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The significance of β-APP immunoreactivity in forensic practice

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The neuropathologist involved in forensic work is not uncommonly confronted with a case in which there is no or only a limited history or, if available, the information is uncertain or is often conflicting. In recent years the immunohistochemical stain β-amyloid precursor protein (β-APP) has been used to assess the extent of axonal injury in a variety of pathological processes but in forensic practice is of greatest utility in the assessment of traumatic brain injury. Diffuse traumatic axonal injury (TAI) in humans has been demonstrated by β-APP immunoreactivity in patients surviving at least 2 h after head injury. However, many of these patients also have an associated ischaemic injury, either focal or diffuse, which may make the interpretation of β-APP immunoreactivity difficult. The present study was designed to evaluate if the published descriptions of the different morphological patterns and distributions of β-APP immunoreactive axons could be used to microscopically distinguish axonal injury attributed to trauma from other causes. To test this hypothesis a total of 73 cases were reviewed. The cases were selected from six different groups based on clinical information. Immunostained sections from each case were assessed 'blind' to the clinical history, and the microscopic pattern and distribution of β-APP positive axons were recorded. Haematoxylin and eosin (H+E) stained sections were then reviewed for each case and a final pathological diagnosis was recorded and compared to the clinical history. 62/73 (85%) cases were correctly correlated with the clinical history and in particular 14/17 (82%) cases of TAI were correctly identified. These findings indicate that the published microscopic patterns of the distribution of β-APP positive axons in TAI and in diffuse ischaemic injury can be used, in conjunction with microscopy of H+E stained sections to determine the cause of axonal pathology in most cases.

Keywords: β-APP, diffuse traumatic axonal injury, immunohistochemistry

Introduction

In forensic practice the pathologist is not uncommonly presented with death in suspicious or unwitnessed circumstances. In such instances it is good practice to examine the brain after formalin fixation for better interpretation of patterns of brain injuries that are suggestive of traumatic brain injury (TBI). However, it is equally important that pathological features are not over or misinterpreted in relation to the cause of death resulting in unnecessary investigation. The lesions associated with traumatic head injury can be classified as either focal or diffuse [1]: focal lesions include skull fractures, haematomas and contusions; diffuse lesions include global ischaemia, cerebral swelling and diffuse traumatic axonal injury (TAI).

Diffuse white matter damage after head injury was first described in detail by Strich in 1956 [2]. The white matter

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damage was described in the context of patients with dementia many years after head injury. Axonal shearing injuries at the time of head injury were suggested as a possible mechanism for axonal disruption. The concept of diffuse axonal injury (DAI) after trauma developed over the following years being described and defined by Adams *et al*. [3,4]. Diagnosis of axonal injury requires identification of axonal swellings and bulbs. This can be performed using haematoxylin and eosin (H+E) stained sections or silver stains, although the most sensitive method is immunohistochemistry against β-amyloid precursor protein (β-APP) [5]. This is a protein that travels from the neuronal cell body to the axonal periphery via fast transport mechanisms. If the axon is disrupted for any reason β-APP accumulates at the point of injury and a swelling develops secondary to cytoskeletal disruption [6]. Accumulation of β-APP has been detected within 2 h after TBI [7–9]; there is no literature relating to the timing of β-APP accumulation in other pathological causes of disruption of axonal transport.

Although the definition of DAI clearly defines the aetiology as being trauma [4] this term has been misused in the literature to describe axonal pathology of any cause. With the application of β-APP immunohistochemistry axonal damage has now been described in a variety of insults including trauma, infarction, HIV [10] and demyelination [11]. As a result current literature recommends that when axonal injury is being described the aetiology should be clearly defined and comment made as to whether the changes are focal or diffuse [12]. Therefore, the pathological entity originally called DAI is now known as diffuse TAI. However the clinico-pathological entity of DAI remains [12].

