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THE SIGNIFICANCE OF DIFFUSE AXONAL INJURY IN A FORENSIC MEDICINE PRACTICE. A REVIEW

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Abstract

Introduction: The knowledge about the diffuse axonal injury (DAI) as a clinic-pathological 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.
Material and method: Here we are presenting the method of detail forensic-neuropathological examination of the brain, followed by the immunohistochemistry method with antibodies against Beta amyloid precursor protein, in a purpose of visualization of the damaged axons in the white brain matter.
Discussion: In this review, upon our previously published scientific results, we are pointing to the significant characteristics of DAI as a distinct clinic-pathological 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 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 medico-legal importance.

Key words: diffuse axonal injury, diffuse brain injuries, closed head injuries

Význam difúzneho axonálneho poškodenia v súdnolekárskej praxi. Review

Abstrakt

Úvod: Znalosť difúzneho axonálneho poškodenia (DAP) ako klinicko-patologickej jednotky dozrela v posledných 30 rokoch. Bolo definované klinicky (okamžité a dlhodobé bezvedomie, ktoré viedlo k úmrtiu alebo závažnému postihnutiu) a patologicky (triáda špecifických zmien DAP). Čo sa týka biomechaniky, DAP sa vyskytuje ako výsledok dlhšie trvajúcej akcelerácie a je plne experimentálne reprodukovateľný.
Materiál a metóda: Prezentujeme metódu detailného súdnolekársko-neuropatologického vyšetrenia mozgu, s následným imunohistochemickým vyšetrením s protilátkami proti Beta amyloidnému prekurzorovému proteínu, za účelom vizualizácie poškodených axónov v bielej mozgovej hmote.
Diskusia: V tomto prehľade, na základe našich predtým publikovaných vedeckých výsledkov, poukazujeme na významné charakteristiky DAP ako na vymedzenú klinicko-patologickú jednotku, ktorá môže spôsobiť vážne poškodenie mozgovej funkcie

a v prostredí forenznej medicíny ju možno nájsť ako konkrétnu príčina smrti. Diskutujeme o jeho patologickom znaku, jeho mechanizme výskytu a udalostiach na bunkovej úrovni, ale aj o dilemách o DAP, ktoré stále existujú vo vede: 1. o prísnych kritériách pre jeho diagnostiku a 2. o jeho biomechanickom význame, ktorý môže mať veľkú súdnolekársku dôležitosť.

Kľúčové slová: difúzne axonálne poškodenie, difúzne poškodenie mozgu, uzavreté kraniocerebrálne poranenia

Introduction

The term *diffuse axonal injury* was introduced in the 1980s and it was defined as a clinic-pathological entity by Adams et al. (1). The grading of the pathological findings of DAI was soon carried out (2). 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, HIV encephalitis, infarcts, hypoglycemia, and some authors have demonstrated the existence of a high degree of axonal damage in cases of intoxication with the opiates (3-6). 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 (7-9).
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 medico-legal autopsy rules (10-13).
Yet, there are still some open questions in the scientific community concerning the DAI entity.
The first group of questions arise from the different criteria for diagnosing DAI: Are there certain differences in the pathological features between axonal damage caused by ischemia and traumatic axonal damage which is the main attribute of DAI?
The second group of questions is especially important from the aspect of the medico-legal 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 death caused by a closed head injury, the task of the forensic doctor is not just to recognize the brain injury as a cause of death, but detailed analyses and a 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 in order 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 medico-legal importance of these vital issues associated with the DAI phenomenon.

DAI as clinic-pathological entity

DAI is the clinic-pathological 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 diffuse damage of the axonal fibres inside the white brain matter, including the fibre tracts and the brain stem (14).
It seems that the most constant clinical indicator of the occurrence of DAI is the impairment of the consciousness i.e. the occurrence of immediate and prolonged coma, with no evidence of the presence of any intracranial lesion. As already described, this coma occurs in almost half of the patients with a severe head injury and its aetiology is considered to be DAI.
"This can't be. The patient is vegetating after severe head injury, but his CT is normal. How should I explain this to his family?" (15). 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.

