Neuropathology and Applied Neurobiology 1997, 23, 339–347

The diagnosis of diffuse axonal injury: implications for forensic practice

J. F. Geddes, G. H. Vowles, T. W. Beer* and D. W. Ellison*

*Department of Morbid Anatomy, Royal London Hospital, Whitechapel, London, and *Department of Histopathology, Southampton General Hospital, Southampton, UK*

J. F. Geddes, G. H. Vowles, T. W. Beer and D. W. Ellison (1997) *Neuropathology and Applied Neurobiology* 23, 339–347

The diagnosis of diffuse axonal injury: implications for forensic practice

The diagnosis of diffuse axonal injury (DAI), which may be of considerable importance in forensic medicine, necessitates widespread sampling of the brain for histology. Because a limited sampling method for screening brains for axonal damage would be of value for medicolegal work, the authors have tested the findings of an earlier study which suggested that a standard set of three blocks from above and below the tentorium could reliably be used in routine practice as a basis for the diagnosis of DAI. A series of 22 previously diagnosed cases of DAI, with a range of survival times, was studied using immunohistochemistry with antibodies to β-amyloid precursor protein (βAPP), the microglial-associated antigen CD68 (PG-M1) and for GFAP. Strict histological criteria were used to assess traumatic damage, and the evolution of the histological changes with increasing survival is described. In four cases, the sampling scheme employed yielded evidence of axonal damage in only one block, and a diagnosis of DAI could have been made in only 13/22 cases. In six of the shortest surviving cases, βAPP positivity in the corpus callosum and brainstem outlined areas of early ischaemia, as well as of traumatic damage, so that interpretation of immunolabelling was not always clear-cut. The findings suggest that DAI cannot be reliably diagnosed on a restricted number of blocks from vulnerable areas, and that the use of βAPP and PG-M1 immunocytochemistry may bring interpretative problems that can only be resolved by taking a larger series of tissue samples for histology.

Keywords: head injuries, diffuse axonal injury, β-amyloid precursor protein, forensic medicine

Introduction

Traumatic white matter damage may occur in mild [8, 15, 18, 22, 25] as well as severe [1] head injury; indeed, it is likely that some damage to axons is present in most fatal head injuries [12, 14]. It appears that there is a spectrum of axonal injury [24, 26], not only at the level of the cellular changes involved but also in terms of the extent of involvement of brain regions. This means in the mildest cases there may be only temporary 'concussive' damage to axons, while secondary axotomy is usual in more severe injury [10, 13]. Similarly, the more severe the head injury, the more likely it is that lesions will be found in the internal capsule, cerebellum and brainstem as well as in the hemispheric white matter [29]. The axonal pathology is by definition *diffuse*, and commonly referred to as 'DAI', when evidence of widespread traumatic damage to axons is detected in cerebral hemispheres, corpus callosum and brainstem; involvement of the cerebellum is variable [2]. The presence of DAI may be inferred from the clinical picture, from radiological findings such as haemorrhages in the corpus callosum and dorsolateral quadrant of the rostral brainstem [9], and — using the same criteria — from macroscopic examination of the sliced brain [17] but the diagnosis can only be confirmed after microscopic examination

Correspondence: Dr J. F. Geddes, Department of Morbid Anatomy, Royal London Hospital, London E1 1BB, UK.

of blocks from a number of different regions of the brain.

While head injury is a common cause of death in the general population, the majority of cases are medico-legal (coroner's) autopsies, and most of the brains are not sent for specialist neuropathological examination. Many are cut fresh, and a diagnosis made on the basis of the macroscopic appearances alone. In some cases of head injury, however, axonal damage may be the only pathological change attributable to trauma, and will be missed if blocks are not taken for histology. The diagnosis of DAI may be of crucial importance in such instances — for example, when there has been prolonged post-traumatic coma in the absence of an intracranial mass lesion. Because a full neuropathological examination is expensive, it would be useful in such cases to have a reliable and cheap way of 'screening' the fixed brain, at sites known to be at risk from such damage. The findings of a recent study of the distribution of pathology in 22 cases of DAI by Ng *et al*. [23] suggested that the diagnosis might be made on limited sampling of corpus callosum, internal capsule and cerebellar peduncles, since these were the sites in which axonal damage was most frequently found.

The ability to date histological changes due to trauma may also be important in forensic work. The evolution of the pathology of DAI has been described in large series by Adams and his colleagues, who used conventional methods such as haematoxylin and eosin (H&E), silver stains, cresyl fast violet and Marchi preparations [1, 2]. However, recent advances in the understanding of the subcellular events in DAI have resulted in the identification of more sensitive indicators of axonal damage, notably β-amyloid precursor protein (βAPP) [27, 28]. Immunocytochemistry with antibodies to βAPP permits detection of axonal damage with only 2 h survival [21]; the technique has now passed into routine neuropathological diagnosis of head injury, although expression of βAPP is also seen in axonal damage due to other causes. In cases of fatal head injury, in which βAPP has been shown to be a reliable marker for axonal damage [21], work has mainly concentrated on the early identification of such pathology. There has not yet been a study of the evolution of the staining patterns of βAPP in DAI with survival beyond a few hours.