An important feature of the original definition of DAI was that it required a history of trauma before the diagnosis could be made [4]. In forensic practice, however, such a definite history is often not forthcoming, or the history proffered does not equate with the nature or severity of the injuries seen at *post-mortem*. Patterns of axonal injury have been described, which were interpreted as highly suggestive of trauma albeit in the absence of a definite history [9,13]. Indeed, the pattern of axonal injury and associated focal lesions has resulted in the definition of three grades of diffuse TAI, each of which is associated with increasingly severe neurological dysfunction [4]. The macroscopic distribution of axonal damage described in relation to trauma includes the corpus callosum, particularly posteriorly, the internal capsule and cerebellar peduncles. Axonal injury attributed to trauma at these sites, however, can be obscured by axonal injury secondary to the vascular complications of brain swelling: infarcts may involve the pericallosal artery (corpus callosum), basal ganglia including internal capsule and the brainstem secondary to compression and displacement. The brainstem damage is usually centrally placed when secondary to brain swelling but may be more extensive. Microscopic assessment can be useful in such cases. In trauma the damaged axons may be scattered or grouped together (Figure 1) but confined to individual white matter bundles. In ischaemic damage the axons show a linear or geographical pattern (Figure 2) not delineated to individual white matter bundles [9,13,14]. However, the authors have been aware of cases in which the characteristic pattern of axonal injury associated with trauma has been mimicked or obscured by the vascular complications of brain swelling and raised intracranial pressure (ICP). Axonal injury in our experience as a consequence of infarction in a swollen brain is common in forensic practice.

In forensic practice the recognition of diffuse TAI is particularly important as it signifies a rotational injury of considerable severity such as may been seen in a road traffic accident, a fall from a height, or an accelerated blow such as may be delivered by a punch or a kick [12]. Therefore, the present study was designed to test the hypothesis that patterns of axonal pathology, as detected by β-APP immunohistochemistry, could be reliably and reproducibly correlated to specific causes such as trauma or vascular injury.

Materials and methods

This study was approved by the Research Ethics Committee of the Southern General Hospital. Previously diagnosed cases were identified from the archives of the Department of Neuropathology, Institute of Neurological Sciences, Glasgow. The inclusion criteria included availability of clinical information including survival time between insult and death, known interval between death and *post-mortem* [*post-mortem* interval (PMI)], full neuropathology report and appropriate blocks of brain tissue embedded in paraffin wax; exclusion criteria were the converse. Cases were assigned to one of six clinical groups; diffuse TAI (17 cases), cardiac arrest (17 cases), hypoglycaemia (13 cases), status epilepticus (12 cases),

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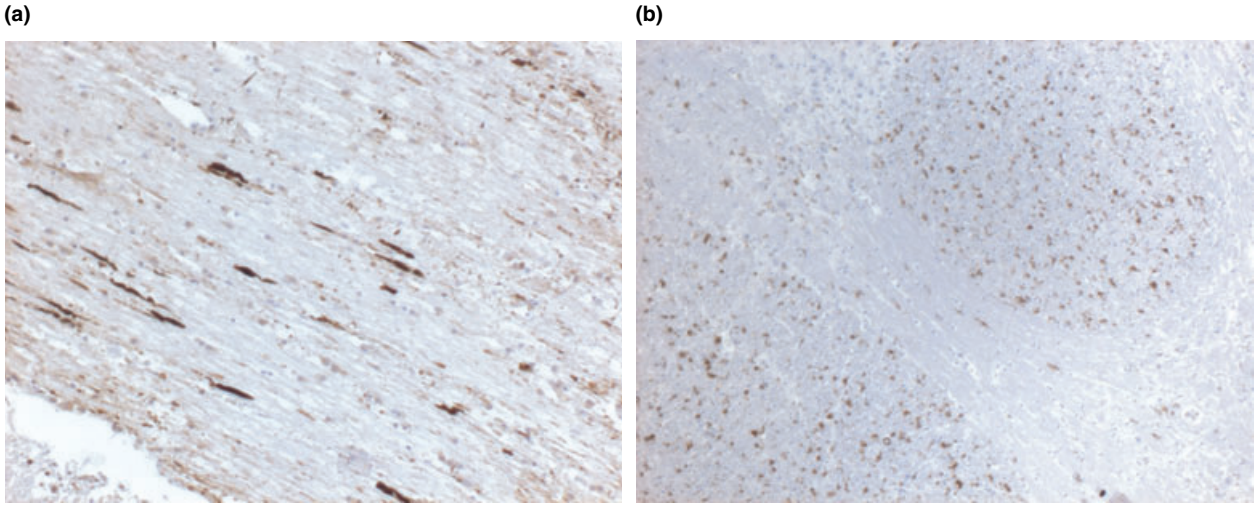

Figure 1. (**a**) β-APP immunohistochemistry demonstrating the characteristic distribution associated with trauma. The axons are scattered within the white matter although they are orientated along white matter bundles (× 200). (**b**) β-APP immunohistochemistry showing TAI confined to white matter bundles (× 50).

