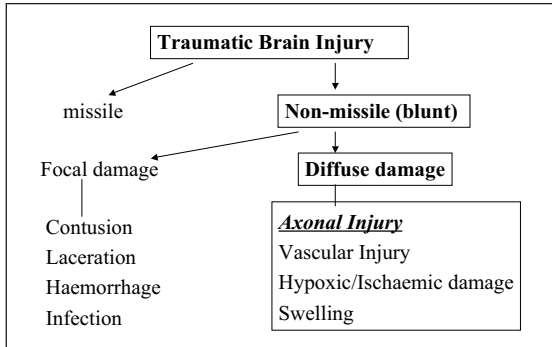


The Significance of Diffuse Axonal Injury: how to diagnose it and what does it tell us?

Traumatic brain injury (TBI) is a leading cause of death and disability worldwide. TBI can be classified as missile and non-missile. The latter can further be subdivided as focal and diffuse damage, which often overlaps (Table 1).¹

Table 1: Classification of Traumatic Brain Injury.



Focal damage includes contusions, which are usually superficial bruises of the brain, affecting the cortex and in more severe cases also the underlying white matter. Contusions often have a triangular shape, with a wide base on the surface of the crest of a gyrus as opposed to ischaemic damage, which tends to be more severe and at the depths of the sulci. They are classified as i) indirect ('contre-coup'), frequently seen in the anterior and inferior surfaces of the frontal and temporal lobes, and ii) direct ('coup') contusions seen at the site of severe impact on the surface of any region of the brain. Lacerations occur when the damage is severe enough to cause tearing of the leptomeninges. Bleeding is common after TBI. Intracranial haemorrhage may develop over a period of time, which may extend into the subarachnoid space causing subarachnoid haemorrhage (SAH); other causes of SAH include skull fracture with tearing and/or dissection of arteries such as the vertebral arteries. Extradural haematoma (EDH) is usually associated with skull fracture and torn meningeal arteries, whereas subdural haematoma (SDH) results from the tearing of the bridging veins in particular those related to the superior sagittal sinus. Focal infection is frequently a complication of skull fracture and contamination with bacteria.

Diffuse damage includes diffuse axonal injury (DAI)(see below), diffuse vascular injury and diffuse hypoxia/ischaemia. Diffuse vascular injury results from shear stress and traction of parenchymal blood vessels resulting in petechial haemorrhages. Diffuse hypoxic-ischaemic damage sometimes accompanies TBI, especially in patients with raised intracranial pressure (over 30 mmHg) and severe long-lasting hypotension.

Classification of axonal injury

Axonal injury is frequently a consequence of traumatic brain injury which may cause other focal damage in the brain like contusions, lacerations or haemorrhage. Depending on the severity of trauma, the axonal injury can be focal, multi-focal or diffuse. In focal and multi-focal axonal injury the damaged axons are seen in one or few locations in the supratentorial parts of the brain, mainly in the corpus callosum and internal capsule, but not in the infratentorial brain regions. Diffuse axonal injury (DAI) is usually associated with rapid angular (rotational) acceleration and deceleration of the brain.

The damaged axons are more widespread and seen in several parts of the brain, including those in the supratentorial and infratentorial brain regions, such as the cerebellum and pons. DAI should be considered as a serious and significant head injury. However, it is graded according to the severity of pathology, clinical presentation and likelihood of survival (Table 2).²

Table 2: Grading of Diffuse Axonal Injury (according to Adams et al.)²

| | Diffuse Axonal Injury | Haemorrhage in corpus callosum | Lesions in dorsolateral rostral brainstem |
|---------|------------------------------|---------------------------------------|--|
| Grade 1 | Present | Absent | Absent |
| Grade 2 | Present | Present | Absent |
| Grade 3 | Present | Present | Present |

The identification of diffuse damage to the axons in the brain should follow a detailed histological examination of many parts of the brain which are more susceptible to axonal injury. These include the frontal parasagittal white matter, parietal lobe (including deep white matter), anterior corpus callosum, posterior corpus callosum, basal ganglia (to include the internal capsule), cerebellum (to include middle cerebellar peduncle) and pons (to include dorsolateral rostral brainstem).^{3,4}

Axonal injury can be caused by immediate (primary) axotomy which occurs at the time of injury or delayed (secondary) axotomy which evolves over a few minutes or hours after impact. In the majority of cases of head injury, secondary axotomy is the major mechanism.⁵ The focal damage to the axonal cytoskeleton is followed by formation of axonal swellings and varicosities proximal to the site of injury. These swellings contain accumulated material which cannot be transported due to disruption of axoplasmic flow.⁶ Therefore, the axonal swelling usually occurs some time after head trauma and indicates some period of survival.

