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Beta-Amyloid Precursor Protein Staining of Nonaccidental Central Nervous System Injury in Pediatric Autopsies

R. ROSS REICHARD,[1] CHARLES L. WHITE III,[1,2] CHRISTA L. HLADIK,[2] and DAVID DOLINAK[3]

ABSTRACT

Immunohistochemical staining for beta–amyloid precursor protein (βAPP) is a well-established marker of traumatic axonal injury in adults. Recent studies have used similar techniques to evaluate nonaccidental central nervous system injury (NAI) in infants and young children. In this prospective study, we report the results of βAPP immunohistochemistry on the brain and spinal cord in 28 pediatric cases of NAI. βAPP-immunoreactive axons were present in 27/28 cases. Vascular axonal injury (VAI) due to brain swelling and secondary vascular compromise was the most common pattern of βAPP immunoreactivity and was detected in 22 of 28 cases. Traumatic axonal injury was detected in 19/28 cases, although only eight of these cases showed brainstem staining, thus fulfilling the criteria for the diagnosis of diffuse traumatic axonal injury (dTAI). TAI and VAI were both present in 16/28 cases. Isolated TAI and VAI occurred in three and five cases, respectively. All children with isolated VAI were <18 months of age. An additional finding highlighted by βAPP immunostaining was a penumbra of axonal injury adjacent to focal lesions, such as lacerations. We conclude that βAPP immunohistochemistry aids in documenting trauma in nonaccidental central nervous system injury in infants and young children and that VAI is a common finding.

Key words: Beta–amyloid precursor protein; Brain; diffuse axonal injury; immunohistochemistry; pediatric

INTRODUCTION

NONACCIDENTAL INJURY (NAI) of the central nervous system (CNS) in children typically involves children ≤3 years of age and is the most common cause of traumatic death in this population (Duhaime et al. 1998). The constellation of pathological findings used to diagnose this entity typically includes all or some combination of subdural, subarachnoid, retinal, and perioptic nerve hemorrhage. In cases of NAI, there is a spectrum of injury that may be found at autopsy, ranging from isolated head and/or neck injury to extensive torso and extremity soft tissue and skeletal injuries of similar or varying age. In this study, we focused specifically on pediatric homicides in which the cause of death was a direct result of CNS injury.

There have been several publications describing various aspects of the neuropathology of NAI in children. In 1969, Lindenberg and Freytag reported contusional tears in the cortex and white matter in infants secondary to an impact. Calder et al. (1984) subsequently reported in a review of 12 cases of NAI that there were three children

[1]Neuropathology and [2]Immunohistochemistry Laboratories, University of Texas Southwestern Medical School, Department of Pathology, Dallas, Texas.
[3]Southwestern Institute of Forensic Sciences, Dallas, Texas.

with white matter pathology in a distribution similar to that seen in adults after closed head injury, including involvement of the brainstem. Traumatic axonal injury of the corpus callosum and cerebral hemispheres was first described in infants <5 months old with closed head injury by Vowles et al. (1987). This report introduced the concept of diffuse axonal injury (DAI) as an integral component of NAI. However, these cases did not have brainstem axonal injury detectable by silver stains and therefore did not meet the specific pathological criteria of DAI as described by Adams et al. (1989).

Relatively recent studies have demonstrated that immunohistochemical staining for beta-amyloid precursor protein (βAPP) may be used to enhance early detection of axonal injury. Specifically, this immunostain enables detection of axonal injury when there is as little as 2–3-h survival time after head trauma (Sherriff et al., 1994; McKenzie et al., 1996). In 1998, Shannon et al. (1998) reported that hypoxic-ischemic injury and βAPP immunoreactivity were present in infants in both NAI and hypoxic encephalopathy. These researchers also reported that βAPP-immunoreactive axons were present in the cervical cord of NAI, but not controls. Gleckman et al. (1999) subsequently reported that βAPP immunostaining enhanced detection of DAI in infants with NAI. However, Geddes et al. (2001b) evaluated a series of 37 infants, 9 months of age or less, and concluded that hypoxic injury, not DAI, is the most common histological finding in NAI. In addition, they evaluated 16 children with NAI, 13 months to 8 years, and concluded that when traumatic axonal injury was present it was similar to DAI in adults. Their article further described axonal damage to the craniocervical junction of the younger population. They theorize that damage to this area results in apnea and begins a fatal cascade of hypoxic brain injury and brain swelling. In summary, there is no consensus regarding the precise nature, etiology, or primacy of βAPP-immunoreactive lesions in NAI.

