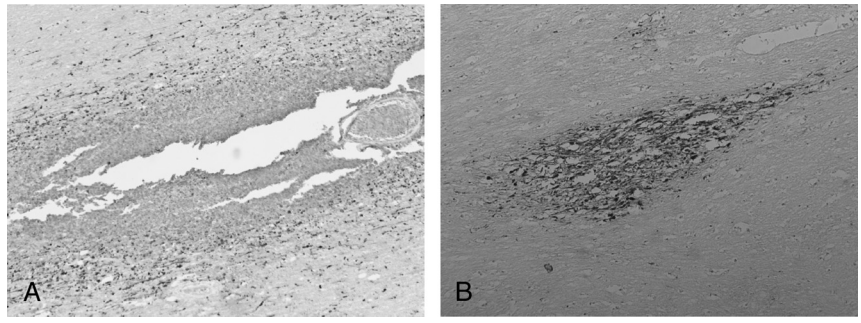

FIGURE 4. A, Intensive ischemic β-APP immunoreactivity can be perceived around the local tissue tearing in the case with an 11-day survival time. B, A typical ischemic appearance of the β-APP expression can be noticed in the case with a 3-day survival time, on a sample from the corpus callosum. There are circumscribed foci of β-APP positive axons, which are never large and are not as neatly shaped as the traumatically damaged axons. They are frequently surrounded by the dirty background (immunohistochemical staining using the monoclonal antibody against β-amyloid precursor protein, clone 4C11, ×200).

In the pathological determination of DAI, in addition to the macroscopic features, such as focal lesions in the corpus callosum (Fig. 1) and dorsolateral quadrant of the rostral pons (Fig. 2), only the detection of single or small groups of scattered and diffusely arranged β-APP–positive axons, seen as "sausage-like" or "varicosity-like" swollen axons or as "retraction bulbs," can be considered as confirmation of the traumatic etiology of axonal damage (Fig. 3). The detection of circumscribed foci or a linear pattern frequently described as a "zigzag" or "Z-shaped" pattern of β-APP–positive axons, which in our experience are never large and are not as neatly shaped as the diffusely arranged traumatically damaged axons, was considered a predominantly hypoxic-ischemic finding and was not considered as a proof of traumatic axonal damage (Fig. 4).[12,52]

As another difference between ischemic and traumatic β-APP expression, it has been stated that the detection of a granulate and a dirty background is typical of cases of hypoxia and ischemia, whereas the detection of well-shaped damaged axons surrounded by clean background is typical in cases of trauma.[9]

One of the big questions or dilemmas concerning DAI which still remain unresolved is about the biomechanical significance of DAI. Which traumatic events are DAI more typical of?

Generally, there are 2 different views in the scientific community regarding this question. The first is represented by authors who stick to the classical descriptions of DAI, and according to these, a DAI is an injury mostly found in road traffic accidents (RTA) and in cases of falling from a considerable height.[2–4,16,29,53] The second view is supported by the authors whose discoveries date after the introduction of the β-APP immunohistochemistry in diagnosing DAI, and according to them, DAI can be found in cases of a simple fall and in cases of a blow as well.[54–58]

This aspect of DAI is of huge forensic and medicolegal importance, and that is the reason why our own results have been put in the context of this discussion.[41] Analyzing the occurrence of DAI through the prism of a traumatic event (RTA, fall, or blow), has shown the highest and almost exclusive (100%) presence of DAI in drivers and also in cases of falling from a considerable height (more than 2 m) (60%). It was not detected in the cases of a simple fall nor in the assault cases. Those results are in accordance with the discoveries of Adams et al,[53] according to who, DAI rarely, if ever, occurs in cases of fall, unless the fall is of a considerable height. Gennarelli[59] also discussed the exclusive occurrence of DAI in RTA as early as 1983.

Sporadic or isolated cases of DAI in the simple falls, that is, falls from somebody's own height or in cases of assault with blows to the head, have been reported occasionally.[53–58] However, this information should be treated with caution for 2 reasons: first, all of these cases have been described after the introduction of the β-APP immunohistochemistry in the diagnosis of DAI, but before the recognition of the other possible reasons for axonal damage, especially ischemia; and second, the diversity of the yielded results greatly depends on the different criteria for diagnosing DAI. Diagnosing of DAI without the use of β-APP immunohistochemistry, or diagnosing it based on the discovery of an axonal damage in one or two brain regions, is not in accordance with the novel standards for diagnosing DAI.[10,50] Furthermore, there are characteristics in the appearance, pattern, and distribution of damaged axons that are indicative of traumatic origin.[10,12–14]

Hence, we have very few cases of DAI resulting from an assault or a simple fall documented in this way, which implicates the necessity of medicolegal precautions in the process of reconstruction of the injury mechanism.

As a conclusion, the information from the reviewed literature undoubtedly shows that DAI is a distinct clinicopathological entity that solely, or as part of the more complex cranial-cerebral entity, can cause serious impairment of the brain function. Speaking in the context of the forensic medicine practice, it can be found as a concrete cause of death. That is the reason why its existence should not be overlooked in the daily practice.

Today, the directions about the postmortal diagnosis of DAI are specified and defined in the forensic-neuropathological science and have been revised many times so far. According to those directions, the 2 main attributes in the pathological diagnosis of DAI are as follows: (1) the diagnosed axonal damage in the white brain matter must be diffuse or precisely it must be multifocal, and (2) it should be widespread in many brain regions, of which at least one should be located above and one below the tentorium. The pattern and the distribution of the damaged axons must suggest its traumatic origin.

The DAI entity can be detected postmortally only by performing a detailed and complete forensic-neuropathological examination of the fixed brain tissue, a fact that has been so far implemented in the international recommendations. The method of choice for the purpose of visualization of the damaged axons is the immunohistochemistry using the antibodies against β-APP.

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