Identification of the cellular response to axonal damage, particularly that of microglia, is also used in the diagnosis of DAI, since with long survival microglial clusters may be the only diagnostic histological feature. Before the development of antibodies to specific microglial antigens, the identification of microglial aggregates in DAI was undertaken using cresyl fast violet on thick sections [2]. Using immunocytochemistry, Aihara and colleagues have demonstrated early microglial activation in mild head injury in the rat [3], while preliminary work has suggested that there is a similar early microglial response in human head injury [4]. To date there has not been an immunohistochemical study of the evolution of the microglial response to trauma.

In the present study we reinvestigate a series of 22 previously diagnosed cases of DAI with known survival times, to see whether a standard set of three blocks taken from above and below the tentorium, in the areas suggested by Ng *et al*. [23], provides a reliable basis for the diagnosis of DAI. For this work, immunocytochemical markers were used to enhance the detection of axonal pathology and cellular reactions to it. In the course of testing the hypothesis on cases with a range of survival times, we became aware that a descriptive study of the evolution of changes seen using these markers would be possible, a description that might eventually — with experience of larger numbers of cases — provide the basis of a more precise scheme for dating DAI than is possible with traditional stains.

Materials and methods

Twenty-two cases of fatal non-missile head injury, previously diagnosed by two neuropathologists (J.F.G. and D.W.E.) as showing DAI, were chosen from the autopsy files of the Royal London and Southampton General Hospitals to give a wide range of survival times. There were 16 males and six females, age range 8–93 years, and the aetiology of the head injury varied: assault (eight), road traffic accident (eight), fall from a height (six). Survival times ranged from 5 h to 4 years. Relevant clinical details are given in Table 1, together with the original grade of DAI assigned to the case, using the criteria of Adams *et al*. [2].

In all cases, the initial diagnosis had been made with the aid of a set of blocks cut from all major regions of the cerebrum, cerebellum and brainstem, sliced after formalin fixation. The routine neuropathological stains and immunohistochemistry considered appropriate at the time of diagnosis had been used. For the present study, a set of three standard blocks — (i) corpus callosum with

Table 1. Clinical details of DAI cases

Case no	*Sex/age*	*Type of head injury*	*Survival time*	*Large intracranial haemorrhage*	*DAI grade**	*Evidence of mass effects /↑ICP*	*Significant ischaemic damage*
1	M32	Fall 30 ft	5 h	No	3	Yes	BZ infarct
2	M23	Assault	24 h	EDH	2	Yes	L pca infarct
3	M48	Assault	36 h	No	3	No	No
4	M25	Assault	36 h	No	3	Yes	No
5	M59	Fall 30 ft	2 days	SDH & ICH	2	Yes	No
6	F18	Accelerated fall	21/2 days	No	3	Yes	Brainstem infarct
7	M39	Assault	21/2 days	ICH	3	Yes	No
8	M28	Fall 15 ft	3 days	Bilateral ICH	3	Yes	No
9	M23	Assault	5 days	ICH	1	Yes	No
10	M43	Fall 30 ft	8 days	No	2	No	No
11	M56	RTA	10 days	SDH & ICH	3	Yes	L mca infarct
12	M63	RTA	11 days	No	3	No	Diffuse neocortical
13	M36	RTA	12 days	No	3	No	Diffuse neocortical
14	F21	Fall 30 ft	2 weeks	EDH	3	No	Diffuse neocortical & putamen
15	F88	Assault	7 weeks	No	1	No	No
16	F93	Assault	7 weeks	No	1	No	No
17	M60	RTA	3 months	No	1	No	Cerebellar infarct
18	F30	RTA	4 months	No	1	No	No
19	M66	Assault	5 months	No	1	No	No
20	M8	RTA	18 months	No	—	No	No
21	F21	RTA	2 years	No	—	No	No
22	M43	RTA	4 years	ICH	—	No	No

*According to the criteria of Adams *et al*. [2]. ↑ICP = raised intracranial pressure; BZ = boundary zone; pca = posterior cerebral artery; mca = middle cerebral artery; EDH = extradural haemorrhage; SDH = subdural haemorrhage; ICH = intracerebral haemorrhage; RTA = road traffic accident.

parasagittal white matter, (ii) posterior limb of the internal capsule, and (iii) rostral brainstem, usually pons, to include the superior cerebellar peduncle — was selected from each case. Where one of these regions had not been included in the original set of blocks used for diagnosis, a new block was cut. Ischaemic damage and/or intracerebral haemorrhage had previously been diagnosed in eight of the 22 cases (see Table 1), and care was taken to avoid areas related to macroscopic foci of haemorrhage or infarction. In three brains (Cases 12–14, survival 11 days to 2 weeks) diffuse neocortical damage was present. In each of these cases, however, the ischaemic damage appeared histologically to be recent (approximately 24 h or less), and so unrelated to the axonal damage in the white matter. In a further five cases in which there were vascular lesions, blocks were taken away from areas of haemorrhage or infarction.

