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Review Articles

The Role of the Beta-Amyloid Precursor Protein in the Diagnosis of Diffuse Axonal Injury

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Diffuse axonal injury (DAI) is a distinct clinical-pathological entity in the closed head injuries, where very often, the macroscopic lesion of the brain tissue cannot be found. Hence, in those cases the microscopic examination is of a huge importance. Using the conventional staining techniques the axonal injuries can be perceived only if there has been a survival of up to 24 hours (hematoxylin and eosin staining), or 12 to 18 hours (silver impregnation methods). With the introduction of immunohistochemistry using antibodies against β-Amyloid Precursor Protein (β-APP), this period has been shortened to 3 hours, even less. In the present paper, a review on the role of β-APP immunohistochemistry in the diagnosis of DAI is presented. This review shows that β-APP immunohistochemistry can be a very powerful tool in diagnosing the axonal injuries, what is of a special significance for the forensic medicine practice.

Key words: diffuse axonal injury, β-amyloid precursor protein, immunohistochemistry, forensic medicine.

In the middle of the 20th century Sabine Strich, one of the most prominent authors in the field of the closed head injuries stated: "If we look back to the literature about the head injuries, very little has been discovered ever since 1900" [2]. However, in the last 20 years of the 20th century, there has been a rapid development in the field of neurotraumatology, mainly as a result of the introduction of the concept of the focal and diffuse brain damage. It has been cleared that the final outcome of a particular closed head injury mainly depends on the occurrence of diffuse brain injuries: diffuse axonal injury (DAI), diffuse vascular injury (DVI), diffuse brain edema and diffuse hypoxic-ischemic brain injury.

DAI is a clinical-pathological entity. Clinically, it is characterized by an immediate and prolonged unconsciousness after a mechanical impact to the head, typically without any lucid interval, leading to severe brain failure, vegetative state and death. Pathologically, DAI is defined by the feature of the diffuse damage of axonal fibers inside the white brain matter, including the fiber tracts and the brain stem [1, 7, 8]. In the last twenty years of the 20th century the knowledge about the Diffuse Axonal Injury (DAI) as a separate clinical-pathological entity matured, featuring it as an almost universal consequence in the fatal closed head injuries [10]

The Process of Diagnosing DAI

The diagnosis of DAI is clinical-pathological. In the neuropathological feature of DAI, a triad of specific pathoanatomical changes has been defined, which is the base for grading of the pathological findings of DAI that was recently conducted [6].

1. Focal lesion in the *corpus callosum*, as the 2nd grade of DAI (**Fig. 1**);
2. Focal lesion in the dorsolateral quadrant of the rostral pons, as the 3rd grade of DAI (**Fig. 2**);

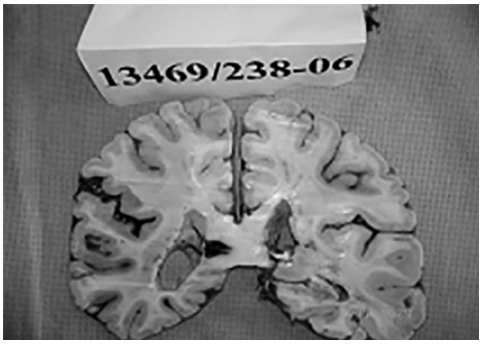

Fig. 1. Focal lesion in the *corpus callosum*, as the 2nd grade of DAI in a case with 6 days survival time

Fig. 2. Focal lesion in the dorsolateral quadrant of the rostral pons, as the 3rd grade of DAI in a case with 7 hours survival time after injury

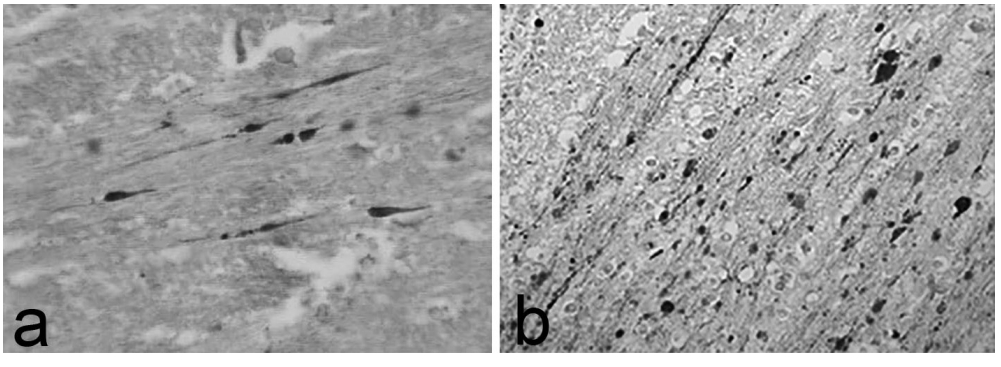

Fig. 3. Employing the method of β-APP immunohistochemistry the visualization of the damaged axons is possible even in cases with several hours of survival time: a – case with 3 hours survival time (×400); b – case with a survival time of 1.5 month (×400)

3. Microscopically detected diffuse axonal damage in the absence of any macroscopical lesion as the 1st grade of DAI (**Fig. 3**).