In this study, we used routine neuropathological diagnostic techniques and βAPP immunohistochemistry to prospectively evaluate 28 consecutive cases of NAI in infants and young children. Specifically, our objective was to investigate the usefulness and limitations of βAPP immunostaining in this type of case. Our results demonstrate that βAPP-immunoreactive axons are virtually ubiquitous in cases of NAI, regardless of clinical history and reported survival interval. Vascular axonal injury (VAI) secondary to brain swelling with vascular compromise and resultant hypoxic-ischemic injury is a common pattern of immunoreactivity, particularly in the brainstem. TAI was also a common finding and was detected in all age ranges, although diffuse traumatic axonal injury (dTAI) per se is less common and more difficult to diagnose in the very young.

MATERIALS AND METHODS

Study Population

This prospective study was based on 28 consecutive brains and spinal cords of infants (0–12 months) and young children submitted from medical examiner autopsy material for neuropathological consultation of nonaccidental CNS injury for July 2000 to February 2002. The cause of death in all cases was "blunt force head injury" or a similar diagnosis such as "craniocerebral trauma." The manner of death in all cases was homicide. In all cases, there was a complete case file: completed autopsy report, toxicology, investigative report, and microbiological and metabolic studies when appropriate. Cases with extra-CNS injury as the cause of death or contributing to the cause of death were excluded. A small number of cases with a survival of >2 weeks were not included, because the focus of this study was evaluation of the role of βAPP immunostaining. We have observed that βAPP immunoreactivity fades with prolonged survival, and lack of staining in this situation may not be an accurate reflection of the initiating event and/or its direct manifestations.

Gross Evaluation and Histological Sampling

A complete neuropathological examination was performed after each brain and spinal cord was fixed in 20% formalin for a minimum of 2 weeks. The minimum standard set of $3 \times 2 \times 0.5$ cm histological sections included body of the corpus callosum with parasagittal white matter, splenium of the corpus callosum, fronto-parietal borderzone, hippocampus, posterior limb of the internal capsule with thalamus, rostral pons, medulla, spinal cord, and cerebellum. Histological samples were taken from both sides, although not necessarily bilateral. Adjacent sections were stained with hematoxylin and eosin (H&E) and βAPP immunohistochemistry. Brain swelling was defined as a brain weight greater than 10% above expected for age, length, and weight, or gross or microscopic evidence of herniation.

Immunohistochemistry

Paraffin sections were cut at 3 μ on a rotary microtome, mounted on positively charged glass slides (POP100 capillary gap slides, Ventana Medical Systems, Tucson, AZ), and air-dried overnight in front of a cool fan. Sections were then deparaffinized in xylene and ethanol, and placed in 200 mL of heat-induced epitope

retrieval (HIER) Biobuffer™ (BioPath Laboratories, Oklahoma City, OK), pH 6.8. The buffer was brought to a boil, after which 50 mL of deionized water was added. The buffer was again brought to a boil for 5 min, and then the slides were allowed to cool in buffer for 20 min, following which they were rinsed thoroughly in deionized water and then buffer. Sections were blocked with unlabeled avidin and biotin (Vector Laboratories, Burlingame, CA) for 15 min each, rinsing between each step with phosphate-buffered saline, pH 7.4. The remaining steps were performed at 4°C, except as noted. Staining was performed manually, with slides in a vertical position using capillary gap methodology. Buffers, blocking serum, secondary antibodies, avidin/biotin complex reagents, chromogen, and hematoxylin counterstain were used as supplied in the ChemMate™ secondary detection kit (Ventana Medical Systems). Optimum primary antibody dilutions were predetermined using known positive control tissues. A known positive control section was included in each run to assure proper staining. Sections were incubated in unlabeled blocking serum for 1 h to block nonspecific binding of the secondary antibody. Sections were then incubated for 1 h with either primary antibody (mouse anti-APP, Chemicon, Temecula, CA) at a 1:400 dilution in buffer, or with buffer alone as a negative reagent control. Following washing in buffer, sections were incubated for 1 h with biotinylated polyvalent secondary antibody solution (containing goat antibodies to rabbit, mouse, and rat immunoglobulin). Sections were then incubated for 25 min with freshly prepared alkaline phosphatase-conjugated avidin–biotin complex. Sections were then washed in buffer and then incubated with two freshly prepared changes of BT Red chromogen (a new fuchsin-type chromogen), 3.5 min each, followed by washing in buffer and then water. Sections were then counterstained at room temperature with Mayer's hematoxylin, dehydrated in a graded series of ethanols and xylene, and coverslipped. Slides were reviewed by light microscopy. Positive reactions with BT Red were identified as red reaction product. Sections were photographed using a CoolSNAP™ digital camera (RS Photometrics, Tucson, AZ).