The three sections from each case were stained with haematoxylin and eosin (H&E), and immunohistochemical stains for βAPP (Boehringer, monoclonal, 1:200), the microglial marker CD68/PG-M1 (Dako, monoclonal, 1:200) and glial fibrillary acidic protein (GFAP, Dako, polyclonal, 1:1000). Immunostaining with each antibody was carried out using a standard avidin–biotin complex, peroxidase-labelling method. Microwave antigen retrieval was used for βAPP and PG-M1.

The reduced set of three blocks was examined from each case. The working histological criteria for assessing the presence of traumatic axonal damage were:

(*short-survival cases*) the presence of βAPP positivity in axons in a distribution that did not appear to represent early infarction. Thus isolated, scattered or small groups of positively stained axons were accepted as likely to be of traumatic aetiology, but *circumscribed* foci demarcated by βAPP immunoreactivity (as illustrated in Figure 1a–c), which might possibly be of vascular rather than traumatic origin, were discounted;
(*cases with >24 h survival*) visible axonal swellings or bulbs on H&E; βAPP positivity in axons in material in

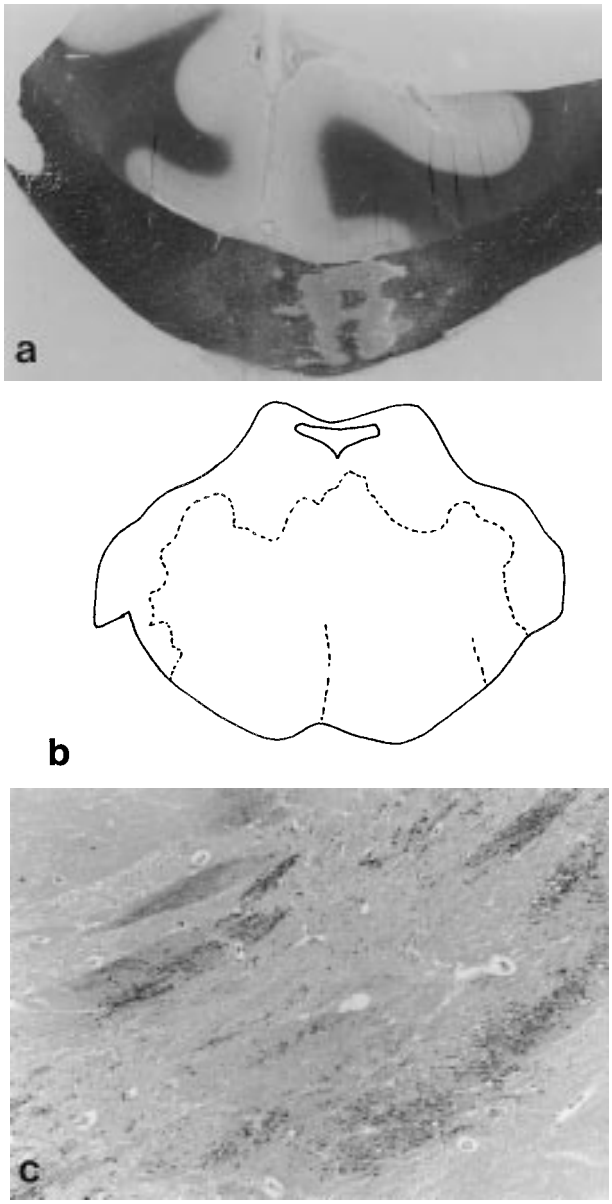

Figure 1. The distinction between traumatic and ischaemic axonal damage may be difficult on βAPP staining, but an area of βAPP positivity with an irregular outline is suggestive of a vascular lesion. a, The irregular shape that a corpus callosum infarct may take is clearly shown in Case 11, in which there was established infarction secondary to brain swelling (10 days survival, luxol-fast blue). b, A tracing of the pattern of βAPP staining in the pontine basis of a fatal head injury case (not included in the present series). The brain showed marked cerebral swelling but no evidence of axonal damage outside the pons. The dotted line marks a band of βAPP positivity around an area interpreted as being ischaemic, rather than traumatic, in origin. Similar smaller lesions, usually in the midline, were seen in the present series. c, Case 6. The neurons in this area in the pons depicted by βAPP immunolabelling showed ischaemic cytoplasmic changes on the H&E stained section.

which there was no infarction, haemorrhage or obvious ischaemic change; microglia around bulbs in the form of early clusters;
(*cases in which bulbs had disappeared*) microglial clusters in the white matter in a case in which there was no other pathology to account for their presence, with or without βAPP positivity;
(*cases with survival >1 year*) evidence of long tract degeneration (large numbers of foamy macrophages and astrocytosis confined to anatomical tracts), together with diffuse gliosis of hemispheric white matter, without significant hypoxic/ischaemic brain damage.