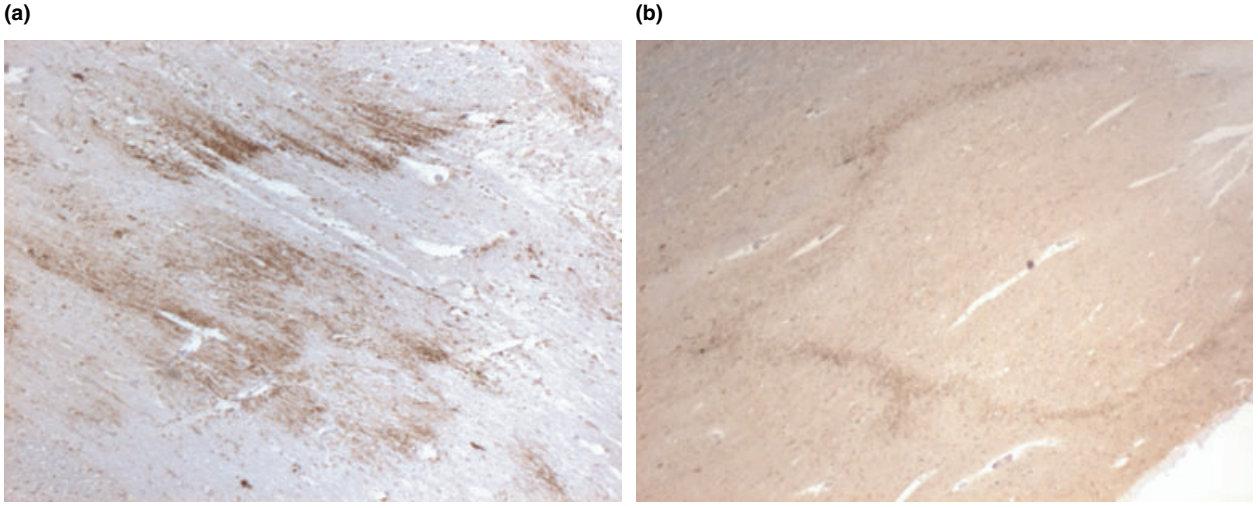

Figure 2. (**a**) β-APP immunohistochemistry demonstrating the characteristic distribution associated with ischaemia in a swollen brain. The axons are arranged in a linear pattern at the edge of the ischaemic tissue (× 50). (**b**) At a lower power the geographical nature of axonal injury secondary to ischaemia becomes apparent. (β-APP immunohistochemistry, ×20).

carbon monoxide poisoning (2 cases) and normal (controls) without neuropathological abnormality (12 cases). The details of the TAI cases are presented in Table 1. The full details of the other cases used in this study have been published elsewhere [14,15].

The histological sections examined from each case in this study were the parasagittal cortex including corpus callosum, internal capsule and hippocampus, all sampled at the level of the lateral geniculate body, the cerebellum including dentate nucleus and the pons including cerebellar peduncles. This represents the minimum recommended sampling when assessing diffuse TAI [1,12]. Tissue was fixed in 10% formal saline for 3–4 weeks prior to blocks being processed in a 60-h cycle (Bayer Diagnostics) and embedded in paraffin wax. Eight-micron-thick sections cut from each of the above named blocks were processed for immunohistochemistry. The sections were pretreated with formic acid (80% solution for 8 min) and subsequently immersed in a 0.3% hydrogen peroxide solution diluted in PBS for 30 min to neutralize endoge-

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Table 1. Dataset of cases previously diagnosed with diffuse traumatic axonal injury