Assessment

All histological sections were evaluated for the presence of hypoxic-ischemic injury using standard neuropathological criteria. White matter βAPP immunoreactivity was assessed and recorded by each observer using a semi-quantitative scoring technique similar to that described by Gentleman et al. (1995). The one exception to the grading system was the occasional isolated, but well-formed, axonal bulb. These findings were noted but not considered as "significant" staining and therefore did not contribute to the score in a given case. When characteristic patterns of axonal immunoreactivity were present on a given slide, this was noted. The observer then recorded a clinicopathological diagnosis of the types of axonal injury for the entire case.

βAPP immunostaining and silver staining techniques both detect axonal injury resulting from diffuse traumatic brain injury. However, the use of immunostains significantly enhances detection of axonal injury in general. Therefore, instead of attempting to compare to two somewhat disparate techniques, we used a classification system based upon βAPP immunostaining. The following clinicopathological diagnostic categories were used: multifocal traumatic axonal injury (mTAI), diffuse traumatic axonal injury (dTAI), vascular axonal injury (VAI), metabolic axonal injury (MAI), and penumbral axonal injury (PAI).

dTAI. dTAI was diagnosed when there was a history of trauma and scattered and/or groups of βAPP immunoreactive axonal swellings/bulbs were present within the corpus callosum, cerebal hemispheric white matter, and brainstem that do not have a pattern consistent with VAI. The immunoreactive axons of d/mTAI are typically scattered or in groups along the long axis of the axon (Fig. 1A–D).

mTAI. mTAI was diagnosed when there was a history of trauma and scattered and/or groups of βAPP immunoreactive axonal swellings/bulbs were present within the corpus callosum, cerebal hemispheric white matter, but not the brainstem, and did not have a pattern consistent with VAI. Non-human primate experiments demonstrate that brainstem lesions do not occur in the absence of supratentorial axonal injury. Furthermore, TAI within the brainstem as a result of acceleration/deceleration injury does not occur in isolation (Gennarelli et al., 1982).

VAI. VAI is a "zig-zag" pattern of axonal immunoreactivity due to vascular compromise, typically due to internal herniation that results in secondary hypoxic-ischemic axonal injury (Fig. 1E,F; Geddes et al., 2000). Therefore, VAI involves the parenchyma in characteristic vascular distributions and implies raised intracranial pressure, and both white and gray matter may be involved. Theoretically, vasospasm or other mechanisms of vessel compromise, such as compression of vessels by swollen hemispheric parenchyma, would result in similar patterns of immunoreactivity. The pattern of VAI is that of clusters of immunoreactive axons that are staining without regard for the course of the axon, that is, there is a well-demarcated area of staining within a long white

matter tract. In addition "punched-out" islands of immunoreactivity were also considered VAI: a perpendicular perspective of the "zig-zag" pattern. These staining patterns may be observed in cases without a history of trauma (Reichard et al., 2003).

MAI. MAI is scattered axonal immunoreactivity identified without a clinical history or pathological evidence of trauma and not occurring in a pattern typical of VAI. The term "metabolic" is used broadly here and may represent the global effects of a specific disturbance, for example, hypoglycemia (Dolinak et al., 2000b) or global hypoxic-ischemic injury, although unusual (Dolinak et al., 2000a). The underlying cause of MAI may not always be identifiable.