The diagnosis of DAI was considered to be established if all three blocks showed evidence of traumatic axonal damage — in other words, fulfilled the original definition of Adams *et al.* [2]. The cases were studied in order of survival, from the shortest to the longest interval between head injury and death, and the pathological changes documented. Assessment of cases was performed blind to previous findings.

Results

Using the diagnostic criteria given above, the yield of traumatic axonal pathology from the three test blocks was variable. Some evidence of white matter damage was found in each case. However, four cases (Cases 1, 8, 9, 11) failed to show any convincing evidence of traumatic axonal damage in two of the sample blocks taken, while axonal pathology was present in all three blocks in only 13 of the 22 cases.

A distinct evolution of changes defined by the three antibodies was seen as survival time increased, some of which are illustrated in Figure 2.

β-Amyloid precursor protein

Staining of the three blocks from the brain of the shortest survivor (Case 1, 5 h) revealed focal linear positivity in the pons only, where groups of axons bearing small axonal bulbs were seen. At 24 h survival (Case 2), the bulbs were large, and strongly positive. There were insufficient early cases to enable us to estimate how long the bulbs continue to enlarge, but it seemed that the size of the largest was the same in Cases 2–8 (24 h to 3 days survival). At 8 days survival (Case 10) a few of the bulbs in the corpus callosum and midbrain were pale, or not