The mechanism of occurrence and the pathogenesis of DAI

Experimental studies have shown that two major mechanisms play a key role in head injuries: 1) the contact phenomenon, and 2) acceleration and deceleration. 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) diffuse axonal injury (DAI) in the white brain matter produced by the strain and tearing of the axonal fibres (16).
Furthermore, it has been observed that ASDH and DAI, as two 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 conditions that produce ASDH (17,18).
In real life, the circumstances for the occurrence of ASDH appear in falls where the head rapidly decelerates against a firm surface, while 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 (18). 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 (18). Analysing 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.

Method of diagnosing the DAI - 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 fibre into the so called „retraction bulb", occurs at the moment of the injury i.e. 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** (19,20). 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, as is the **β–amyloid precursor protein (β-APP).**

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 (21). 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). 46 Hence, in normal circumstances β-APP is not accumulated to the degree to 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 (21).

The introduction of the immunohistochemical technique that utilizes antibodies against β-APP, represented a watershed in the elucidation of the DAI phenomenon (4,21-23). This method enables the visualization of damaged axons as early as 2 to 3 hours post injury comparing with method of routine haematoxylin and eosin (H&E) staining and methods of impregnation with silver. This proved to be a highly specific and an extremely sensitive method targeting selectively damaged axons. 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. 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.

Upon the aforementioned, for the purpose of visualization of damaged axons, additionally to the conventional haematoxylin and eosin staining, immunohistochemical staining was performed with the application of antibodies to β–APP: antigen retrieval in citrate buffer (pH 5.0), incubation with antibody against β–APP (Mouse anti-alzheimer precursor protein A4 monoclonal antibody, clone 22 C 11, diluted 1:200, Chemicon International, Temecula, CA) overnight at 4C. The enzyme complex used was ABC (Universal VECTASTAIN ABC-Peroxidase kit, Vector Labs, Burlingame, CA) with a secondary antibody - biotinylated anti-mouse IgG (Biotinylated Anti-mouse IgG, produced in horse, Vector Labs). Diaminobenzidine (Peroxidase Substrate Kit (DAB) Vector Labs) was used for visualization.

The process of DAI diagnosing is explained in detail in the Discussion section.

Discussion

Neuropathological findings of DAI

Described by Adams in 1989, a triad of specific pathoanatomical changes has been defined 2:

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

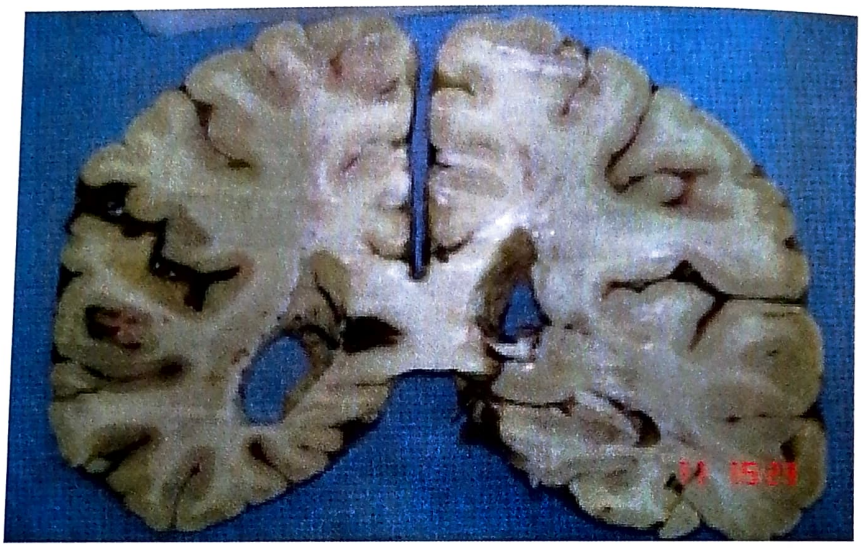

Fig. 1 Focal lesion in the corpus callosum, grade II of DAI

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 (Figure 1) and a focal lesion in the dorsolateral quadrant of the rostral brain stem has been detected in 10 cases (Figure 2) (12).