PAI. PAI is a descriptive phrase used to describe axonal immunoreactivity adjacent to isolated focal lesions, regardless of the etiology, such as lacunar infarcts, cortical lacerations, or an abscess (Fig. 1I). In other words, axonal damage around a laceration or infarct would be PAI, not TAI or VAI. The term PAI enabled observers to indicate the presence of an area of axonal immunostaining, often with a qualifier, without implying a more global process. For example, a cortical laceration is present with PAI, but there is not m/dTAI.

Multiple categories of axonal injury may be present in a single case.

RESULTS

See Table 1 for the complete data for each case. The results are summarized in Table 2. Of the 28 infants and young children in this study, 27 contained βAPP-immunoreactive axons. The survival period ranged from none to 13 days. The recorded length of survival was from the time when the individual presented to medical attention to when they were declared dead. All cases with resuscitation revealed white matter immunostaining. Only case 18, "found dead," revealed no βAPP-immunoreactive white matter. The longest survival interval was 13 days, case 17. In this case, there was extensive βAPP immunoreactivity with areas characteristic of VAI. However, the staining in a significant number of axonal processes had faded and precluded more specific interpretation.

DISCUSSION

The diagnosis of nonaccidental CNS injury in the pediatric population requires a detailed multidisciplinary evaluation. The pathological findings observed in isolation may not be pathognmonic of NAI, but when assessed within the context of complete case information, may be diagnostic. In this study, we address a specific component of the neuropathological examination, βAPP immunohistochemistry. The results indicate that βAPP-immunoreactive white matter is very common in pediatric NAI. Furthermore, the findings illustrate the utility of βAPP immunostaining in documenting injury and understanding the pathophysiology of NAI.

Interpreting βAPP immunohistochemical staining in the brain and spinal cords in cases of NAI requires integration of all available information. However, the clinical history provided by the caretaker is often inaccurate and may be intentionally misleading. These patients usually present gravely ill, and therefore it may be difficult to determine the exact initiating process. Other researchers have used the clinical presentation of apnea as a piece of evidence that respiratory compromise may be the initial clinical manifestation of traumatic injury (Geddes et al., 2001a,b). Although global hypoxic–ischemic injury (GHII) from apnea may be a critical component in this process, it is difficult to confirm the exact temporal sequence of events. Reviewing the clinical history and presentation of the cases in our study cardiopulmonary arrest likely reflects the terminal phase of a cascade of events that are traumatically initiated.

Careful review of the records in this study revealed that the caretakers typically provided a history of either minor trauma or that the child was found unresponsive or dead. For example, "fell in tub" or "put to bed" was the history in multiple cases. A history of vomiting and seizure activity was provided in several cases and suggests significant CNS pathology. However, whether these symptoms reflect raised intracranial pressure, post-traumatic events, or contemporaneous illness cannot be determined. In the cases we report, most presented comatose, in full cardiopulmonary arrest, or dead. A single case presented with a Glasgow Coma Score (GCS) of 9 and then rapidly became bradycardic with agonal breathing and progressed to brain death. The remaining cases presented with a GCS of <7 and rapidly deteriorated to brain death. Intracranial monitors were not routinely placed, but brain swelling and anoxic brain injury were typically documented by imaging studies. Although respiratory compromise likely plays a significant role in these deaths, it is clear that the children are frequently brought to medical attention in a state of severe neurological dysfunction. The complexity of interpreting the history provided by the caretakers in most cases precludes much of the usefulness of this information, and reliance on such potentially inaccurate historical events could lead to inaccurate interpretation of findings.