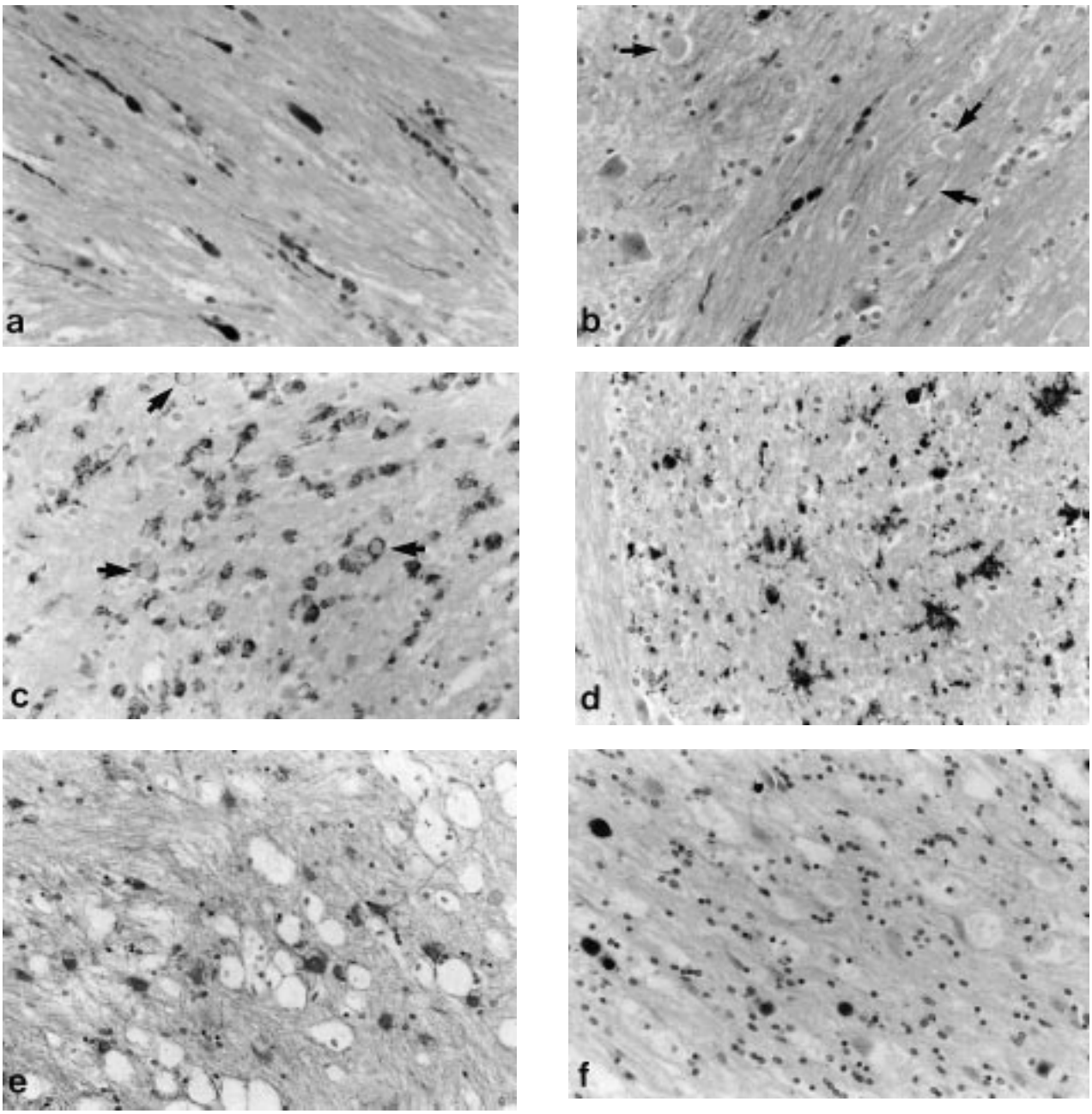

Figure 2. Aspects of the evolution of histological appearances with increasing survival. a, Axonal bulbs positive for βAPP in the internal capsule at 36 h (Case 4). b–d, Case 11, 10 days survival; b, βAPP staining of the pons, axonal positivity, but some bulbs (arrows) are no longer positive; c, PG-M1 staining, internal capsule, microglia aggregated around axonal bulbs (arrows); d, Microglial nodules have formed in the corticospinal tracts in the pons. e, Dense astrogliosis with macrophages in the internal capsule (GFAP, Case 20, 18 months survival). f, Case 21, 2 years survival: granular deposits of βAPP lie between axons in the degenerating corpus callosum. Magnification of all pictures: × 128.

staining, although adjacent axons were still strongly positive (Figure 2). In four cases of survival between 10 days and 2 weeks (Cases 11–14), variability of βAPP expression in axonal bulbs was quite marked in different parts of the same brain. In Case 14, for example, axons remained stained, but all the bulbs in the transverse fibres of the pons were negative, while those in the substantia nigra were still strongly positive. With longer

survival still, βAPP-immunoreactivity disappeared from axons as well as from bulbs. In one of the two cases with survival of 7 weeks (Cases 15 and 16), occasional axonal bulbs were detectable, and occasional small granular clumps of βAPP-positive material, some in macrophages, were found between axons which no longer stained (Figure 2). In four of the five longest surviving cases ($\geqslant 6$ months) none of this granular staining was found; however, in one case with 2 years survival (Case 21), scattered granular βAPP-positive debris, apparently extracellular, was detected in the severely atrophic corpus callosum (Figure 2).

Staining of neuronal cytoplasm with βAPP was noted in a few cases. In neocortical neurons, this positivity correlated with acute cytoplasmic changes (cytoplasmic shrinkage and eosinophilia) seen on H&E; in three other cases, positive-staining neurons were seen adjacent to swellings: cytoplasmic staining in the nuclei basis pontis was associated with bulbs (Case 13) or with profuse granular βAPP staining (Case 17), and in Case 14 neurons of the substantia nigra expressing βAPP were close to numerous axonal bulbs. In three of the oldest subjects (Cases 15, 16, 17), occasional neocortical senile plaques were demonstrated by the stain.

In six of the shortest surviving cases (Cases 2, 4, 5, 6, 7, 8), foci of wavy linear βAPP positivity with axonal bulbs were seen, typically in the corpus callosum and/or pons, demarcating circumscribed areas which in most cases showed no changes either on H&E or with a PG-M1 immunostain (Figure 1). In all six brains there was evidence of mass effect/raised intracranial pressure in the form of subfalcine and transtentorial herniation, and these foci of βAPP positivity were thought to represent early infarction secondary to brain swelling and/or shift, and to be unrelated to the traumatic damage seen elsewhere. In one such area neuronal nuclear pyknosis and cytoplasmic eosinophilia were seen on the corresponding H&E-stained section, suggesting that this was indeed an early ischaemic focus (Case 6). In five cases there appeared to be coexisting traumatic and vascular damage in the same blocks.

Microglial-associated antigen CD68/PG-M1

There were no easily detectable changes in either microglial morphology or numbers with survival up to 24 h in this series. At 36 h survival (Cases 3 and 4) there was a subjective increase in size and number of microglia, in areas where axons were damaged, and to a lesser extent in areas where there was no βAPP positivity. Aggregation of microglia around axonal bulbs (Figure 2) was first seen at 5 days (Case 9). Microglial clusters (stars) were first detected in this series at 10/11 days (Cases 11 and 12, Figure 2), and were present in considerable numbers at 2 weeks survival (Case 14). They were seen in all cases of survival between 7 weeks and 5 months, but were no longer identifiable in our one case who survived 18 months (Case 20); here they were obscured by large numbers of foamy macrophages through the white matter. In the eight longest surviving cases (Cases 15–22), the appearance of foamy macrophages together with diffuse microglial proliferation, in a strictly anatomical distribution in corpus callosum and long tracts, was a marker of secondary degeneration. This was confirmed in four cases (Cases 15, 16, 19 and 20) by Marchi stains on additional blocks, which showed myelin breakdown products in the same distribution.

Scattered microglial nodules seen in the hemispheric white matter of Case 4, who survived only 36 h, were thought to represent pre-existing traumatic axonal damage.

Glial fibrillary acidic protein

No changes were observed in the white matter on GFAP up to 5 days survival. Beyond 8 days survival there was marked reactive astrocytosis, but no aggregation of astrocytes round individual bulbs or areas of damage. However with survival of over 3 months, marked diffuse astrocytic gliosis was seen in degenerating corpus callosum, hemispheric white matter, internal capsule and long tracts (Figure 2e). The astrocytes were evenly spread through the white matter; there were no features that could be considered specific to DAI.