Case	*Age (years)*	*Gender*	*Survival*	*PMI*	*TAI grade*	*Vent*	*Weight-g (swelling)*	*Hernia*	*BS haem*	*Raised ICP*	*Hypoxic brain damage*	*Cause of death*
1	47	M	36 h	16 h	3	–	1600 (–)	–	+	–	Widespread	Cardioresp. arrest
2	23	M	8 h	48 h	3	+	1620 (–)	–	+	–	Widespread	Head injury
3	73	F	4 weeks	24 h	3	–	1150 (–)	–	+	–	–	Pneumonia
4	76	M	5 weeks	23 h	1	–	1200 (–)	–	–	–	–	Pneumonia
5	17	M	2 weeks	84 h	3	+	1570 (–)	–	+	–	–	Pneumonia
6	41	M	5 h	39 h	3	–	1330 (–)	–	+	–	–	Head injury
7	23	M	4 weeks	82 h	2	+	1480 (–)	–	–	–	–	Pneumonia
8	46	M	48 h	1 weeks	1	–	1600 (–)	–	–	–	–	PTE
9	11	M	4 days	60 h	2	+	1580 (+)	–	–	+	Widespread	Head injury
10	17	M	3 days	72 h	3	+	1400 (–)	Axial	+	+	Hippocampi	Head injury
11	65	M	5 month	4 days	3	+	1440 (–)	–	–	–	–	Pneumonia
12	16	M	7 days	47 h	3	+	1640 (+)	Axial	+	+	Widespread	Head injury
13	9	F	10 h	56 h	3	+	1560 (+)	Axial	+	+	–	Head injury
14	31	M	27 days	4 days	1	+	1680 (–)	–	–	–	Hippocampi	PTE
15	15	F	8 days	23 h	3	–	1340 (+)	Axial	+	+	Widespread	Head injury
16	16	F	7 days	46 h	1	+	1500 (+)	Axial Tonsil	+	+	Widespread	Head injury
17	22	M	8 days	4 days	2	+	1490 (+)	–	–	+	Hippocampi	Pneumonia

PMI, *post-mortem* interval; TAI, traumatic axonal injury (Grade 1, 2 or 3); vent, ventilated; BS haem, brainstem haemorrhage; ICP, intracranial pressure; h, hours; PTE, pulmonary thrombo-embolism; swelling, present (+) or absent (–).

Table 2. Diffuse traumatic axonal injury cases

Case	*IHC diagnosis*	*Second diagnosis*	*Clinical brain swelling*	*Clinical diagnosis*
1	TAI 3	TAI 3	Absent	TAI 3
2	TAI 3 + ↑ICP	TAI 3 + ↑ICP	Absent	TAI 3
3	TAI 3	TAI 3	Absent	TAI 3
4	TAI 1	TAI 1	Absent	TAI 1
5	TAI 3	TAI 3	Absent	TAI 3
6	TAI 3	TAI 3	Absent	TAI 3
7	TAI 2	TAI 2	Absent	TAI 2
8	Normal	TAI 1	Absent	TAI 1
9	TAI 2	TAI 2	Absent	TAI 2
10	TAI 3 + ↑ICP	TAI 3 + ↑ICP	Present	TAI 3 + ↑ICP
11	Normal	TAI 3	Absent	TAI 3
12	TAI 2 + ↑ICP	TAI 2 + ↑ICP	Present	TAI 2 + ↑ICP
13	↑ICP	Metabolic + ↑ICP	Present	TAI 3 + ↑ICP
14	TAI 1	TAI 1	Absent	TAI 1
15	TAI 3 + ↑ICP	TAI 3 + ↑ICP	Present	TAI 3 + ↑ICP
16	TAI 1 + ↑ICP	TAI 1 + ↑ICP	Present	TAI 1 + ↑ICP
17	TAI 2 + ↑ICP	TAI 2 + ↑ICP	Present	TAI 2 + ↑ICP

IHC, immunohistochemistry; TAI 1, diffuse traumatic axonal injury grade 1; TAI 2, diffuse traumatic axonal injury grade 2; TAI 3, diffuse traumatic axonal injury grade 3; ↑ICP, raised intracranial pressure; +, and.

nous peroxide activity. Sections were incubated overnight at 4°C with a monoclonal antibody against the N-terminus of the human APP molecule (clone 22C11, Böehringer, Ingleheim, Germany; dilution 1 : 50) diluted in a PBS solution containing 2% horse serum, 0.3% triton X-100 and 0.1% sodium azide. Sections were then incubated with an antimouse biotinylated IgG antibody (Vector Laboratories, Peterborough, UK) for 1 h and the antibody complex revealed using the avidin biotin peroxidase method (Vector Elite kit, Peterborough, UK) using diaminobenzidine as a substrate. All sections were lightly counterstained with Meyer's haematoxylin. From each block a second section was cut at 8 microns and stained with H+E.

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Each case was reviewed 'blind' to the clinical diagnosis by each of the authors. After review of the immunostained sections a provisional pathological diagnosis was documented based on the distribution of axonal injury. The H+E stained sections were then reviewed and a second pathological diagnosis was made based on both the H+E appearances of the brain and the distribution of β-APP axonal immunoreactivity. The pathological diagnoses were then compared with the clinical diagnosis for assessment of the specificity of the patterns of axonal injury (Tables 2–7).