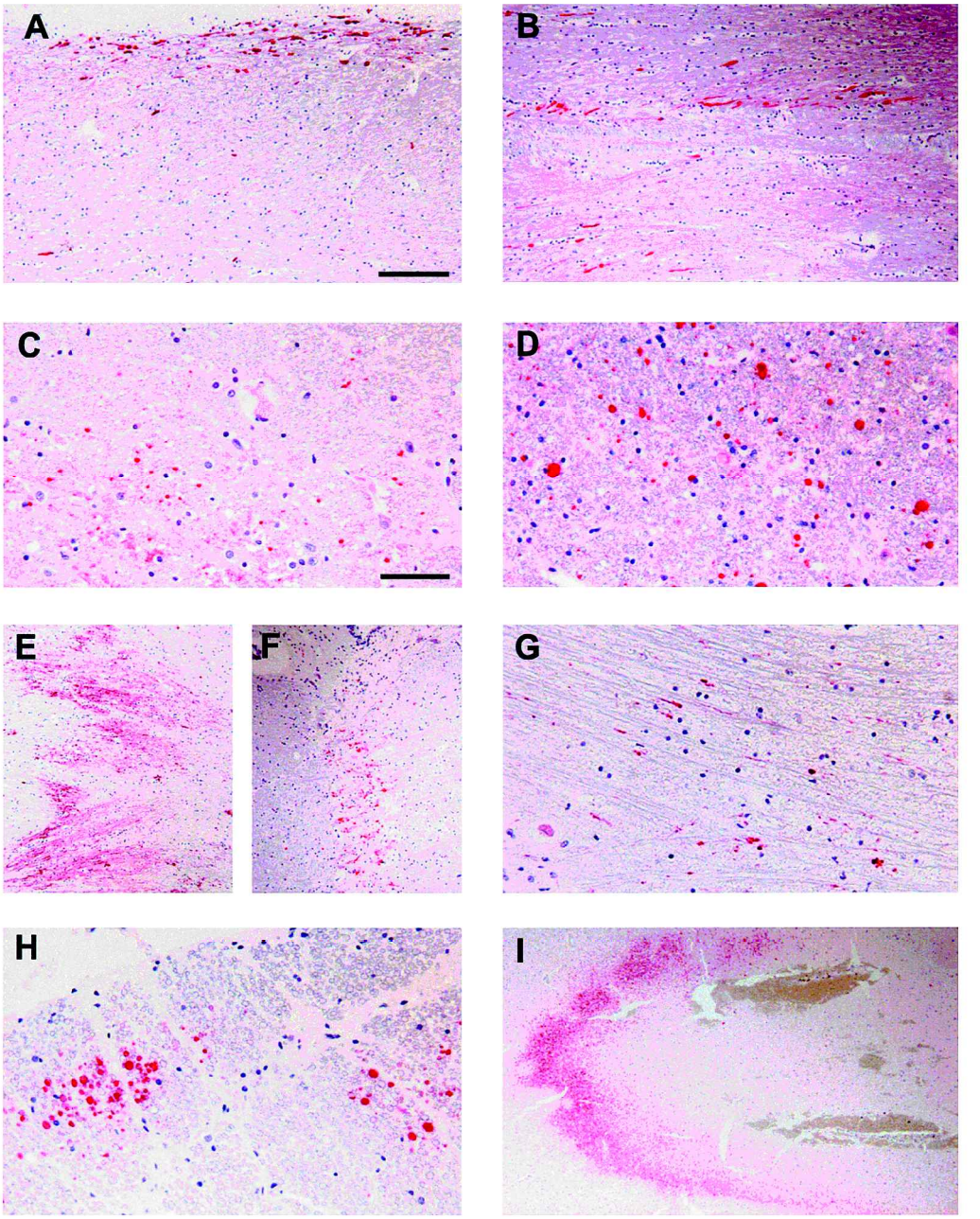

FIG. 1. βAPP immunostaining findings in NAI. (A–C) Diffuse traumatic axonal injury. (**A**) Corpus callosum, body (case 26). (**B**) Internal capsule (case 26). (**C**) Superior cerebellar peduncle, cross-sections of axonal swellings bulbs (case 26). (**D**) Medullary pyramid, cross-section of axonal swellings/bulbs (case 12). (**E**) Midline VAI, pons (case 21). (**F**) Midline VAI, medulla (case 4). (**G**) Inferior olive axonal swellings (case 4). (**H**) Cervical spinal cord lateral corticospinal tract (case 12). (**I**) Cortical laceration with PAI (case 6). Bar = 400 μ (A,B,E,F,I); 200 μ (C,D,G,H).