These findings, with survival times for the cases in the series, are summarized in diagrammatic form in Figure 3.

Discussion

Many of the more challenging problems encountered in forensic practice lie in the field of neuropathology. One such problem is DAI, which on occasion is the only significant pathology found after head injury, and which

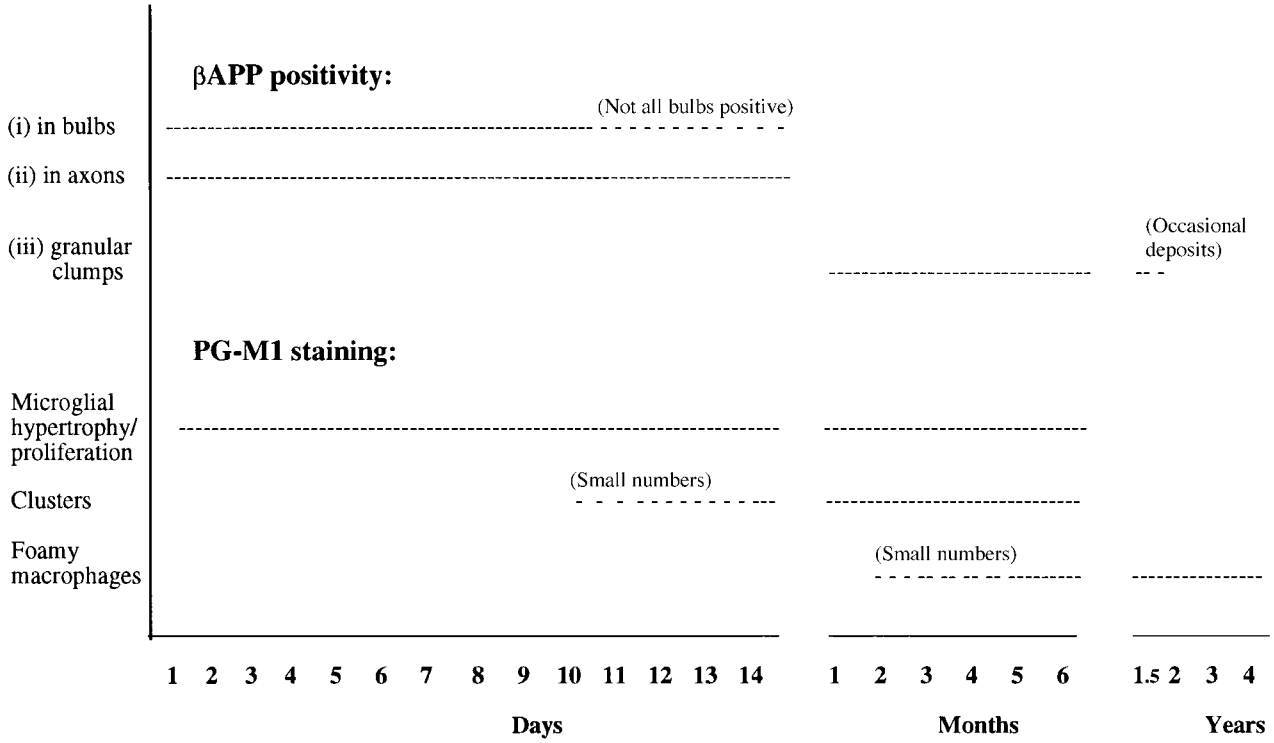

Figure 3. Diagrammatic representation of the evolution of staining patterns seen with anti-βAPP and anti-CD68 (PG-M1) in this series.

is (by definition) a diagnosis only made after widespread sampling of the brain for histology. The idea of a simple sampling technique that could be used for 'screening' head-injured brains for DAI would be attractive for those who routinely examine large numbers of head injuries for medico-legal purposes. However, this small study shows that even when blocks are taken from areas vulnerable to traumatic axonal damage, diagnostic pathology may be missed. While evidence of white matter damage was found in every case, in only 13 (59%) could we have made a diagnosis of DAI as originally defined — that is, in cerebral hemispheres, corpus callosum and brainstem [2] — using the three test blocks. In four cases there was evidence of axonal damage in only one block, despite the fact that the original examination of multiple blocks had shown involvement of areas above and below the tentorium. The posterior limb of the internal capsule showed evidence of axonal damage or long tract degeneration in 18 cases, but the most consistently useful block was the one from the rostral brainstem, which displayed traumatic axonal injury in either the superior cerebellar peduncle or the transverse pontine fibres/middle cerebellar peduncle in all but two cases.

The clear conclusion from this work is that a larger number of samples must be taken in order to diagnose DAI. But how many, and from which areas? This is a question that needs to be addressed, particularly if patterns of axonal injury may vary from patient to patient, according to forces involved in the injury [17]. We conclude that a *minimum* set of samples should include two blocks of corpus callosum (anterior and posterior) together with adjacent parasagittal white matter, one block of the posterior limb of the internal capsule, one block of cerebellar hemispheric white matter with middle cerebellar peduncle, and at least one of the upper brainstem, preferably to include the superior cerebellar peduncle. Such a set would cover all the principal anatomical sites affected by traumatic axonal injury [1, 23], and so decrease the possibility of missing the diagnosis.