Results

A comparison of pathological diagnoses to clinical diagnoses for each group is presented in Tables 2–7. An overview of the results is presented in Table 8.

Diffuse TAI

Seventeen cases of diffuse TAI were included in the study. A correct diagnosis of diffuse TAI +/– raised ICP was made from assessment of β-APP stained slides in 13/17 cases. Of

Table 3. Cardiac arrest cases

Case	*Survival time*	*IHC diagnosis*	*Second diagnosis*	*Clinical brain swelling*	*Clinical diagnosis*
1	3 h	Normal	Ischaemia	Absent	Cardiac arrest
2	10 h	Normal	Ischaemia	Absent	Cardiac arrest
3	20 h	↑ICP	Ischaemia + ↑ICP	Present	Cardiac arrest + ↑ICP
4	23 h	↑ICP	Ischaemia + ↑ICP	Present	Cardiac arrest + ↑ICP
5	24 h	Normal	Ischaemia	Absent	Cardiac arrest
6	26 h	Normal	Ischaemia	Absent	Cardiac arrest
7	47 h	↑ICP	Ischaemia + ↑ICP	Present	Cardiac arrest + ↑ICP
8	47 h	↑ICP	Ischaemia + ↑ICP	Present	Cardiac arrest + ↑ICP
9	3 days	Normal	Ischaemia	Absent	Cardiac arrest
10	4 days	Normal	Ischaemia	Absent	Cardiac arrest
11	4 days	Normal	Ischaemia	Absent	Cardiac arrest
12	5 days	Normal	Ischaemia	Absent	Cardiac arrest
13	5 days	↑ICP	Ischaemia + ↑ICP	Present	Cardiac arrest + ↑ICP
14	6 days	Normal	Ischaemia	Absent	Cardiac arrest
15	3 days	↑ICP	Ischaemia + ↑ICP	Present	Cardiac arrest + ↑ICP
16	2 days	↑ICP	Ischaemia + ↑ICP	Present	Cardiac arrest + ↑ICP
17	4 days	↑ICP	Ischaemia + ↑ICP	Present	Cardiac arrest + ↑ICP

IHC, immunohistochemistry; ↑ICP, raised intracranial pressure; +, and.

Table 4. Hypoglycaemia cases

Case	*Survival time*	*IHC diagnosis*	*Second diagnosis*	*Clinical brain swelling*	*Clinical diagnosis*
1	10 h	Normal	Hypoglycaemia	Absent	Hypoglycaemia
2	14 h	TAI 1	Hypoglycaemia	Present	Hypoglycaemia + ↑ICP
3	23 h	Normal	Hypoglycaemia	Absent	Hypoglycaemia
4	31 h	↑ICP	Hypoglycaemia + ↑ICP	Absent	Hypoglycaemia
5	48 h	↑ICP	Hypoglycaemia + ↑ICP	Present	Hypoglycaemia + ↑ICP
6	3 days	TAI 1 + ↑ICP	Hypoglycaemia + ↑ICP	Present	Hypoglycaemia + ↑ICP
7	5 days	TAI 1 + ↑ICP	Hypoglycaemia + ↑ICP	Present	Hypoglycaemia + ↑ICP
8	5 days	↑ICP	Hypoglycaemia + ↑ICP	Present	Hypoglycaemia + ↑ICP
9	5 days	TAI 1	Hypoglycaemia	Absent	Hypoglycaemia
10	7 days	Normal	Hypoglycaemia	Absent	Hypoglycaemia
11	14 days	Normal	Hypoglycaemia	Absent	Hypoglycaemia
12	21 days	TAI 1	Hypoglycaemia	Absent	Hypoglycaemia
13	26 days	↑ICP	Hypoglycaemia + ↑ICP	Absent	Hypoglycaemia

IHC, immunohistochemistry; TAI 1, diffuse traumatic axonal injury grade 1; ↑ICP, raised intracranial pressure; +, and.