βAPP immunoreactivity was present in 27/28 cases, regardless of the reported length of post-event survival as reported by caretakers or by medical records. For example, three of four cases with no recorded survival interval demonstrated βAPP-immunoreactive white matter, indicating some period of post-injury survival. Presumably, in the one case without immunoreactive white matter, the child died rapidly as a result of the initial blunt force head injury. We have found that βAPP-positive axons are not normally present in the CNS of infants and

Table 1. Case Data

Case	Age	LOS	SDH	SAH	BS	GHII	Borderzone	CC-Body	CC-LGB	IC/Thalamus	Hippo	Midbrain	Pons	Medulla	Spinal cord	Cerebellum	mTAI	dTAI	VAI	PAI	MAI	Extra-CNS	Skull Fx.	Lac/cont
1	6w	10d	+	+	+	+	+++	NE	NE	+++	NE	NE	+++	+++	+++	+++	+	−	+	−	−	−	−	+
2	2m	R,2.5h	−	+	−	−	+	++	++	++	−	NE	−	+	−	−	−	−	+	−	−	−	−	+
3	2m	R,1h	+	+	+	+	−	NE	NE	−	+++	NE	+++	NE	+++	+++	−	−	+	−	−	−	−	−
4	5m	2d	−	−	+	+	NE	+++	NE	+++	NE	NE	+++	+++	+++	+++	−	−	+	−	−	−	−	−
5	6m	24h	+	+	+	+	+	+	++	−	+	NE	+	−	−	+	−	−	−	−	+	−	−	−
6	7m	R,1h	+	+	+	−	−	−	+++	+++	+	−	−	−	−	−	+	−	−	+	−	−	−	+
7	7m	2d	+	+	+	+	−	+	NE	+	−	−	−	+	−	−	−	−	−	−	+	−	−	−
8	10m	7d	+	+	+	+	+++	++	+++	+++	+++	++	++	+	−	+	+	+	+	−	−	−	+	−
9	11m	18h	+	−	+	+	−	+++	++	+++	+++	−	++	+++	−	−	+	−	+	−	−	−	−	−
10	12m	R	+	+	−	−	−	+	+	+++	+	−	−	++	++	++	+	−	+	−	−	−	−	−
11	18m	4d	+	+	+	+	++	+++	++	+++	++	−	−	−	−	NE	−	−	+	+	−	−	−	−
12	18m	R,>2.5h	+	+	−	−	++	+++	+++	++	+	NE	+++	+++	+++	−	−	−	+	+	−	−	−	+
13	22m	48h	+	+	+	+	−	+++	+++	++	+++	NE	+++	+++	+++	+++	+	+	+	−	−	+	−	−
14	23m	35h	+	−	−	+	+++	++	+++	+++	+++	NE	++	−	−	+++	+	−	+	−	−	−	+	−
15	26m	N	+	+	−	−	−	++	−	+	−	NE	−	−	−	−	+	−	−	+	−	−	+	+
16	27m	36h	+	+	+	+	+++	+++	+++	+++	++	+++	+++	+++	+++	++	+	+	+	+	−	−	−	−
17	27m	13d	+	+	+	+	+++	NE	NE	+++	+++	+++	+++	+++	NE	+++	NI	NI	+	−	−	−	−	+
18	30m	N	+	−	−	−	−	−	−	−	−	NE	−	−	−	−	−	−	−	−	−	+	−	+
19	32m	>24h	+	−	+	+	+	+++	+++	+++	+++	NE	+++	++	++	++	+	+	+	+	−	−	+	+
20	34m	6d	+	−	+	+	++	+++	+++	++	++	NE	+	−	−	++	+	−	+	−	−	−	−	−
21	36m	2d	+	+	+	+	+++	+++	+++	+++	−	+++	+++	+++	+++	+	+	−	+	−	−	−	−	−
22	36m	5d	+	+	+	+	+++	+++	+++	+++	+++	NE	++	++	+	+	+	−	+	−	−	−	+	+
23	37m	7d	+	+	+	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	−	+	−	−	−	−	−
24	37m	18h	+	+	+	+	++	+++	+++	+++	++	+++	+++	+++	−	+	+	+	+	−	−	+	−	+
25	38m	21h	+	−	+	+	+++	−	+++	+++	+	+++	+++	−	−	++	+	+	+	−	−	−	−	−
26	4y	N	+	+	+	−	++	++	+++	+++	+++	+++	++	+++	−	++	+	+	+	−	−	+	+	+
27	5y	N	+	+	+	−	++	+++	+++	+++	++	+++	+++	NE	++	++	+	−	+	−	−	−	+	+
28	7y	R	+	+	−	−	+++	+++	NE	+++	+++	NE	+++	−	−	+++	+	+	+	+	−	−	+	+