The present study shows not only that it is possible to underdiagnose DAI when it is in fact present, but that it might also be possible to overdiagnose the condition, particularly in cases of short survival, if βAPP positivity were interpreted uncritically. The use of βAPP immunocytochemistry means that it is now possible to detect axonal damage at an extremely early stage [6, 21, 27],

well before changes can be demonstrated by any other stain. However, βAPP positivity merely indicates interruption to fast intra-axonal transport [28], regardless of aetiology, and it is important to exclude other causes of white matter pathology before attributing changes seen in axons to trauma. In the present study, terminal vascular events, usually secondary to brain swelling, have been frequently seen, and may be the most problematic. As a result of brain swelling and shift, vessels become compromised, and secondary infarction in the corpus callosum and deep grey matter, particularly the territory of the anterior choroidal artery, occurs. Vascular damage to the brainstem is extremely common in the presence of raised intracranial pressure [19]. Awareness of this fact will prevent overinterpretation of βAPP immunoreactivity when the staining pattern appears to outline an area that could be vascular in origin. Similarly, sampling and diagnosis of traumatic axonal damage should take account of — and preferably avoid — established areas of haemorrhage and infarction, at the edges of which there will inevitably be disruption of axonal transport and hence upregulation of βAPP expression. In the present study, care was taken with long-surviving cases to exclude long-tract degeneration which might be due to infarction, rather than DAI.

Even if it appears clear that βAPP expression in axons is likely to be traumatic in origin, it is difficult to know what significance to put on the finding at a very early stage — i.e. at a time before secondary axotomy could have occurred. Recent work suggests that axonal disconnection may not be an inevitable consequence of injury, and that some damage may be repaired [12]. Given the likelihood that some form of axonal pathology underlies most concussive head injuries [11], care should be taken before interpreting early traumatic axonal damage — even if widespread — as irreversible and thus as confirmation of the *severity* of a head injury. It is possible only with fully developed swellings that one can be certain that the detected damage is irreversible.

The evolution of the microscopical changes as revealed by βAPP, CD68 and GFAP are described in this study. While the temporal sequence of changes (Figure 3) was similar to that originally described for traditional staining methods by Adams *et al*. [2], the numbers of brains examined for each period of survival do not enable us to give more than an approximate indication of the timing of changes. Indeed, even in this relatively small series using a small number of blocks, it appeared that progression is not necessarily uniform throughout the same brain — an observation that has some support from experimental head injury [7, 24]. For example, axonal bulbs seemed to be more developed in one area than in others, and there appeared to be variability in the time at which bulbs no longer stained positively for βAPP. It may be that there is a spectrum of evolving axonal pathology after head injury [5, 7, 16, 20], and that precise dating of histological changes, which is often an issue in forensic cases, may not be possible, beyond the broad outline of 'days', 'weeks', 'months', 'years', originally given by Adams *et al*. [2]. As an example, with the microglial marker, occasional microglial clusters were identified as early as 10 to 11 days, which is much earlier than can be reliably demonstrated with a Nissl stain, but it was not until about 2 weeks survival that they were present in any numbers.

Finally, different problems of interpretation may be posed by finding scattered microglial clusters in hemispheric white matter in a subject who has had only a short survival after fatal head injury, as seen in Case 4 in this series. In our experience, this is not an uncommon finding in a population that includes a high number of assault cases, and appears to indicate previous, presumably mild, head trauma. In many forensic cases an accurate 'history' is lacking, but it is important to rule out other possible causes — e.g. a previous episode of global hypoxia, or viral encephalitis, particularly HIV encephalitis — before ascribing the finding to traumatic axonal damage.

Summary

In conclusion: A simple restricted sampling technique such as the one used in this study does not provide a reliable basis for the diagnosis of DAI. Early detection of βAPP positivity should be interpreted with extreme caution. With very short survival times of less than 24 h, the most useful statement about βAPP immunostaining may be that it is absent, *in a properly sampled brain*; this suggests that there is no DAI. With survival over 24 h, however, a panel of immunohistochemical markers comprising βAPP in combination with a microglial marker such as CD68, provides a satisfactory means of diagnosing traumatic axonal damage; for longer survival cases GFAP should be included. Even with these

immunostains, however, it is unlikely that precise dating of the histological changes will be possible.