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Table 5. Status epilepticus cases

Case	*Survival time*	*IHC diagnosis*	*Second diagnosis*	*Clinical brain swelling*	*Clinical diagnosis*
1	6 h	Normal	Ischaemia	Absent	Status epilepticus
2	18 h	↑ICP	Ischaemia + ↑ICP	Absent	Status epilepticus
3	22 h	↑ICP	Ischaemia + ↑ICP	Absent	Status epilepticus
4	23 h	Normal	Ischaemia	Absent	Status epilepticus
5	4 days	Normal	Ischaemia	Absent	Status epilepticus
6	4 days	↑ICP	Ischaemia + ↑ICP	Present	Status epilepticus + ↑ICP
7	4 days	Normal	Ischaemia	Absent	Status epilepticus
8	4 days	↑ICP	Ischaemia + ↑ICP	Present	Status epilepticus + ↑ICP
9	6 days	↑ICP	Ischaemia + ↑ICP	Present	Status epilepticus + ↑ICP
10	7 days	Normal	Ischaemia	Absent	Status epilepticus
11	8 days	↑ICP	Ischaemia	Present	Status epilepticus + ↑ICP
12	15 days	Normal	Ischaemia	Absent	Status epilepticus

IHC, immunohistochemistry; ↑ICP, raised intracranial pressure; +, and.

Table 6. Carbon monoxide poisoning cases

Case	*Survival time*	*IHC diagnosis*	*Second diagnosis*	*Clinical brain swelling*	*Clinical diagnosis*
1	5 days	TAI 1	Ischaemia	Absent	CO poisoning
2	7 h	Normal	Ischaemia	Absent	CO poisoning

IHC, immunohistochemistry; TAI 1, diffuse traumatic axonal injury grade 1; CO, carbon monoxide.

Table 7. Cases with no significant neurological abnormality

Case	*IHC diagnosis*	*Second diagnosis*	*Clinical brain swelling*	*Clinical diagnosis*
1	Normal	Normal	Absent	No significant abnormality
2	Normal	Normal	Absent	No significant abnormality
3	Normal	Normal	Absent	No significant abnormality
4	Normal	Normal	Absent	No significant abnormality
5	Normal	Normal	Absent	No significant abnormality
6	Normal	Normal	Absent	No significant abnormality
7	Normal	Normal	Absent	No significant abnormality
8	Normal	Normal	Absent	No significant abnormality
9	Normal	Normal	Absent	No significant abnormality
10	Normal	Normal	Absent	No significant abnormality
11	Normal	Normal	Absent	No significant abnormality
12	Normal	Normal	Absent	No significant abnormality

IHC, immunohistochemistry.

the four remaining cases one (case 11) was correctly diagnosed after subsequent assessment of the H+E stained sections. In this case the survival was such (5 months) that no β-APP positive axons remained. Instead the diagnosis was made by the distribution of macrophages throughout the white matter indicating a pattern of Wallerian degeneration consistent with diffuse TAI. In a second case (case 13) the pattern of β-APP was thought to be most consistent with infarction secondary to raised ICP. H+E stained sections demonstrated global ischaemia. As such this case was thought to represent axonal injury secondary to infarction associated with brain swelling. However, on review the clinical history was more consistent with high grade diffuse TAI complicated by raised ICP. The third case (case 2) was thought on both immunohistochemistry and H+E stained sections to represent diffuse TAI grade 3 with raised ICP. Raised ICP was not documented in the original clinicopathological diagnosis as ICP had not been mea-

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Table 8. Summary of results

Clinicopathological diagnosis	*Total number of cases*	*Total β-APP diagnosis consistent with CPC DX*	*Total β-APP & H+E diagnosis compared with CPC DX*
TAI	17	13 (76%)	14 (82%)
Cardiac Arrest	17	0 (0%)	17 (100%)
Hypoglycemia	13	0 (0%)	8 (62%)
Status epilepticus	12	0 (0%)	10 (83%)
CO poisoning	2	1 (50%)	1 (50%)
Normal	12	12 (100%)	12 (100%)
All Cases	73	26 (36%)	62 (85%)

β-APP, beta-amyloid precursor protein; CPC, clinicopathological; DX, diagnosis; H+E, haematoxylin and eosin; TAI, diffuse traumatic axonal injury; CO, carbon monoxide.

sured during life and *post-mortem* examination of the brain did not show any definite evidence of raised ICP [16]. This, however, in our opinion does not exclude the possibility of raised ICP during life sufficient to cause vascular complications. In this regard we strongly recommend that at *post-mortem* the pathologist assess the tightness of the dura as this may be the only evidence that ICP has been raised in life. The final case (case 8) showed no β-APP immunoreactivity although eosinophilic spheroids, thought to represent axonal swellings, were seen on H+E. Although the clinicopathological diagnosis was of diffuse TAI grade 1 there was some uncertainty regarding the clinical diagnosis at the time of the original report.