Detection of axonal injury

Several histological methods can detect damaged axons with variable degrees of sensitivity and depend on minimum survival time of patients after head trauma (Table 3). The most widely used and most reliable method is amyloid precursor protein (APP) immunohistochemistry.⁷ APP (Table 4) is a membrane glycoprotein which is

Table 3: Methods and their time dependency to detect axonal injury

| | |
|----------------------------|----------|
| Silver | 15-18h |
| H&E | 24h |
| Immunohistochemistry: GFAP | 5d < |
| CD68 | 36-48h |
| Neurofilament | 60 min |
| Chromogranin A | |
| Cathepsin D | |
| SNAP-25 | |
| APP | < 35 min |



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Table 3: Methods and their time dependency for detection of axonal injury (AI) in paraffin embedded human brain tissue. (Silver: silver impregnation technique; H&E: Haematoxylin and eosin stains; The other methods listed are immunohistochemical. GFAP: Glial Fibrillary Acidic Protein; SNAP-25: Synaptic Protein-25; APP: Amyloid Precursor Protein).

Table 4: The role and significance of Amyloid Precursor Protein (APP).

| Amyloid precursor protein (APP) |
|---|
| <ul style="list-style-type: none"> • ubiquitous membrane glycoprotein coded on chromosome 21 • alternate splicing and proteolytic processing - > 3 major subtypes |
| <i>Physiological/pathological significance:</i> <ul style="list-style-type: none"> • cell-adhesion • ?neuroprotective (via secreted N-terminal fragments) • amyloidogenic proteolytic pathways ▶ β-Amyloid plaque • fast anterograde axoplasmic transport ▶ synaptic function ▶ accumulation proximal to AI • APP is a sensitive marker of AI of any cause • non-specific for traumatic aetiology of AI |
| <i>In axonal injury:</i> <ul style="list-style-type: none"> • size and staining pattern correlates with survival time • helpful but not sufficient for exact timing of injury |

produced in cell bodies and neurones. Its major physiological role is in cell adhesion, neuroprotection and synaptic function. This is the same protein which is implicated in β-amyloid plaque formation in Alzheimer's disease following an abnormal proteolytic cleavage by an enzyme called gamma secretase. To this end, head injury is a known environmental risk factor for Alzheimer's disease, in particular in subjects carrying the apolipoprotein E (APOE) ε 4 allele as an additional genetic risk factor.⁸

In normal circumstances, APP travels from the neurone to the peripheral axons via fast transport mechanism which cannot be detected by immunohistochemistry. This transport is an ATP-dependent process, and the speed is influenced by axonal diameter and age of the individual. If there is axonal disruption for any reason, including trauma, the APP can accumulate at the site of injury and can be demonstrated by immunohistochemistry. It is important that very small amounts of protein are detected in these cases, so antigen retrieval techniques (e.g. microwaving), careful optimisation of the immunohistochemical method and selection of the antibody are crucial. When such issues are addressed, APP immunohistochemistry can be detected within one hour survival period, and in some cases following 35 minutes survival period.⁹ With an optimised method, including microwave and citrate pre-treatment of histological sections, APP immunohistochemistry can detect axonal injury with post TBI survival times of less than 60 minutes and a minimum of 35 minutes.⁹

The APP intensity increases with time up to 24 hours. After that, the staining may become more granular, slightly pale after a few days and disappears after one month or less.

It has been demonstrated that wide sampling and APP immunohistochemistry can determine the cause of axonal injury in most cases.¹⁰

Differential diagnosis

It is important to emphasise that axonal injury can not only be caused by trauma but by different mechanisms such as ischaemia, hypoglycaemia, inflammation, haemorrhage, drugs, alcohol and even ageing.¹¹ Therefore, APP immunohistochemistry should be considered to be a sensitive, but not specific, marker of axonal injury. The distinction between traumatic and other causes of axonal injury can be difficult and, in many cases, only a probability can be established. The most frequent problem is to distinguish hypoxic-ischaemic damage to the axons from that caused by trauma. This is made more complicated by virtue that ischaemia and hypoxia are frequent occurrences and sometimes considered an integral part of head injury. Many cases of head injury are associated with subdural or extradural haematoma which can cause brain shifting and herniation and subsequent axonal injury due to vascular damage.

In traumatic head injury, the APP immunohistochemistry usually reveals well-defined fusiform swellings of different sizes, beaded and thickened filaments and globules which, in some places, are seen along white matter tracts (such as those seen in the internal capsule and corpus callosum) with no granular background (Figure 2). In cases of hypoxia and ischaemia or other vascular damage, the APP immunohistochemistry is usually associated with heavy deposition in ill-defined areas, and sometimes a geographical pattern (following the areas of ischaemia) with a heavy granular background.¹² (Figure 3).

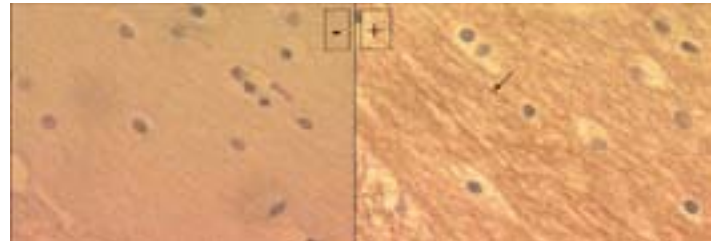


Figure 1: Immunohistochemical detection of APP without (left) and with (right) antigen retrieval. The accumulation of APP proximal to the site of axonal injury is visualised only after application of antigen retrieval techniques.