w, weeks; m, months; y, years; LOS, length of survival; d, days; h, hours; R, resuscitation (±1 hr); N, none; SDH, subadural hemorrhage; SAH, subrachnoid hemorrhage; BS, brain swelling; GHII, global hypoxic-ischemic injury; CC-B, corpus callosum body; CC-LGB, corpus callosum level of lateral geniculate body; IC/Thal, internal capsule and thalamus; Hippo, hippocampus; mTAI, multifocal traumatic axonal injury; dTAI, diffuse traumatic axonal injury; VAI, vascular axonal injury; PAI, penumbral axonal injury; MAI, metabolic axonal injury; Extra-CNS, extra-central nervous system injury; Skull Fx., skull fracture; Lac./Cont., laceration and/or contusion; NE, not examined; NI, not interpretable.

Table 2. Results Summary

Subdural hemorrhage	26
Subarachnoid hemorrhage	21
Brain swelling	21
Global hypoxic-ischemic injury	18
Skull fracture	8
CNS laceration/contusion	13
mTAI	19
dTAI	8
VAI	23
PAI	7
MAI	2

young children (Reichard et al., 2003). In this study, there is an insufficient number of well-documented cases of traumatic injury to definitively determine the minimal length of survival necessary to highlight damaged axons; however, based upon the limited number of cases, it appears to be comparable to the 2–3-h survival interval necessary in adults.

The most common type of axonal injury identified was VAI, with 22 cases. Geddes et al. (2000, 2001a,b) has described it as occurring secondary to the vascular compromise resulting from internal herniation due to raised intracranial pressure. Complicating the understanding of this process is that brain swelling is inherently difficult to assess in children, particularly in the very young, where clinical signs and gross pathological changes of internal herniation are difficult to appreciate. In our study, VAI was present in both the youngest (6 weeks) and the oldest (7 years old) decedents and at many ages between these. Brain swelling was detectable in 16 of these cases. VAI occurred in isolation in five cases; of these, three had brain swelling, and four were less than 6 months of age. The axonal injury appreciated with βAPP immunostaining may highlight secondary changes of raised intracranial pressure (VAI) even when brain swelling is not grossly apparent.

VAI has been reported to have a characteristic "zigzag" or "geographic" pattern of immunoreactivity, and to occur in characteristic regions within the CNS. Specifically, it has been described in the diencephalon and brainstem of infants and young children (Geddes et al., 2001a,b) and in the pons of adults (Oehmichen et al., 1999). Our data revealed midline brainstem (Fig. 1E,F) and/or inferior olive white matter (Fig. 1G) immunoreactivity in 18 cases, of which 16 had evidence of brain swelling. Of course, these cases also had evidence of trauma, but we concur with Oehmichen et al. (1999) that midline brainstem axonal immunoreactivity reflects early ischemic damage. Perhaps these findings parallel the pathophysiological process of secondary brainstem hemorrhages more commonly seen in adults. In two pediatric cases from our files without any evidence of trauma, there were similar staining patterns in the brainstem. Based on these data, we suggest that midline brainstem βAPP immunoreactivity in infants and young children most likely reflects axonal injury secondary to vascular compromise due to raised intracranial pressure as opposed to direct traumatic axonal injury. In contrast, the lack of the characteristic VAI staining pattern within the inferior olives suggests that the caudal displacement of the brainstem results in mechanical injury of these axons (Fig. 1G). In addition to these findings, we frequently noted VAI within areas in the characteristic distribution of dTAI, including the corpus callosum, internal capsule and superior cerebellar peduncle (Fig. 1A–C). These findings illustrate that the simple presence of βAPP-immunoreactive axons is not sufficient in itself for the diagnosis of dTAI. Specifically, TAI within the brainstem was only diagnosed when there were scattered and/or groups of axonal swellings/bulbs, not in the pattern of VAI or located within the midline or hilus of the inferior olive. However, VAI and TAI can be diagnosed in the same case, or even within the same histological section. The presence of extensive VAI may have precluded the identification of TAI in some cases and thus resulted in an underappreciation of dTAI. The pattern and location of axonal staining must be interpreted within the context of the entire case to make an accurate diagnosis.