Cardiac arrest

Seventeen cases of cardiac arrest were examined. The amount and distribution of ischaemic damage was assessed in the H+E stained sections. In cases of short survival the features were those of the ischaemic cell process. In longer surviving cases the features were largely those of reactive changes in microglia and astrocytes and the presence of macrophages. In the cases surviving for many weeks or months the appearances were those of cavitation and scarring attributed to gliosis as seen for example in sclerosis of the hippocampus. Examination of β-APP stained sections resulted in a diagnosis of no significant abnormality (nine cases) in which there was no immunostaining, or a diagnosis of infarction secondary to raised ICP (eight cases). Examination of H+E stained sections in conjunction with the β-APP stained sections resulted in the diagnosis of global cerebral ischaemia +/− raised ICP. The clinical history was required to make the diagnosis of cardiac arrest in all cases.

Hypoglycaemia

Thirteen cases of hypoglycaemia were examined. Examination of β-APP stained sections resulted in a diagnosis of no significant abnormality (four cases), diffuse TAI grade 1 (three cases), diffuse TAI grade 1 with raised ICP (two cases) and raised ICP (four cases). Examination of H+E stained sections demonstrated the distribution of irreversible neuronal damage in keeping with hypoglycaemia in all the cases [15,17]. In each there was laminar necrosis of the neocortical ribbon, which was maximal in the superficial layers. Necrosis of dentate fascia, CA1 and CA4 sectors of the hippocampus was also seen, and there was variable involvement of thalamic nuclei. In contrast, the Purkinje cell complement in the cerebellum was entirely normal and no abnormalities were seen in the nuclei of the brainstem. Therefore, assessment of β-APP stained sections in association with H+E stained sections resulted in correct clinicopathological correlation in six cases. In two cases (cases 4 and 13) there was a pattern of β-APP immunoreactivity suggestive of raised ICP but no clinical documentation of raised ICP, similar to cases 2 and 3 of the status epilepticus group and case 2 of the diffuse TAI group. In five cases (cases 2, 6, 7, 9 and 12) the pattern of β-APP immunoreactivity suggested diffuse TAI grade 1 with raised ICP being superimposed in two (cases 6 and 7).

Status epilepticus

Twelve cases of status epilepticus were examined. Microscopy of β-APP stained sections resulted in a diagnosis of no significant abnormality (six cases) or raised ICP (six cases). Examination of H+E stained sections demonstrated global cerebral ischaemia in all cases. The microscopic

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diagnosis was therefore of ischaemic encephalopathy in all cases with associated raised ICP in six cases. The clinical history was required to make the diagnosis of status epilepticus in all cases. In two cases (cases 2 and 3) the pattern of β-APP immunoreactivity and H+E analysis suggested a diagnosis of global ischaemia with raised ICP but raised ICP was not described clinically and was not noted at neuropathological examination of the brain. These cases are similar to case 2 of the diffuse TAI group in that β-APP immunoreactivity suggested raised ICP but this was not recognized clinically.

Carbon monoxide poisoning

Two cases of carbon monoxide poisoning were examined. Examination of β-APP stained sections resulted in a diagnosis of diffuse TAI grade 1 (one case) or no significant abnormality (one case). Examination of H+E stained sections showed global cerebral ischaemia in both cases. The clinical history was required in both cases to make the diagnosis of carbon monoxide poisoning.

No significant abnormality

Twelve cases without significant neurological abnormality were examined in this study. No β-APP staining was seen in any of the cases, and H+E stained sections revealed no abnormality. All cases were correctly diagnosed as normal.

Discussion

The neuropathologist can be confronted with medicolegal autopsies in which there is no clinical history and either minimal or no gross *post-mortem* or neuropathological findings. In these situations the pathologist may think it appropriate to thoroughly sample the central nervous system and, with the aid of suitable stains, attempt to identify and define the nature (and maybe the cause) of any pathology. In these cases microscopic examination may reveal a metabolic abnormality or provide evidence of trauma. This study was designed to assess the value and the limitations of β-APP immunohistochemistry by evaluating the usefulness of the published descriptions of traumatic, ischaemic and metabolic axonal pathology in determining causation.

The clinicopathological entity of DAI has evolved over many years of clinical and experimental research. The relatively recent application of the immunohistochemical stain β-APP has improved the evaluation of axonal injury. The diagnosis of diffuse TAI can now be made in patients surviving 2–3 h after injury. However, as experience with β-APP immunostaining has increased so have the number of conflicting reports about the interpretation of axonal injury. It is well known that β-APP is a nonspecific marker of axonal injury resulting in problems differentiating between axonal injury attributed to trauma, ischaemic injury or other metabolic causes. Some researchers have reported that hypoxia alone will produce axonal injury in a pattern that may mimic trauma [18], although other authors have not been able to reproduce this finding [14].