References

1 Adams JH. Head injury. In *Greenfield's Neuropathology*, Eds JH Adams, LW Duchen, 5th edn. London: Edward Arnold, 1992: 106–152

2 Adams JH, Doyle D, Ford I *et al*. Diffuse axonal injury in head injury: definition, diagnosis and grading. *Histopathology* 1989; 15: 49–59

3 Aihara N, Hall JJ, Pitts LH, Fukuda K, Noble LJ. Altered immunoexpression of microglia and macrophages after mild head injury. *J Neurotrauma* 1995; 12: 53–63

4 Bazzaz AA, Bridges LR, Sivaloganathan S. Microglial activation in head injury: an immunohistochemical study using antibodies to CD68 (PG-M1) and CR3/43. *Neuropathol Appl Neurobiol* 1995; 21: 446–7

5 Blumbergs PC, Scott G, Manavis J *et al*. Staining of amyloid precursor protein to study axonal damage in mild head injury. *Lancet* 1994; 344: 1055–6

6 Blumbergs PC, Scott G, Manavis J *et al*. Topography of axonal injury as defined by amyloid precursor protein and the sector scoring method in mild and severe closed head injury. *J Neurotrauma* 1995; 12: 565–72

7 Christman CW, Grady MS, Walker SA, Holloway KL, Povlishock JT. Ultrastructural studies of diffuse axonal injury in humans. *J Neurotrauma* 1994; 11: 173–86

8 Elson LM, Ward CC. Mechanisms and pathophysiology of mild head injury. *Semin Neurol* 1994; 14: 8–18

9 Gean AD. *Imaging of Head Trauma*. New York: Raven Press, 1994: 220–41

10 Gennarelli TA. Cerebral concussion and diffuse brain injuries. In *Head Injury*, Ed PR Cooper, 3rd edn. Baltimore: Williams & Wilkins, 1993: 137–58

11 Gennarelli TA. Mechanisms of brain injury. *J Emerg Med* 1993; 1: 5–11

12 Gennarelli TA. The spectrum of traumatic axonal injury. *Neuropathol Appl Neurobiol* 1996; 22: 509–13

13 Gennarelli TA. Types and amount of axonal injury in traumatic brain injury. *Neuropathol Appl Neurobiol* 1996; 22 (Suppl 1): 44

14 Gentleman SM, Roberts GW, Gennarelli TA *et al*. Axonal injury: a universal consequence of fatal closed head injury? *Acta Neuropathol* 1995; 89: 537–43

15 Goodman JC. Pathologic changes in mild head injury. *Semin Neurol* 1994; 14: 19–24

16 Grady MS, McLaughlin MR, Christman CW *et al*. The use of antibodies targeted against the neurofilament subunits for the detection of diffuse axonal injury in humans. *J Neuropathol Exper Neurol* 1993; 52: 143–52

17 Graham DI, Gennarelli TA. Trauma. In *Greenfield's Neuropathology*, Eds DI Graham, PL Lantos, 6th edn. London: Arnold, 1996: 197–262

18 Graham DI, Lawrence AE, Adams JH *et al*. Pathology of mild head injury. In *Mild to Moderate Head Injury*, Eds JT Hoff, T Anderson, T Cole. Boston: Blackwell Scientific, 1989: 63–75

19 Graham DI, Lawrence AE, Adams JH, Doyle D, McLellan DR. Brain damage in non-missile head injury secondary to high intracranial pressure. *Neuropathol Appl Neurobiol* 1987; 13: 209–17

20 Maxwell WL, Povlishock JT, Graham DI. A mechanistic analysis of non-disruptive axonal injury. *J Neurotrauma* in press

21 McKenzie KJ, McLellan DR, Gentleman SM, Graham DI. Is beta-APP a marker of axonal damage in short-surviving head injury? *Neuropathol Appl Neurobiol* 1996; 22: 160

22 Mittl RL, Grossman RI, Hiehle JF *et al*. Prevalence of MR evidence of diffuse axonal injury in patients with mild head injury and normal head CT findings. *AJNR* 1994; 15: 1583–9

23 Ng HK, Mahaliyana RD, Poon WS. The pathological spectrum of diffuse axonal injury in blunt head trauma: assessment with axon and myelin strains. *Clin Neurol Neurosurg* 1994; 96: 24–31

24 Povlishock JT. Are the pathobiological changes evoked by traumatic brain injury immediate and irreversible? *Brain Pathol* 1995; 5: 415–26

25 Povlishock JT, Becker DP, Cheng CLY, Vaughan GW. Axonal change in minor head injury. *J Neuropathol Exper Neurol* 1983; 42: 225–42

26 Povlishock JT, Erb DE, Astruc J. Axonal response to traumatic brain injury: reactive axonal change, deafferentation, and neuroplasticity. *J Neurotrauma* 1992; 9: 189–200

27 Sherriff FE, Bridges LR, Gentleman SM, Sivaloganathan S, Wilson S. Markers of axonal injury in *post mortem* human brain. *Acta Neuropathol* 1994; 88: 433–9

28 Sherriff FE, Bridges LR, Sivaloganathan S. Early detection of axonal injury after human head trauma using immunocytochemistry for beta-amyloid precursor protein. *Acta Neuropathol* 1994; 87: 55–62

29 Teasdale GM. Head injury. *J Neurol Neurosurg Psychiatry* 1995; 58: 526–39

Received 9 October 1996
Accepted after revision 10 January 1997