Our results indicate that TAI is common in NAI of all ages. Although dTAI does occur at all ages, it is more frequently diagnosed in children over 1 year of age. For example, we have observed two examples of dTAI in infants as a result of a high-speed motor vehicle accident: a 1-year-old and a 26-day-old infant. Our results indicate that TAI in children frequently occurs in conjunction with VAI (16/19 cases). In addition, 7/26 cases were noted to have immunostaining adjacent to focal lesions (PAI; Fig. 1I). Histological sections of all focal lesions were not routinely evaluated; therefore, this type of immunoreactivity may be underrepresented in our data table. These results clearly illustrate that multiple types of axonal injury can, and do occur in a single case.

Understanding the mechanism of injury and resultant pathophysiology of NAI from the perspective of the postmortem examination is extraordinarily challenging. Geddes et al. (2001a,b) has reported that the presence of axonal damage of the corticospinal tracts within the pons and medulla of infants suggests that neck injury may be a significant site of damage. We did not appreciate distinct corticospinal tract involvement within the pons; however, a mixture of descending fibers was βAPP-immunoreactive in cases of dTAI. In several cases, there

were βAPP-immunoreactive axons in the region of the lateral corticospinal fibers of the spinal cord (Fig. 1H). Axonal damage within the medullary pyramids was identified in three cases of dTAI; however, only case 12 revealed this finding without supratentorial evidence of TAI (Fig. 1D). In this 18-month-old child, there was extensive supratentorial VAI. A pathophysiologically analogous finding was that of cervical spinal cord disruption and supratentorial VAI in case 4, a 5-month-old child. In addition, VAI was present in three children, all less than 5 months of age, without any concomitant TAI. Interestingly, case 26 (4 years old), revealed both dTAI and βAPP-immunoreactive axonal swellings within the pyramids. These findings demonstrate that, although certain types of axonal injury may be more common in different age ranges, overlap does occur.

The data in this study add to our understanding of NAI, but also raise many questions. The presence of TAI and VAI in our series is consistent with the findings of other researchers. The presence of SDH in virtually all cases is consistent with reports of this lesion as a marker of traumatic CNS injury in infants and young children. However, it has been demonstrated in non-human primates that, as the duration of acceleration/deceleration increases, the severity of axonal injury increases and the likelihood of SDH decreases (Graham, 1997), although there are clearly cases that have both SDH and dTAI. This is consistent with our finding of a relatively low incidence of dTAI compared with the high frequency of SDH. We have observed dTAI in cases of severe accidental injury; however, in contrast to severe head injury in adults, TAI within the brainstem is relatively uncommon. The brainstem may be injured in children, for example, axonal swellings/bulbs within the medullary pyramids, but the distribution may be different than in adults. These findings suggest that brainstem involvement in severe head injury is different in infants and young children than adults. Furthermore, our data revealed brain swelling in all cases with significant survival. This finding is in contrast to that of adults with dTAI without mass lesion, in which brain swelling is distinctly uncommon (Lee et al., 1998). Brain swelling in the pediatric population may be a result of post-traumatic cerebral edema or global hypoxic-ischemic injury from either compromise of the airway or some other mechanism. The rapidity of the brain swelling makes the presence of the subdural hemorrhage, or its breakdown products, as a cause seem less likely.

This study demonstrates that axonal injury as a result of trauma and hypoxic-ischemic injury secondary to vascular compromise are common in NAI. Our findings are consistent with those of other researchers, although we found slight differences in distribution of injury and the relationship with age. Clearly, as the infant CNS and skeletal system mature, different responses to trauma would be expected. Establishing characteristic patterns of injury on limited case numbers is challenging. However, reporting and confirming pathological findings with sensitive tools such as βAPP immunohistochemistry is an important step in understanding and diagnosing NAI accurately and consistently.

The interpretation of βAPP-immunoreactive axons in NAI requires the pathologist to recognize patterns of immunoreactivity and distribution of injury. The integration of these findings into an understanding of traumatic CNS injury and its associated complications within the total case context allows more accurate diagnosis, and will aid in planning future investigations directed at elucidating the mechanisms and pathophysiological processes involved in NAI, not only in infants and young children, but also in adults.

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Address reprint requests to:
Ross Reichard, M.D.
922 Varian Way
Palo Alto, CA 94304

E-mail: Ross@ud.net