In our study a total of 73 cases were examined with both β-APP immunohistochemistry and H+E staining. The observers first recorded a diagnosis based on β-APP immunoreactivity, and then recorded a second diagnosis based on subsequent examination of H+E stained sections. Using β-APP immunohistochemistry the observers were able to correctly separate only 26 of 73 cases into traumatic, metabolic or no significant abnormality groups. When H+E stained sections were then reviewed 62 of 73 cases were correctly classified into one of these three groups. Therefore, examination of β-APP and H+E stained sections together, in an appropriately sampled brain, allowed a diagnosis to be made in 85% of cases.

The β-APP immunohistochemistry in diffuse TAI suggests that, when considered with the anatomical distribution, the microscopic patterns of axonal injury described are reliable indicators of traumatic injury. In only one case, which was typical of the clinical entity DAI, was diffuse TAI obscured by axonal pathology secondary to the vascular complications of brain swelling. In such cases, where no clinical history is available, it is important that the pathologist is aware of the limitations of β-APP immunohistochemistry and neither confirms nor denies the presence of diffuse TAI.

In one case (TAI case 8) axonal spheroids were seen with H+E staining but no β-APP immunoreactivity was seen. There is increasing awareness of different mechanisms of axonal damage in TAI [19], some of which do not show β-APP immunoreactivity. These studies have yet to impact on diagnostic practice but in future a panel of antibodies may be required to fully assess TAI [20].

In cases of ischaemic and hypoglycaemic (metabolic) encephalopathy the microscopic pattern of β-APP immu-

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noreactivity was useful as a marker of raised ICP. H+E stained sections were required to demonstrate the pattern of neuronal injury, which allowed differentiation between ischaemia and hypoglycaemia. The clinical history was required to differentiate between cardiac arrest, status epilepticus and carbon monoxide poisoning in cases of ischaemic encephalopathy. In some cases, however, β-APP immunoreactivity demonstrated a pattern that was considered to be indicative of raised ICP but there had been no clinical history of raised ICP or definite pathological evidence of cerebral swelling or herniation. In all these cases ICP was not directly measured during life and therefore no definitive comments can be made in relation to raised ICP. It is possible that β-APP immunoreactivity is a useful marker of early stages of raised ICP with associated vascular complications developing before there is macroscopic evidence of brain herniation.

In hypoglycaemic encephalopathy five cases showed a pattern of axonal injury that was considered to be consistent with diffuse TAI grade 1. On review of the H+E sections it became clear, however, that the principal pathology was that of hypoglycaemia. Therefore, there does appear to be a pattern of axonal pathology associated with hypoglycaemia that is not of a typically ischaemic pattern and can mimic trauma. This confirmed the findings of a previous study [15]. The pattern of β-APP immunoreactivity in one case of carbon monoxide poisoning also resembled diffuse TAI grade 1.

Therefore while β-APP immunohistochemistry was of value in demonstrating patterns of axonal damage that could be interpreted as being secondary to trauma or the vascular complications of raised ICP, it should not be interpreted in isolation. Some insults, in particular hypoglycaemia, can produce a pattern of injury that mimics trauma, but assessment of H+E stained sections should allow the correct diagnosis to be made. This highlights the importance of examining both immunohistochemical preparation and H+E stained sections from every case. H+E stained sections allowed a diagnosis of metabolic brain injury, either ischaemic or hypoglycaemic, to be made in each case, and provided essential information in one case of diffuse TAI.

In summary, this study has demonstrated that when the brain is appropriately sampled the described patterns of axonal injury seen after trauma and vascular complications of raised ICP are distinguishable except in very extreme cases of brain swelling. In such cases we suggest that the pathologist is unable to comment on the presence or absence of diffuse TAI. Hypoglycaemia and carbon monoxide poisoning can produce a pattern of axonal injury that may mimic diffuse TAI grade 1, but assessment of H+E stained sections will demonstrate lesions typical of these entities. β-APP immunohistochemistry may also highlight vascular complications of raised ICP in brains that are only mildly swollen. When available brain examination should always be in conjunction with the clinical history. However, when the history is not available or is unreliable, β-APP immunohistochemistry can provide useful information in relation to the type of brain injury.

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