Fig. 2 Focal hemorrhagic lesion in the dorsolateral segment of the rostral brainstem and in the cerebellar peduncles

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

A new dilemma that arose in the recent years is: Is DAI a distinct clinic-pathological entity of traumatic origin which must be diagnosed by strict criteria?
In order 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 (7-10,14). 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 located above, and one below the tentorium, especially including axonal tracts, such as the corpus callosum and the internal capsule (14). 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 ante-mortem 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 (14).
All the current discussions are directing to the difference between terms: ***Axonal injury (AI)*** as a nonspecific term referring to the axonal damage of any etiology with no any specific biomechanical relevance (14), and ***diffuse axonal injury*** **(DAI)** as a distinct clinic-pathological 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 diffuse axonal damage throughout the brain, including the brainstem.
Based on the above-mentioned 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 aetiology. In the pathological determination of DAI, in addition to the macroscopic features such as focal lesions in the corpus callosum (Figure 1) and dorsolateral quadrant of the rostral pons (Figure 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 aetiology of axonal damage (Figure 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 (Figure 4) (14,17,18).

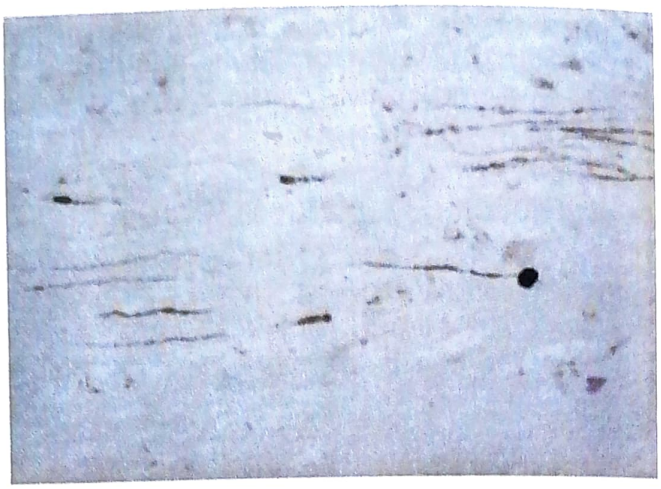

Fig. 3 Typically traumatic pattern and distribution of β-APP immunoreactive damaged axons: the scattered β-APP positive axons and the clearly present retraction bulbs can be noticed as well (x200, Immunohistochemical staining using the monoclonal antibody against β-Amyloid Precursor Protein, clone 4C11)

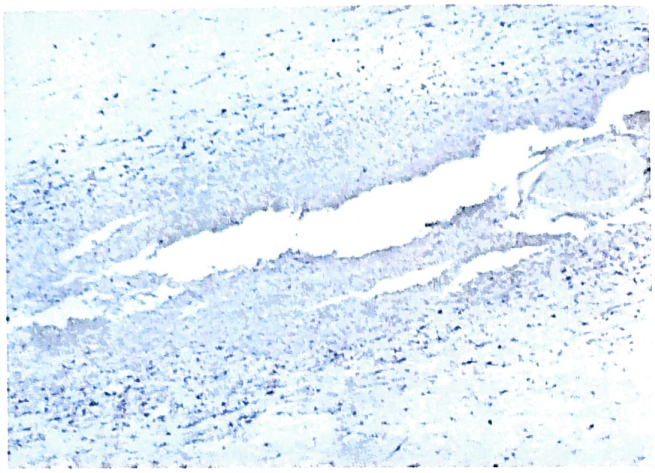

Fig. 4 A typical ischemic appearance of the β-APP expression, frequently described as "geographical" can be noticed on the wide planes and with the dirty background in case with a 3 days' survival time (x200, Immunohistochemical staining using the monoclonal antibody against β-Amyloid Precursor Protein, clone 4C11)

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?

This aspect of DAI is of huge forensic and medico-legal importance and that is the reason why our own results have been put in the context of this discussion (17,18). Analyzing the occurrence of DAI through the prism of a traumatic event (RTA, fall or blow) it was 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 meters) (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., according to who, DAI rarely, if ever, occurs in cases of fall, unless the fall is of a considerable height (23).

As a conclusion, the information from the reviewed literature undoubtedly shows that DAI is a distinct clinic-pathological 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 post mortal 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 two main attributes in the pathological diagnosis of DAI are: (1) the diagnosed axonal damage in the white brain matter must be **diffuse**, 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 post mortally 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 visualisation of the damaged axons is the immunohistochemistry using the antibodies against β-APP.

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