The main attribute in the pathological determination of DAI is the widespread and diffuse axonal damage throughout the white brain matter including the white matter bundles.

Events on the Cellular Level and Methods for the Visualization of Axonal Damage

The first considerations of the scientists pointed out that tearing of the axons with a subsequent retraction of the torn fiber into a ball, the so called "retraction ball", occurs at the moment of injury, i.e. the **primary axotomy**. However, later studies showed that axonal damage is not an immediate but rather delayed consequence of the impairment of axoplasmic transport (**secondary axotomy**) [14, 15]. With the impairment of the axonal transport, the axon is starting to swell. Such swollen axons can be seen microscopically as "sausages" or "varicosities". After that, a degeneration of the whole axon takes place with the formation of a typical "retraction balls". All these changes occur in a time period of about 12 to 24 hours, which is the reason why they cannot be visualized with the conventional histological methods. Employing the method of haematoxylin and eosin staining, the damaged axons can be seen in the time period of at least 24 hours after the injury (**Fig. 3**). Using the methods of silver impregnation, they can be detected 12 to 18 hours post injury [6, 8].

The Role of the β-amyloid Precursor Protein

The disruption of the axonal transport causes a deposition of some substances that are the normal contents of axolemma and normally are moving through it by anterograde axonal transport, as is the **ß-amyloid precursor protein (β-APP)**.

β-APP is a transmembrane glycoprotein, widely distributed in the central nervous system and involved in many normal cellular functions. In the neuron, β-APP is synthesized in the pericarion and then, by a fast anterograde transport (100-400 mm/day) it moves through the neuron [13]. In normal circumstances β-APP is not accumulated to such a degree to be detected in the tissue. However, in the condition of a structural axonal injury, an accumulation of β-APP happens to a degree to be detected by immunohistochemistry [13]. The immunohistochemical technique using antibodies to β-APP [9], enables the visualization of damaged axons as early as 2 to 3 hours post injury, and sometimes even earlier [12]. This method proved to be a highly specific and extremely sensitive, targeting selectively damaged axons. Application of antibodies to β-APP makes the visualization of axons possible, even in cases of short survival (2-3 hours), unlike the conventional methods. Our own results on a series of 60 cases with closed head injuries have shown that β-APP-immunoreactivity can be observed about 2-3 hours post injury, and the longest survival time has been 1.5 month where β-APP has been still visible [3, 5].

Pattern and Distribution of the ß-APP Immunoreactivity as Indicator for the Traumatic Axonal Damage

Some time ago, when for the visualization of the damaged axons only conventional staining techniques were available, the axonal damage in the white brain matter was considered as an indicator of traumatic damage of the brain. With the introduction of the immunohistohemistry in the process of diagnosing of DAI [9] it became clear that the axonal damage is not occurring only as a result of trauma, but can be caused by other conditions, such as: hypoxia and ischemia, multiple sclerosis, HIV-encephalitis, infarcts, hypoglycemia [16], as well as intoxication.

Nowadays, all the efforts are aimed at establishing diagnostic criteria for DAI [3, 5]. It is widely accepted that for diagnosing DAI, a correlation between a well-documented clinical history of a head trauma and certain neuropathological features is essential. Pathologically, there must be found a widespread diffuse axonal damage in no less than three different brain regions, at least one of which located above and one beneath the tentorium, including also the tracts of axonal fibers as are *corpus callosum* and the internal capsule [7].

Furthermore, prominent authors have reported that there are certain differences in the pathological findings indicative for axonal damage. Thus: "Linear or geographical patterns of β-APP accumulation are more likely to represent damaged axons at the edge of a focus of early ischemia, while scattered or groups of immunoreactive axons, particularly involving single white matter bundle (*corpus callosum*, internal capsule), probably indicate traumatic damage" [8]. In this respect, there are differences in the appearance, distribution and pattern of the axonal damage that is indicative of a traumatic or ischemic etiology. In the pathological determination of DAI, in addition to the macroscopic features like focal lesions in the *corpus callosum* and dorsolateral quadrant of the rostral pons, only the finding of single positive axons or small groups of scattered and diffusely arranged β-APP positive axons, seen as "sausage-like" or "varicosity-like" swollen axons or as "retraction balls", can be considered as confirmation of the traumatic etiology of axonal damage [11]. A finding of circumscribed foci or a linear pattern frequently described as a "zigzag" or "Z-shaped" pattern of β-APP positive axons [4], which in our experience are never big 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 [3, 5].

Conclusion

From this review it becomes clear that β-APP immunohistochemistry is a powerful tool in the hands of forensic pathologists which undoubtedly proves that:

1. There has been an injury of the brain. With the appropriate sampling of the brain and appropriate interpretation, it can point out the origin of the axonal damage – is it traumatic or ischemic.
2. The injury of the brain has occurred during life or intravitally, which is of a big medico-legal relevance.
3. There has been the time of survival of at least 2-3 hours post injury.
4. The time course of the axonal pathology can point to the age of the injury.

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