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Scientific correspondence

Axonal injury in head injuries with very short survival times

The timing of head injuries resulting in axonal damage is a controversial area of forensic neuropathology. Using beta amyloid precursor protein (beta APP) immunocytochemistry to detect axonal injury, times of 105–180 min [1] and approximately 2 h [2] are stated in relevant authorities. However, a study of paediatric head injury reported beta APP positive nerve fibres 35–45 min after injury [3]. Furthermore, a recent study by Hortobagyi *et al*. [4] provided convincing evidence that traumatic axonal damage in the brain can be detected as early as 35 min following head injury using immunocytochemistry for beta APP. We have recently undertaken a study which provides evidence corroborating the findings of Hortobagyi *et al*.

The project was undertaken following the relevant guidelines [5] and with the authorization of the Grampian Research Ethics Committee. A total of 47 cases in which death had been either due to, or associated with, a head injury and where the survival time appeared short from the available records, were selected from the archives of the Department of Pathology at Aberdeen Royal Infirmary. Of these 47 cases most were road traffic accident fatalities where the deceased was dead at the scene of the accident. Forty of the cases were dead at the scene with a presumed survival of probably a few minutes. Four cases were described as dead at the scene but with projected maximum survival times taken as the difference between the time at which the deceased was found dead and the time last seen alive (three at less than 2 h, one at less than 5 min). A further three cases acted as internal quality controls in that they had known survival time of sufficient length to allow for the development of clearly identifiable changes of traumatic axonal injury using beta APP immunocytochemistry (one at 2 h 40 min, one at 6 h and one at 12 h 40 min). Blocks of paraffin-embedded brain tissue were retrieved from the archives. The blocks available were limited but usually included one or more of the following areas: corpus callosum, internal capsule and pons. For some cases midbrain and pontomedullary junction were available. Sections were cut and stained by a standard automated immunocytochemical method using a Zymed monoclonal antibody for beta APP at a 1/1000 dilution. Immunostaining was evaluated on a 7-point scale as follows:

0 – no staining
1 – positive neuronal staining
2 – faint blush of staining in axons +/– positive neuronal staining
3 – occasional beaded axons
4 – obvious swollen beaded axons
5 – axonal spheroids visible at low power
6 – intense axonal spheroids

The assessment was carried out on sections from all of the blocks available for each case and the mean score for the case as a whole calculated. The three internal quality control cases all showed a beta APP score of 5 or 6. Of the cases dead at the scene with a known survival time, two of the cases showed Grade 3 or 4 staining and two Grade 1 staining. Of the remaining 40 cases dead at the scene with the presumed short survival time, all showed some degree of staining although in seven of the cases this was only Grade 1 (that is, only neuronal staining). Of the remaining 33 cases, 17 showed Grade 2 staining, 14 Grade 3 and one each Grades 4 and 6.

Much of the staining in our cases was relatively subtle, that is, Grade 2 or 3. The interpretation of lesser degrees of staining is problematic particularly if the axonal pathology may be vascular in aetiology [2]. It could be argued that Grade 2 immunostaining (a faint blush in axons – Figure 1A) might be nonspecific and/or due to local vascular factors and therefore not of any significance in terms of the assessment of traumatic axonal injury. However, our Grade 3 immunostaining (Figure 1B) seems to equate to what Hortobagyi *et al.* describe as 'small granules' and 'axonal beads'. It is highly unlikely that the survival in most of our cases would have exceeded 35 min and therefore our results are broadly comparable with those of Hortobagyi *et al.*

It is likely that most, if not all, of the axonal damage identified by beta APP immunocytochemistry in our cases

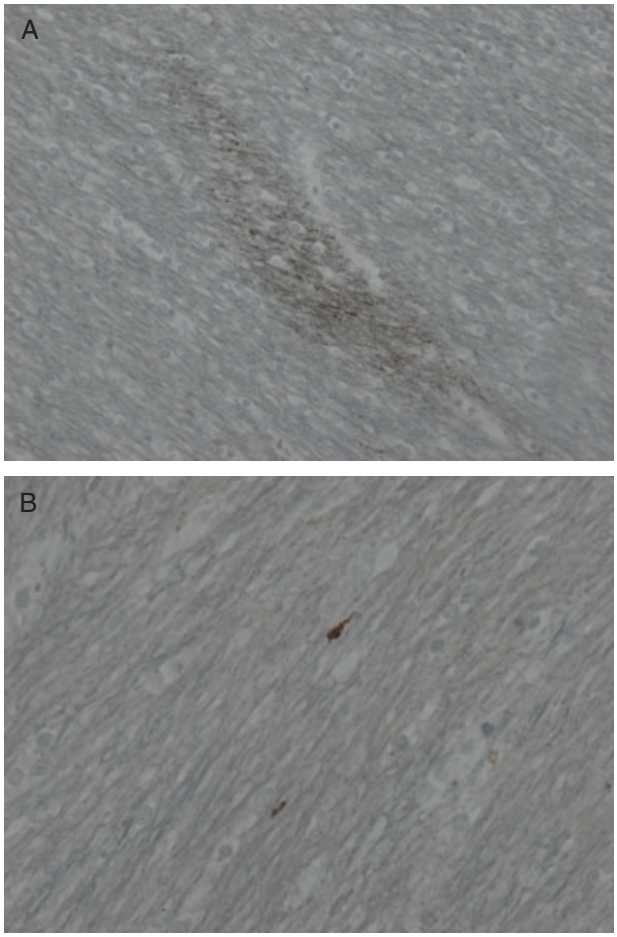

Figure 1. Beta APP immunostaining (A) Grade 2 (B) Grade 3.

was traumatic in aetiology, but given the limited number of anatomical sites from which blocks were available for study it is not possible to conclude whether the axonal injury was focal or diffuse.

In summary, therefore, our study provides further evidence of the usefulness of beta APP immunocytochemistry as a marker of axonal injury in forensic neuropathology practice. Using this technique, axonal injury can be identified in the brains of short survival (that is, less than 35 min) head injury cases.

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