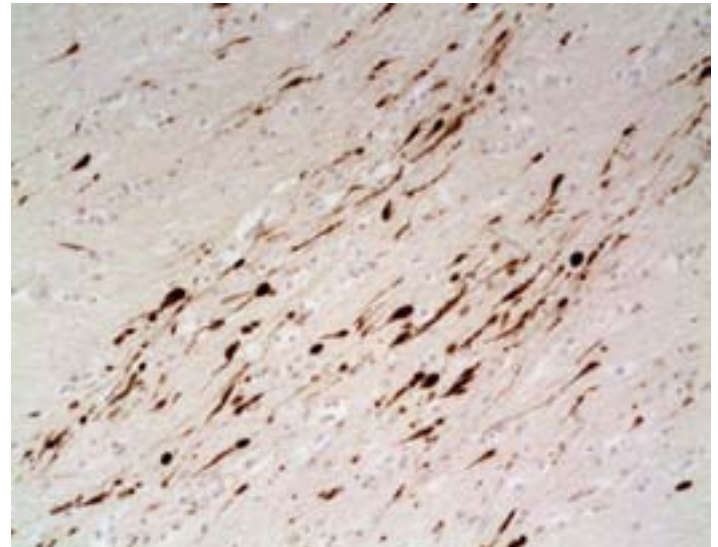


Figure 2: APP immunohistochemistry in a case of severe brain trauma. Note the strong labelling of damaged axons on a clear background with fusiform swellings, thickened filaments and globules.



Figure 3: APP immunohistochemistry in a case of acute cerebral ischaemia. There is a heavy granular staining revealing an ill-defined, geographical pattern on a 'dirty' background.

The 'shaken baby syndrome'

One of the rare causes of head injury in children is non-accidental injury (shaken baby syndrome or shaken-impact baby syndrome). The brain usually shows no evidence of contusions or lacerations but swelling and oedema associated with ischaemic damage. Therefore, the damaged axons in these cases are more frequently seen in a pattern consistent with ischaemic damage than traumatic damage.^{13,14} There are two possible explanations for the mechanism of brain swelling and hypoxia in shaken baby syndrome.¹⁵ The first is an alteration in the blood brain barrier leading to oedema and increased intracranial pressure followed by ischaemia. However, recently it has been proposed that focal damage to

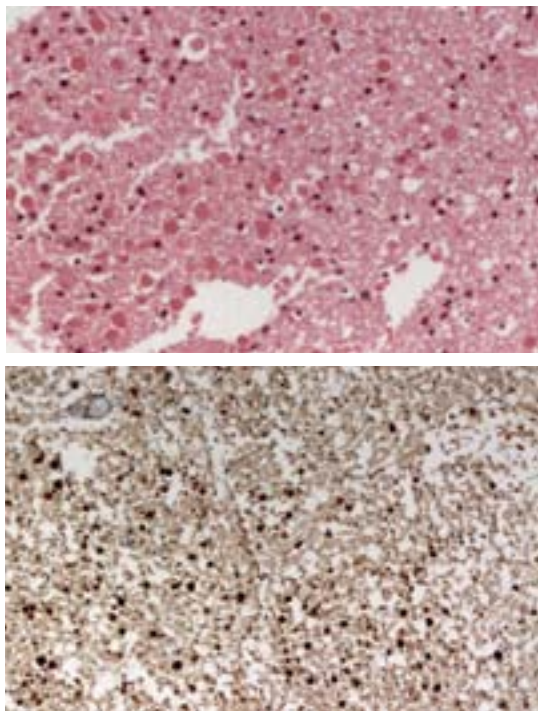


Figure 4: Histological appearances at the level of an injured cranio-cervical junction in a case of paediatric non-accidental injury ('Shaken Baby Syndrome').
 a) (top image) Haematoxylin & Eosin stain reveals the dilated axons as pink globules.
 b) (bottom image) Immunohistochemistry highlights the dilated axons, rich in accumulated APP.

the medulla may cause cardio-respiratory arrest and ischaemia; the axonal damage can be demonstrated in a proportion of victims by APP immunohistochemistry (Figures 4a & b).

Conclusions

Diffuse axonal injury is a significant traumatic brain injury which involves widespread damage to axons in supra- and infratentorial parts of the brain and is graded 1-3, according to the severity of pathology and the likelihood of survival. It should be differentiated from focal or multi-focal axonal injury. APP immunohistochemistry is the most sensitive tool to detect damaged axons. However, it is not specific as it could occur in any other condition which causes damage to the axons, such as ischaemia. APP immunohistochemistry can detect axonal injury within one hour and as early as 35 minutes after trauma, which has medico-legal implications.

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